Desiccation stress of insect-killing nematodes induces the accumulation of a novel heat-stable protein

Solomon AHARON [1], Salomon RAFFI [2], Paperna ILAN [1] and Glazer ITAMAR [3]

[1] *Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences of the Hebrew University of Jerusalem, Rehovot 76100, Israel*
[2] *Department of Virology, ARO, The Volcani Center, Bet Dagan, 50250, Israel*
[3] *Department of Nematology, ARO, The Volcani Center, Bet Dagan, 50250, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Infective juveniles (IJ) of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae can actively locate, infect and kill, with the cooperation of a symbiotic bacterium, a wide range of insect species. Both *Steinernema* and *Heterorhabditis* pass through four juvenile stages in the insect host before maturing. Only the IJ (third-stage larva) can survive outside the insect host and move from one insect to another. These parasitoid nematodes are considered among the most promising alternatives to chemical control of insect pests. However, their sensitivity to desiccation stress and other environmental constraints reduces their field efficacy. Therefore, in order to understand better the molecular mechanisms which elicit resistance to desiccation stress in entomopathogenic nematodes, we identify a novel water-stress-related protein which is induced in *Steinernema feltiae* (IS-6) during the process of inducing the IJ into a quiescent anhydrobiotic state. The protein was found to be heat-stable with a molecular mass of 47 kDa (designated Desc47). Desc47 accumulated about 10-fold (from 7.84 $\pm$ 1.85 to 74.09 $\pm$ 4.35% relative content level [RCL]) in dehydrated clumps of IJ, which lost 34.4% of their initial water content (WC) (from 65.1 $\pm$ 1.7% to 42.7 $\pm$ 0.72%) in a desiccation-tolerance-inducing treatment (97% relative humidity [RH] for 3 days). The appearance of Desc47 was accompanied by trehalose accumulation (from 300 to 600 mg trehalose/g protein). Trehalose is known to be accumulated to high concentrations by organisms that naturally survive dehydration. A second cycle of IJ dehydration did not alter the RCL of Desc47 (79.3% for the first cycle and 73.3% for the second cycle). Desc47 retained its high RCL (69.7%) in rehydrated active IJ for 3 days, reaching 51.2% of its initial RCL only after a week. No homology to other known proteins was found by mass-spectrometry electrospray-ion-trap analysis. However, from five obtained sequences of the protein (ranging from 10 to 21 amino acids), the 21-amino-acid peptide N V A S D A V E T V G N A A G Q A G (D/T) A V showed 82% similarity and 59% identity to the *Caenorhabditis elegans* late embryogenesis abundant (LEA) homologue protein, and 79% similarity and 53% identity to the LEA group 3-like protein from the fern *Onclea sensibilis*. LEA proteins are a diverse group of water-stress-related proteins, heat-stable, that are expressed in maturing seeds, under cold stress and during water deficit in the vegetative tissues of higher plants. In plants, accumulation of LEA is also accompanied by the accumulation of soluble sugars such as sucrose. These water-stress-related proteins and soluble sugars have been suggested to play a major role in desiccation tolerance. Our findings in the present study may indicate a common molecular mechanism in nematodes and plants which elicits resistance to desiccation stress.

Root-knot nematode project expressed sequence tag (EST)

Pierre ABAD, Philippe CASTAGNONE-SERENO and Marie-Noëlle ROSSO

INRA, Unité Santé Végétale et Environnement, Nematology Department, B.P. 2078, 06606 Antibes Cedex, France

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

With the aim to identify root-knot nematode genes involved in plant parasitism, *e.g.*, migration in the root tissue, feeding cell induction, virulence phenotype, we are developing a large scale EST project (5000 clones). A cDNA library (Zap vector) was constructed from invasive second-stage juveniles of *Meloidogyne incognita.* Before sequencing, the quality of the library was checked by analyzing the number of primary recombinants ($> 10^6$) and the length of the inserts (> 1 kb). Here we report the data obtained from 300 sequenced clones. The mRNAs could be grouped in three main categories: *i*) sequences with no homology in databases; *ii*) genes encoding housekeeping proteins such as those involved in translation or key enzymes in metabolic pathways, and, more interestingly; *iii*) genes putatively involved in nematode parasitism *e.g.*, genes encoding proteins involved in protection against oxidative stress. The proportion of genes encoding potentially secreted proteins was evaluated. These preliminary results will be discussed in relation to current knowledge of plant-pathogen interactions.

Effect of temperature and pH on development of nematode trapping fungi

Aniko ANTAL [1], Erzsebet DORMANNS-SIMON [2] and C. Johannes KOK [3]

[1] *Pannon University of Agricultural Sciences Georgikon Faculty, Department of Entomology, Deak F. str. 57, 8360 Keszthely, Hungary*
[2] *Csongrad County Plant Health and Soil Conservation Station, Biological Control and Quarantine Development Laboratory, P.O. Box 99, 6800 Hodmezovasarhely, Hungary*
[3] *IPO Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The purpose of these studies was to determine the effect of environmental factors on potential biological agents. The experiments were carried out by nematode trapping fungi belonging to *Arthrobotrys* and *Monacrosporium* genera, suitable for biological control of *Meloidogyne* nematodes. The trials were carried out *in vitro* on PDA plates. The examined temperatures ranged from 5 to 35°C, in 5°C intervals. The pH intervals ranged from 3 to 11. At 5°C the isolates practically did not grow. At 10°C, growth was poor, except for the *Monacrosporium* strains that showed better activity. In the interval from 15 to 30°C, the growth of practically all strains was fast. At 35°C, development stopped and some strains were damaged by this temperature. Altogether it was established that *Monacrosporium* isolates preferred lower temperatures compared with *Arthrobotrys* isolates. Concerning the effect of pH, the best growth was observed at pH 7. The isolates did not grow at pH 3, 4, and 5. At pH 6, fast development was observed initially but then growth slowed down. At pH 8, 9, 10, and 11 development was slow and differences between isolates were not significant.

Influence of climatic and crop management conditions on *Xiphinema index* populations and GFLV spread in Castilla-La Mancha (Spain) vineyards

María ARIAS [1], Jesús FRESNO [2] and Antonio LÓPEZ-PÉREZ [1]

[1] *Dpto Agroecología, Centro de Ciencias Medioambientales, CSIC. Serrano 115 dpdo, 28006 Madrid, Spain*
[2] *Dpto de Mejora Genética y Biotecnología, SGIT-INIA, Ctra de la Coruña Km 7.5, 28040 Madrid, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A study on *Xiphinema index* and GFLV incidence has been carried out in Castilla-La Mancha (Spain) vineyards, where the climatic conditions and traditional crop management prevent the pathogen development. Many plants died because of the dry climatic conditions (rainfall rates about 300 mm) in 1988-1996. Therefore, different irrigation systems were introduced to grow plants and increase their production. The influence of climatic and crop management conditions are analyzed by comparing the situation in vineyards with drop irrigation or under traditional management and between different rainfall periods. Results show that in vineyards where irrigation had been introduced, yellow mosaic, sometimes plus veinbanding, symptoms appear, GFLV dispersal and *X. index* populations increases occur, while in those under traditional management disease and pathogens remain stable for years. On the other hand, in vineyards with virus and nematode presence, only isolated wood and fanleaf symptoms in small patches were observed in dry periods, the yellow mosaic and veinbanding symptoms becoming patent since 1996 to 1999 when the climatic conditions changed (rainfall rates over 600 mm). It is concluded that on introducing irrigation, the risk of these pathogens increasing must be considered before the introduction of new management techniques.

Durable resistance management of soil-borne quarantine nematode pests *Meloidogyne chitwoodi* and *M. fallax*

Jaap BAKKER [1], Wladyslaw GOLINOWSKI [2], Richard JANSSEN [3], Joka KLAP [4], Didier MUGNIÉRY [5], Mark S. PHILLIPS [6], Michaella SCHLATHÖLTER [7] and J.G. Hans VAN DER BEEK [3]

[1] *Laboratory of Nematology, Wageningen University and Research Centre, P.O. Box 8123, NL-6700 ES Wageningen, The Netherlands*
[2] *Department of Botany (SGGW), Faculty of Agriculture, Warsaw Agricultural University, Rakowiecka 26/30, P-02-528 Warsaw, Poland*
[3] *Plant Research International, Wageningen University and Research Centre, P.O. Box 16, NL-6700 AA Wageningen, The Netherlands*
[4] *Barenburg Research, Duitsekampweg 60, NL-6874 BX Wolfheze, The Netherlands*
[5] *Laboratoire de Zoologie, Institut National de la Recherche Agronomique, Domaine de la Motte, P.O. Box 29, F-35160 Le Rheu, France*
[6] *Department of Nematology, Scottish Crop Research Institute, Invergowrie, DD2 5DA Dundee, United Kingdom*
[7] *P.H. Petersen Saatsucht Lundsgaard, Streichmühler Strasse 8, D-24977 Grundhof, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The EU-funded project QLRT-1999-1462 DREAM (Durable Resistance Against *Meloidogyne*) aims to contribute to sustainable production systems by developing a strategy for durable resistance management for these soil-borne pests. This is necessary in order to solve the world-wide problem of the use of very toxic chemicals to control the soil-borne pests. This study will use the two quarantine organisms *Meoidogyne chitwoodi* and *M. fallax* which are an important economic threat and for which no adequate durable alternative exists. The objective will

be achieved by integrating expertise in breeding, nematology, botany and molecular biology into one project, novel by its European dimension. The project combines three areas of research: *i*) identification and incorporation of resistance in important arable crops; *ii*) study of durability of the resistance and *iii*) optimising production systems by rotation schemes. The main results expected are: resistant germplasm; well characterised pathogen collections; reliable selection methods; knowledge on the stability of resistance, molecular markers linked to resistance and (a) virulence; reliable tools for breeding methods, resistant germplasm, genetic maps, and advice about improved rotation schemes.

Development and evaluation of new sampling methods for fields with infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*)

Thomas H. BEEN and H. Corrie SCHOMAKER

Plant Research International, Postbox 16, 6700 AA, Wageningen, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

An extended version of the computer program 'SAMPLE' was used to develop new sampling methods for the detection of infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). By combining a model for the medium scale distribution of cysts, which provides the expected population densities at each position within the focus, and a model for the small-scale distribution within square meters, sampling procedures can be simulated. SAMPLE 4 also takes into consideration the bivariate normal distribution of the parameters (length and width gradient) of the medium scale distribution to calculate average detection probabilities over the range of possible shapes of a standard infestation focus (central population density 50 cysts/kg soil). This infestation focus is small enough to use resistant potato varieties as a control measure without noticeable yield reductions in a 1:3 year cropping frequency of potatoes. In that way the 'worst case scenario' used in earlier approaches, requiring large soil samples, could be avoided. Several new versions of high performance detection methods were developed. For ware potato growers sampling methods were developed whose intensity (amount of soil collected) depend on the cropping frequency of the farmer. SAMPLE V4 can be considered a tool to develop sampling methods on demand for every possible combination of characteristics required for use by seed and ware potato growers (recommendations for optimum control measures leading to maximum returns, IPM) and by governments (legislation, quarantine and export protection).

Biofumigation, solarization and nematode control

Antonio BELLO, José-Antonio LÓPEZ-PÉREZ, Luisa DÍAZ-VIRULICHE and Rafael SANZ

Dpto Agroecología, Centro de Ciencias Medioambientales, CSIC. Serrano 115 dpdo, 28006 Madrid, Spain

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The action of gases discharged by the decomposition of organic matter is studied for the control of nematodes and other phytopathogenic soil-borne organisms. Effectiveness is similar to conventional biofumigants, differing from polarisation in that temperatures over 30°C are not required. Therefore it can used in different seasons of the year and in areas having low temperatures, as well as on extensive crops. On the other hand, biofumigation acts in depth, in the case of nematodes solving problems of vertical dynamics, which is a problem to all mobile organisms, a problem caused when the soil is heated in polarisation. Results of the application of biofumigation techniques in extensive crops are shown under low temperature conditions and without the application of plastic covers. This is different from polarisation, although both techniques can be complementary, increasing their effectiveness in the case of phytopathogenic nematodes.

Gonad morphology and evolutionary relationships within plant parasitic nematodes

Wim BERT and Etienne GERAERT

University of Gent, Zoological Institute, Ledeganckstraat 35 B-9000 Gent, Belgium

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The morphology of the female reproductive system is important in nematode systematics. In this study the cellular morphology of the gonads of 60 different species of tylenchids is described and compared. Previous research suggested that uterine cells arranged in three rows represent a derived condition within Tylenchoidea; this is supported in this study. According to our results the Criconematoidea appear to have a close common ancestor with Tylenchidae. The economically very important sedentary endoparasitic nematodes display markedly different gonad morphology for each genus; this sheds a new light on the evolutionary relationships of this group. Within the genera, all species share typically similar gonad morphology except for *Meloidogyne ichinohei* that is different from other *Meloidogyne* species. DNA studies also demonstrated this species as being quite divergent from the other *Meloidogyne* species. The results, obtained by our methods to study the cellular morphology of the reproductive system, lead to a better understanding of the relationships within the plant parasitic nematodes.

Functional analysis of gene expression in giant cells

David BIRD [1], Ann GREENE[1,†], Hinanit KOLTAI [1], Jennifer SCHAFF [1], Jessica WATKINS [1] and Joseph KIEBER [2]

[1] *Plant Nematode Genetics Group, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA*
[2] *Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

We have identified a large suite of genes expressed in *Meloidogyne incognita*-induced tomato giant cells, and are interested in the regulatory cascades they define. These sequences are available in GenBank. Because root-knot nematodes produce biologically-active cytokinin, one approach we are pursuing is to map the spatial and temporal induction of cytokinin-responsive promoters during giant cell initiation, and during gall formation. Our initial experiments have employed the *IBC6* promoter (*Plant Cell* 10, 1009-19, 1998) driving GFP expression in tomato hairy roots. To account for the differential accumulation of detectable GFP mRNA *vs* GFP protein (10 min *vs* 10 hr) we are using *in situ* transcript localization as well as fluorescence microscopy. To assemble regulatory cascades it will be necessary to establish functional relationships (*e.g.*, by genetic epistasis), and we have begun to examine expression of selected giant cell genes in various backgrounds created by transgenic ablation. Null mutations established by anti-sense expression or co-suppression are confirmed by sensitive, *in situ* transcript localization. The use of constitutive (35S), giant cell specific (TobRB7) and glucocorticoid-inducible promoters (*Plant J.* 11, 605-12, 1997) will permit the role of these genes in the induction and maintenance of the pathological state to be established.

How nematode secretions work in plant cells

Teresa BLEVE-ZACHEO [1], A. LEONE [2], L. ROBERTSON [3], M.T. MELILLO [1] and C. FENOLL [1]

[1] *Istituto di Nematologia Agraria, Amendola 165/A, 70126 Bari, Italy*
[2] *Departamento de Biologia, UAM, 28049 Madrid, Spain*
[3] *Departamento Medio Ambiente, UCLM, Avda Carlos 111, S/N, 45071 Toledo, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The availability of secretions obtained by stimulation of J2 juveniles of *Globodera rostochiensis* allowed us to investigate, using the microinjection technique, whether secretions had the capacity to interact with injected cells. Cortical cells in the differentiating zone of root apices of potato and tomato explants were injected with a solution containing 0.02-0.4 μg/μl of secretions added to Lucifer yellow as a tracer. Injected roots collected and fixed at different times after injection were analysed at light and electron microscope level. Sectioned roots observed at light microscope revealed that the least amount of injected secretions were sufficient to induce a syncytium, as a conglomerate of fused protoplasts with dissolution of adjoining cell walls. Ultrastructural analyses of syncytia demonstrated cytological changes such as granular cytoplasm, abundant mitochondria and endoplasmic reticulum, plastids with starch grains, small vacuoles, and enlarged amoeboid nuclei. These cytological changes were similar to those induced by feeding nematodes on host roots. Microinjection of either buffer alone or heat-inactivated secretions did not give any syncytial induction. These preliminary studies provided the first direct experimental evidence that artificial injection of nematode secretions can interact with host cells and can induce a structure similar to feeding sites.

Virulence and avirulence in root-knot nematodes in Crete on tomato

Vivian C. BLOK [1], Miles R. ARMSTRONG [1], Emmanuel A. TZORTZAKAKIS [2] and Mark S. PHILLIPS [1]

[1] *Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Scotland DD2 5DA, UK*
[2] *Plant Protection Institute, National Agricultural Research Foundation, P.O. Box 1802, 71110, Heraklion, Crete, Greece*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In a survey of vegetable growing areas of Crete, the majority of the infestations were found to be *Meloidogyne javanica*. Most of these populations were avirulent on tomato carrying the *Mi* gene; however a few virulent populations were also identified. In an AFLP comparison of both virulent and avirulent populations, a very high mean similarity between these populations was found, suggesting that both virulent and avirulent populations probably derived from the same founder populations. We have been comparing expressed gene sequences of both virulent and avirulent populations using cDNA AFLPs and suppressive subtractive hybridisation to identify pathogenicity factors. We have also been using these techniques to identify host genes with altered expression during the compatible and incompatible responses.

Neuropeptide type sequences and activities in *Globodera* spp.

Vivian C. BLOK [1], Konstantina BOUTSIKA [1], Lydia CASTELLI [1], Mark S. PHILLIPS [1], Geert SMANT [2] and John T. JONES [1]

[1] *Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, Scotland DD2 5DA, UK*
[2] *Nematology Department, Wageningen Agricultural University, Wageningen, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The anatomy of the nervous system of nematodes is conserved, generally conforming to that described for *Caenorhabditis elegans*. However, there is an apparently rich diversity in putative signalling molecules. Several ESTs were identified with high homology to neuropeptide-type sequences during a small scale project with *G. rostochiensis*. FMRF-like motifs occur in these sequences and protease cleavage sites for processing neuropeptides are present. This sequence was also found to be expressed in *G. pallida*. One of the neuropeptide genes was shown to be expressed in the nerve ring using *in situ* hybridisation.

The feasibility of altering nematode behaviour by exogenous exposure to neuropeptides was also assessed in a plate assay. An increase in movement of PCN J2 was observed when exposed to one of the peptides suggesting that plant parasitic nematodes may be vulnerable to small peptide molecules and that such peptides may be useful for novel control applications.

Bursaphelenchus species in conifers in Europe and their determination

Helen BRAASCH [1], Kai METGE [2] and Wolfgang BURGERMEISTER [2]

[1] *Department for National and International Plant Health, Kleinmachnow Branch, Federal Biological Research Centre for Agriculture and Forestry, Stahnsdorfer Damm 81, D-14532 Kleinmachnow, Germany*
[2] *Institute for Plant Virology, Microbiology and Biosafety, Federal Biological Reseach Centre for Agriculture and Forestry, Messeweg 11, D-38104 Braunschweig, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Information on the distribution and reliable identification of the various *Bursaphelenchus* species in European conifer trees is of increasing importance due to the recent introduction of the pinewood nematode (*B. xylophilus*) into Portugal and the survey in Europe necessary to control its possible spread. Results from an EU-supported research project on European *Bursaphelenchus* spp. (Fair CT 95-83) and a list of European species with details on their distribution and hosts are presented. Key factors for the distribution of *Bursaphelenchus* species are the presence of host plants and the availability of suitable insect vectors. Different *Bursaphelenchus* species distribution has been found in Central and Southern Europe. The species are grouped by their morphological and molecular biological features. Appropriate identification methods based on selected morphological characters and rDNA sequence differences revealed by ITS-RFLP technique are emphasised.

Resistance of vegetable crops to *Meloidogyne* spp.: suggestion for a crop rotation system

Regina M.D.G. CARNEIRO [1], Onivaldo RANDIG [2] and Angela Diniz CAMPOS [2]

[1] *EMBRAPA/Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-970 Brasília, DF, Brazil*
[2] *EMBRAPA/Clima Temperado, C.P. 403, 96001-970 Pelotas, RS, Brazil*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A greenhouse test was carried out to determine the reproductive potential, resistance or susceptibility of *Meloidogyne javanica, M. incognita* race 3, *M. arenaria* race 2 and *M. hapla*, on 24 vegetable crops belonging to 14 botanical species. Sweet peppers (*Capsicum annuum*) cv. All Big and Híbrido Tongo, spicy dry cress (*Rorippa nasturtium*) and kale (*Brassica oleracea* var. *acephala*) cv. Manteiga are resistant to *M. javanica*. Cauliflowers (*Brassica oleracea* var. *botrytis*) cvs Teresópolis Gigante and Bola de Neve, spicy dry cress and kale cv. Manteiga are resistant to *M. incognita* race 3. Sweet peppers cvs All Big and Híbrido Tongo, spicy dry cress and kale cv. Manteiga are resistant to *M. arenaria* race 2. Squashes (*Cucurbita pepo*) cvs Caserta and Branca de Virgínea, melons (*Cucumis melo*) cvs Híbrido Piel de Sapo and Yellow Start, spinach (*Spinacea oleracea*) cvs Viro Flay and Nova Zelândia, cucumbers (*Cucumis sativus*) cvs SMR58 and Marketer and lettuce (*Lactuca sativa*) cv. Crespa Rápida are resistant to *M. hapla*. A crop rotation system alternating these vegetables is suggested for the growers in Rio Grande do Sul State, Brazil.

Screening the Commonwealth Potato Collection for novel sources of resistance to the Potato Cyst Nematode *Globodera pallida*

Lydia CASTELLI, Gavin RAMSAY, Sharon J. NEILSON and Mark S. PHILLIPS

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Professor Jack Hawkes' collection (the Birmingham Potato Collection) of wild species of potatoes has been recently incorporated into the Commonwealth Potato Collection, supplementing the range of species held. The material originates from several expeditions to the Americas and represents a potentially valuable source of resistance to the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*, both of which are serious pathogens of potatoes. The germplasm screened in this study included taxa mainly from Mexico, Bolivia, Argentina and Peru.

Seeds from 199 accessions, comprising 66 different species and focusing predominantly on material from the Birmingham Potato Collection, have been screened for resistance to *G. pallida* (Pa 2/3) which is the more important of the two species in the UK. A total of 10 seedlings for each outbreeding accession and 3 seedlings for each inbreeding accession were tested. Forty of the outbreeding and 28 of inbreeding accessions were found to be uniformly resistant, with a further 17 segregating for resistance. These accessions were from 34 different species of wild potato, of which 11 are believed to be novel sources, following an extensive search through all available databases on known sources of resistant to *G. pallida*. To identify genotypes with broad spectrum resistance the accessions will also be screened for resistance to *G. rostochiensis* as well as the temperate root knot nematode *Meloidogyne chitwoodi.*

Soil populations dynamics of *Heterodera schachtii*, using resistant cultivars of sugar-beet

Georges CAUBEL [1], Catherine PORTE [1] and Hervé MARZIN [2]

[1] *Institut National de la Recherche Agronomique, Zoologie, 35650 Le Rheu, France*
[2] *Laboratoire National Protection Végétaux, 35650 Le Rheu, France*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In France, *Heterodera schachtii* affect the level and regularity of sugar-beet yields, especially in short rotations. Since 1996, sugar-beet cultivars with resistance conferred by *Beta procumbens* are registered. In infested soils, root yield increase is 10-20%. The objective of this study was to evaluate the population effect over multiple years at different representative locations. Influence of resistant sugar-beet on nematode population dynamics was investigated in 14 naturally infested fields, with a range of initial population densities (*Pi*), using susceptible (Carat, Roberta) in comparison with resistant cultivars (Evasion, Nemakill, Mercure, Anema). Results indicated that the influence on soil *H. schachtii* population densities of these cultivars, resistant or not, is always correlated with *Pi*. At low initial infestations, final populations (*Pf*) are increased and at very low infestations, below 5 juveniles/g, multiplication may be high, even with a resistant cultivar. Reproduction rate was negatively correlated with *Pi* across environments of both resistant and susceptible cultivars. The relative performance of resistant and susceptible cultivars can be reliably predicted based on preplant egg densities. By use of a resistant cultivar, soil infestation decreased only at *Pi* higher that the economic threshold.

Magnetic capture of nematodes using antiserum and lectin coated Dynabeads

Qing CHEN, Lee ROBERTSON, John T. JONES, Vivian C. BLOK, Mark S. PHILLIPS and Derek J.F. BROWN

Mycology, Bacteriology and Nematology Unit, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The effective use of integrated management strategies requires simple, rapid and reliable identification and quantification of the plant parasitic nematodes present in a soil. Unfortunately, plant parasitic nematodes are small and extremely difficult to identify. Previous studies have demonstrated the potential for antibody-assisted identification of nematodes. We present an extension of this technology, using antibody or lectin coated magnetic beads (Dynabeads) to recover target nematodes from samples. First, we identified lectins and antisera that bound specifically and reproducibly to the whole surface of *Globodera rostochiensis* and *Meloidogyne arenaria*. We found that binding to the entire surface of the nematode was a prerequisite for efficient extraction that was related to strength of binding of the antisera to the nematode (as determined by immunofluorescence microscopy). Factors affecting the extraction efficiency when using lectin coated beads are currently under further investigation. This study expands the use of Dynabeads from cell and molecular biology to parasitology and shows, in principle, that Dynabeads coated with a probe of suitable specificity can be used to extract specific nematodes from test samples. This technology may ultimately be useful in "non-expert systems" for detection and quantification of nematode species.

Stem nematode resistance in white clover and variation in nematode virulence

Roger COOK and K. Anthony MIZEN

Institute of Grassland and Environmental Research, Aberystwyth, SY23 3EB, Wales, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Stem nematode (*Ditylenchus dipsaci*) is widespread on white clover (*Trifolium repens*) in grassland in England and Wales. White clover populations and cultivars are heterogeneous for many characters. Resistant germplasm has been selected by assessing symptoms after inoculation of meristems with nematodes. Twenty seven cultivars and 4 breeders' lines had a range from 0 to 30% resistant plants. The very large leaved cv. Aran had 64% resistant plants. None of this germplasm is suitable for use in long-term, grazed swards without further selection. We have developed white clover populations for use as resistant (R) and susceptible (S) standards in experiments and developed two resistant cultivars. Before releasing these, we are investigating variation in virulence characteristics of white clover stem nematode populations. Resistant clovers selected with a UK population were also resistant to populations from France and New Zealand but were fully susceptible to a population from Switzerland. This appears to be evidence for variation in virulence in natural populations of the nematode.

Bacterial symbionts in *Xiphinema americanum* s.l.

August COOMANS [1] and Tom VANDEKERCKHOVE [2]

[1] *Biology Department, Institute of Zoology, University of Gent, Belgium*
[2] *Biology Department and Department of Biochemistry, Physiology and Microbiology, University of Gent, Belgium*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The best known example of this phenomenon is *Wolbachia pipientis* in parasitoid wasps and other arthropods. *Wolbachia* has also been found in some nematodes, but in *Xiphinema americanum* s.l. other symbionts are present. They belong to a new clade of Verrucomicrobia. In the female the bacteria occur in the intestine and the ovaria; they are most obvious in the ovarial wall. A number of them enter the maturing oocytes where they further divide till about 250 bacteria are present in the egg. There they aggregate near the anterior pole and apparently become enclosed in the descendants of the E-founder cell. Indeed, endosymbionts are stored in intestinal cells, but not in genital cells of at least the first three juvenile stages. The transfer to the ovaria is supposed to occur during the last moult of the nematode. Molecular analyses show that the symbionts are species specific and that the symbiosis is a very old and stable one.

Selection of Belgian *Verticillium chlamydosporium* isolates for the control of *Meloidogyne javanica* in greenhouse crops

G. DE DEYN [1], T. DERYCKE [1], M. MOENS [1] and N. VIAENE [1]

[1] *Agricultural Research Centre, Department of Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

We obtained 92 isolates of *Verticillium chlamydosporium*, a fungus parasitizing nematode eggs, from 46 soil samples taken at 11 locations in Belgium. Fungal isolates should be virulent, establish well in the host plant rhizosphere and be easily produced when used in an integrated control strategy. *In vitro* virulence against *Meloidogyne javanica* of ten isolates was tested using egg masses. The percentage of infected eggs per egg mass varied from 49 to 96%. Test egg masses should be similar in composition, and contain few empty and fully developed eggs. We compared the composition of 50 egg masses produced on tomato roots in the glass house, 8, 10, 12 and 15 weeks after soil infestation. In a test performed in spring, 4 to 48% of eggs were empty. In summer, 32 to 61% of eggs were empty. The ability of 23 isolates to colonise plant roots was assessed *in vitro* on germinating barley seeds. Colonisation ranged from 0.5 to 87.6% of the total root length. Fifty isolates were tested *in vitro* for their dictyochlamydospore production in a wheat:sand mixture. At 25°C twenty isolates did not produce any dictyochlamydospores. The isolates scoring well for all three criteria were selected for pot tests.

Measurement of the infection potential of nematode-transmitted virus in soil

Ate DE HEIJ [1], Frans C. ZOON [1], F. Astrid DE BOER [1] and Cees J. ASJES [2]

[1] *Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands*
[2] *Bulb Reseach Centre, P.O. Box 85, 2160 AB Lisse, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Tobacco rattle virus (TRV) is transmitted by trichodorid nematodes and causes considerable losses in flowerbulbs and potato. Quantification of the infection potential of soil (IPS) is needed to assess the risk of infection in crops and effects of treatments in field and laboratory experiments. Counting vector nematodes is of little use, because not all nematodes are viruliferous. Therefore a method was designed to estimate the number of nematodes able to transmit TRV to a bait plant. Soil samples were divided over pots in replicated dilution series. Petunia bait plants were grown in each pot and subsequently root sap was tested in an ELISA. The most probable number (MPN) of virus-transmitting nematodes per unit soil was calculated by likelihood approximation of the number of positive bait plants in each dilution. MPN values in field experiments with tulip and gladiolus explained about 45% of the variance in the fraction diseased plants. Sources of variance in the method itself, in the sampling and in disease expression, are studied in order to decide whether development of IPS measurement for risk analysis at field scale is worthwhile.

Biochemical characterisation of pharyngeal secretions from *Heterodera schachtii* stage 2 juveniles

Jan DE MEUTTER [1], Tom TYTGAT [2], Greetje GHEYSEN [1], August COOMANS [2] and Godelieve GHEYSEN [1,3]

[1] *VIB, Dept of Plant Genetics, University of Gent, Belgium*
[2] *Institute of Zoology, University of Gent, Belgium*
[3] *Faculty of Agricultural and Applied Biological Sciences, University of Gent, Belgium*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Heterodera schachtii belongs to the group of sedentary plant parasitic nematodes which causes a lot of agricultural damage. In the roots of a host plant stage 2 juveniles induce specialised feeding cells, most probably by injecting secretions from the pharyngeal glands. Despite many efforts, the triggers responsible for this feeding site formation are still unknown. This research is aimed to gain insight into the composition and the function of the pharyngeal secretions from *Heterodera schachtii.* Therefore the culturing and the purification techniques were upscaled to assure a sufficient supply of material. A rapid nematode sterilisation method, based on a disinfectant treatment, was developed which did not compromise the production of secretions. An efficient way for producing secretions and different concentration methods for the recovery of secretory proteins in dilute solutions are presented and evaluated. The methodology presented should allow sequencing of proteins separated by PAGE.

Distribution of *Bursaphelenchus xylophilus* in pine logs and consequences for sampling

Lutgard DE WAEL [1], Nancy DE SUTTER [1], Renaat MOERMANS [1], Marc LINIT [2] and Maurice MOENS [1]

[1] *Agricultural Research Centre, Crop Protection Department, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium*
[2] *University of Missouri-Columbia, Department of Entomology, Columbia, Mo, 65211, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* is vectored by *Monochamus* spp. and is reported to cause severe pine wilt in several Asian countries and parts of North America. In the European Union, the nematode is classified as a quarantine organism. To develop appropriate sampling strategies, nematode distribution was monitored in 10 PWN-infected *Pinus sylvestris* logs (3-8 m long) cut into planks. A total of 5387 samples were collected with a joiner drill and extracted with a technique based on centrifugation. The concentration of nematodes in the trees did not follow any consistent pattern over the trees. The distribution of nematode numbers per 5 g of wood curls was skewed to the right (mean: 14.76; median: 4.00; st. dev.: 28.96). The proportion of the infected samples was 0.63. The presence/absence of PWN was related to observable characteristics (grub holes, colour, knots and resin) of the plank. The highest probability for detecting PWN is related to grey stained wood parts (0.80), followed by the presence of a grub hole (0.77). The lowest probability was observed in parts with obvious resin presence (0.38). The combination of characteristics did not increase the probability of detection. Sampling sites not showing any of the aforementioned characteristics still had a probability for detecting PWN of 0.65 on average.

Pathogenicity of the root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* on faba bean (*Vicia faba* L.)

Mauro DI VITO, Giovanni ZACCHEO and Fabio CATALANO

Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

An experiment was undertaken to investigate the effect of a range of inoculum densities of two Italian populations of *Pratylenchus neglectus* and *P. thornei* on the growth of faba bean in pots. Eighty-four clay pots of 1000 cm^3 each, filled with steam sterilized sandy soil, were used for each nematode species. Pots were sown with a single pregerminated seed of faba bean cv. Aquadulce. At plant emergence a suspension of juveniles and adults of one of the nematode species was poured into six holes around seedling roots to give initial population densities of 0, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 or 256 specimens/cm^3 soil. Data of fresh top weight of plants fitted the Seinhorst's model $y = m + (1 - m)z^{P-T}$. According to this model, tolerance limits (T) of faba bean to *P. neglectus* and *P. thornei* were 2 and 2.4 specimens/cm^3 soil, respectively. Minimum relative yields (m) of faba bean were 0.4 and 0.5 for fresh top plant weight for *P. neglectus* and *P. thornei*, respectively, and occurred at $Pi \geqslant 128$ specimens/cm^3 soil. Maximum reproduction rates (Pf/Pi) were 17.9 and 3.9-fold, at lowest Pi, for *P. neglectus* and *P. thornei*, respectively.

Mapping of the pepper *Me 3* gene conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.) and comparison with the tomato- and potato-nematode resistance genes location

Caroline DJIAN-CAPORALINO [1], Lucette PIJAROWSKI [1], Ariane FAZARI [1], Manuella SAMSON [1], Laurent GAVEAU [1], Véronique LEFEBVRE [2], Carole CARANTA [2], Anne BLATTES [2], Alain PALLOIX [2] and Pierre ABAD [1]

[1] *Unité Santé Végétale et Environnement, INRA, Antibes, France*
[2] *Unité de Génétique et Amélioration des Fruits et Légumes, INRA, Montfavet, France*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Me3, single dominant gene of the PM687 line of *Capsicum annuum,* confers heat-stable resistance to root-knot nematodes (RKN). We have located it using doubled-haploid (DH) lines and F_2 progeny from a cross between the susceptible cultivar Yolo Wonder and the highly resistant line PM687. Bulked-segregant analysis with DNA bulks, from susceptible or resistant DH lines, was performed to identify AFLP markers linked to *Me3*. Four repulsion-phase and two coupling-phase markers complementary to the *Hind*III and *Mse*I sites were located between 0.5 and 26.3 centimorgan (cM) on both sides of the gene. Analysis of the F_2 progeny (162 plants) with the coupling-phase marker located 0.5 cM from *Me3* in the DH progeny, confirmed its closed position. An other resistance gene to RKN, present in PM687 *Me4*, was shown to be linked to *Me3*, nearly 10 cM from it. In order to localize *Me3* and *Me4* on the intraspecific pepper linkage map, two AFLP markers were mapped. The *Me3* nearest marker linked to resistance was 2.7 cM from RFLP-CT135. PCR-specific markers obtained from two AFLPs will be used in pepper breeding for marker-assisted selection. We investigate map position homologies between *Me3* and two other nematode resistance genes, *Mi-3* and *Gpa2* which mapped in the telomeric region of the short arm of the tomato and potato chromosome 12.

Effects of edible fungi oyster mushroom (*Pleurotus ostreatus*) on *Aphelenchus avenae* and *Steinernema feltiae*

Ewa DMOWSKA

Institute of Ecology Polish Academy of Sciences, Dziekanow Lesny, 05-092 Lomianki, Poland

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The oyster mushroom is able to capture and consume nematodes. Hyphae of older fungi produce nematotoxin. When nematodes come in contact with this substance, they become immobilised. Then hyphae penetrate into nematode body and digest it. Some authors proved that the oyster mushroom is capable of destroying the bacterial feeding nematode, *Panagrellus redidivivus*, and preparasitic larvae of several animal parasites. In this study survival of two species, *Aphelenchus avenae* and *Steinernema feltiae*, affected by oyster mushroom, was estimated. These two species were analysed because they could play important roles in commercial growing of the oyster mushroom. *A. avenae*, a fungal-feeding nematode, can be a pest of edible mushroom, and the entomopathogenic species *S. feltiae* could be applied in biological control of pests of the oyster mushroom, flies belonging to the family *Sciaridae.* The results of the laboratory tests indicated that *A. avenae* was more susceptible than *S. feltiae* to the nematotoxin produced by the oyster mushroom.

New *Musa* hybrids with partial resistance to *Radopholus similis*

Carine DOCHEZ, Paul R. SPEIJER†, John HARTMAN and Dirk VUYLSTEKE

International Institute of Tropical Agriculture (IITA), Eastern and Southern Africa Regional Centre (ESARC), P.O. Box 7878, Kampala, Uganda
†*Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Several *Musa* hybrids were tested for nematode resistance and compared to the reference cultivars Yangambi-km 5 (resistant), Gros Michel (partially resistant) and Valery (susceptible). Genotypes included the diploid hybrids 2521S-31, -47 and -50, 1411S-2 and -10, 2569S-1 and -2 and the tetraploid hybrids 2094S-1 and TMHx 660K-1. Genotypes were planted in wooden boxes containing sterilised sawdust. From each plant, three roots were selected and a plastic container was placed around each of them. Inoculation was done by pouring a suspension of 50 females of *Radopholus similis* on each individual root. Eight weeks after inoculation, the roots were harvested and the number of nematodes counted, including all vermiform developmental stages and sexes. From these counts, the reproduction ratio was calculated. All genotypes showed a lower reproduction ratio than Valery. The genotypes Gros Michel, 2521S-31 and -47, 1411S-10, 2569S-2, 2094S-1 and TMHx 660K-1 showed a reproduction ratio that was not significantly different from Yangambi-km 5. Genotypes with low reproduction ratios support low *R. similis* densities and are therefore interesting genotypes for further evaluation. Except for the East African highland banana hybrid TMHx 660K-1, all the other hybrids have the highly *R. similis*-resistant Pisang Jari Buaya in their pedigree.

The scope and limitations of a computer programme for the integrated control of potato cyst nematodes

Martin ELLIOTT [1], Mark S. PHILLIPS [1], James W. MCNICOL [2] and David L. TRUDGILL [1]

[1] *Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK*
[2] *Biomathematics and Statistics Scotland, SCRI, Invergowrie, Dundee, DD2 5DA, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Field trials at different sites prepared with plots spanning a range of population densities of potato cyst nematode (PCN, *Globodera pallida*) provided data for developing a computer-based programme for modelling PCN population dynamics and yield losses. Potato yields and PCN multiplication were well described by density dependent equations which were made potentially predictive by incorporating functions for consistent site and cultivar differences in tolerance. The current programme allows for differences in site and cultivar tolerance, yield potential and resistance. The effects of different management strategies can be examined by changing the effectiveness of nematicides and/or resistant cultivars as well as the duration and rates of PCN decline during the years between potato crops. The programme has demonstrated the difficulties many farmers face in managing *G. pallida*, the need to prevent small populations from increasing and the problems of decreasing large populations. The effectiveness of integrated management has been shown to be very sensitive to differences in the percentage "kill" by nematicides and rates of population decline between potato crops. Even quite low levels of partial resistance have been shown to be useful when combined with nematicides. The challenge now is to devise realistic sampling strategies for generating data to allow the model to be applied in specific situations.

Development of aseptic culture systems of *Radopholus similis* for *in vitro* host-pathogen studies

Annemie ELSEN, Ruth STOFFELEN and Dirk DE WAELE

Laboratory of Tropical Crop Improvement, K.U. Leuven, K. Mercierlaan 92, 3001 Heverlee, Belgium

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Radopholus similis is one of the most damaging nematodes in bananas. Chemical control is currently the most used method, but nematode control through genetic improvement is widely encouraged. The objective of this study was to establish an aseptic culture system for *R. similis* and to determine whether *R. similis* can infect and reproduce on *in vitro* banana plantlets and *Arabidopsis thaliana*. In a first part a suitable aseptic culture system was developed using alfalfa callus. *Radopholus similis* could penetrate and reproduce in the callus: six weeks after inoculation with 25 females the reproduction ratio was 26.3 and all vermiform stages were present. The reproduction ratio increased up to 223.2 after 12 weeks. Results of a greenhouse test showed that *R. similis* did not lose its pathogenicity after culturing on alfalfa callus. In a second part the infection and reproduction of the nematodes cultured on the callus was studied on both *in vitro* banana plantlets and *A. thaliana*. It was shown that *R. similis* could infect and reproduce on both banana and *A. thaliana*. Furthermore, nematode damage was observed in the root system of both hosts. These successful infections open new perspectives for rapid *in vitro* screening for resistance of the existing banana cultivars and antinematode proteins expressed in *A. thaliana*.

Aphelenchoidoidea nematodes of phytopathological interest and their distribution in Spain

Miguel ESCUER, María ARIAS and Antonio BELLO

Dpto Agroecología, Centro de Ciencias Medioambientales, CSIC. Serrano 115 dpdo, 28006 Madrid, Spain

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Previous references of plant parasitic nematodes belonging to the superfamily Aphelenchoidoidea in Spain have been reviewed and new samples have been studied, due to their interest as pathogens to rice crops, edible mushrooms, ornamental plants, strawberry crops and pine tree woodlands and for having quarantine species. *Aphelenchoides bicaudatus, A. blastophthorus, A. composticola, A. fragariae* and *A. ritzemabosi* have been found in several Spanish areas. *A. ritzemabosi* has only appeared in La Palma Island, in the Canary Island. Quarantine nematodes *A. besseyi*, a pathogen of rice crops, and *Bursaphelenchus xylophilus*, causal agent of pinewilt diseases affecting several conifer species and recently reported from Portugal, have not been found.

VAM fungi increase nematode resistance, growth, and nutrient uptake of field grown apple trees

Thomas FORGE, Andrea MUEHLCHEN, Clemens HACKENBERG, Gerry NEILSEN and Thierry VRAIN

Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, VOH 1ZO, Canada

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The effects of Vesicular Arbuscular Mycorrhizal (VAM) fungi on the development of *Pratylenchus penetrans* and growth of Ottawa 3 apple rootstock were studied. Six species of fungi were evaluated in two greenhouse experiments, and two species were selected for a 2-year field experiment. *Glomus mosseae* increased total dry weights of nematode-inoculated and non-inoculated plants in both initial greenhouse experiments, and *G. intraradices, G. versiforme* and *G. etunicatum* each increased dry weights in one of the two experiments. Plants inoculated with *G. mosseae* and *G. intraradices* supported significantly fewer *P. penetrans* per g root than *G. etunicatum* and *G. clarum*, but were not different from controls. Colonisation of roots by VAM was not affected by nematode inoculation. *G. mosseae* and *G. intraradices* increased rootstock growth and nutrient uptake in the field. After two years in the field, numbers of *P. penetrans* per g root and per 50 ml root zone soil were significantly lower for *G. mosseae*-inoculated plants than for control plants.

Cuticle structure among the Tylenchs

Etienne GERAERT

Department of Biology, Gent University, Ledeganckstraat, 35, 9000 Gent, Belgium

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

It is well known that cuticle ultrastructure differs among the various tylenchs. When the cuticle is rather thin a similar structure is found throughout the several systematical groupings. When the cuticle is thicker or becomes thicker some special patterns are found that can be used to characterise some systematical subdivisions: in the superfamily Criconematoidea a thick cuticle is the result of an additional layering of the cortex as well in *Criconema, Hemicycliophora* as in *Tylenchulus.* In the family Hoplolaimidae and in the subfamily Heteroderinae the thick cuticle is the result of an additional layer or layers underneath the striated layer. In all other tylenchs the striated layer is the basal layer.

Ultrastructure of syncytia after micro-injection of barnase

Wladyslaw GOLINOWSKI [1], Petra VOSS [2], Miroslaw SOBCZAK [1], Stephan OHL [3] and Florian M.W. GRUNDLER [2]

[1] *Department of Botany, Warsaw Agricultural University, Rakowiecka 26/30, 02528 Warsaw, Poland*
[2] *Institute of Phytopathology, Hermann-Rodewald Str. 9, 24118 Kiel, Germany*
[3] *ZENECA-MOGEN, Einsteinweg 97, 2333CB Leiden, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Syncytia induced by *Heterodera schachtii* in roots of *Arabidopsis thaliana* were used for microinjection. As a control, lucifer yellow (LYCH) dissolved in PBS was taken. After injection, LYCH spread within the syncytia and was readily taken up by the associated juveniles. At the ultrastructural level, these control injections were found to cause conspicuous swirling and accumulation of the syncytial endomembranes and the formation of numerous vesicles. Injections of barnase together with LYCH caused a mortality of about 70% of the associated nematodes. Already one day after microinjection, degenerating syncytia were found. Their protoplasts were strongly accumulated so that only remnants of cytoplasm were found in wide areas of the former syncytium. Four days after injection, the treated syncytia contained only membraneous material, vesicles and osmiophilic granules. Degeneration was strictly confined to the treated syncytia. In some cases degeneration of both syncytia and nematodes was retarded. The cytoplasm of these syncytia appeared to be still alive but was almost free of ribosomes.

Pathogenic potential of the root-knot nematode, *Meloidogyne incognita*, on rice in Venezuela

Nicola GRECO [1], Renato CROZZOLI [2], Franco LAMBERTI [1] and Antonio BRANDONISIO [1]

[1] *Istituto di Nematologia Agraria, C.N.R., Via Amendola 165/A, 70126 Bari, Italy*
[2] *Instituto de Zoología Agrícola, Facultad de Agronomía, Universidad Central de Venezuela, Apdo 4579, 2101-A Maracay, Venezuela*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

An experiment was conducted in a glasshouse to relate densities of three Venezuelan populations of *Meloidogyne incognita* with rice growth. Nematode densities were 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 eggs and juveniles/cm^3 soil for the Zulia population and 0, 2, 8, 32, 64, and 128 eggs and juveniles/cm^3 soil for the Barinas and Lara populations. Tiller height was recorded at five plant stages and fresh plant weight and nematode populations in roots and soil were determined at harvest. The Lara population was the most aggressive; plants in pots inoculated with 128 nematodes/cm^3 soil were yellowing at emergence and died 40 days later. Tolerance limits for shoot weight and tiller height of rice were 8, 6.6, 6.6 and 4, 2.4 and 5.5 eggs and juveniles/cm^3 soil for the Zulia, Barinas and Lara populations, respectively. A minimum relative yield of 0 could have been achieved at 520-420, 420-256, 156-226 eggs and juveniles/cm^3 soil, for plant weight and shoot height, for the Zulia, Barinas and Lara populations, respectively. Nematode maximum reproduction rates were 50, 19.5, and 57-fold while equilibrium densities were 119, 256, and 62 eggs and juveniles/cm^3 soil, respectively, for the populations from Zulia, Barinas and Lara.

Toxicity of 1,3-dichloropropene (Telone II) to potato cyst nematodes

Ivan GROVE and Patrick HAYDOCK

Plant Nematology and Soil Pests Group, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Telone II, a soil fumigant containing 94% 1,3-dichloropropene in both the *cis* and *trans* isomers, is applied in the field at 225 l/ha using specialist equipment. Experiments determined air-vapour and soil-water phase toxicity of Telone II to *Globodera* spp. Air-vapour phase fumigation at 4.37 mg.litre.day 1,3-D almost completely inhibited hatching of a mixed *G. rostochiensis* and *G. pallida* population over 14 weeks. In a sandy loam soil the mixed *G. rostochiensis* and *G. pallida* population was prevented from hatching for up to 7 weeks into the experiment by 25.26 mg.litre/soil.day 1,3-D (equivalent to 50.5 l/ha field application). In conclusion, both air-vapour and soil-water phase fumigation with Telone II, shows high toxicity to PCN. Telone II will control PCN at dosages below the current field application rates when used in a controlled environment. Field applications of Telone II, however, should remain at 225 l/ha until further research can identify whether there is scope to reduce application rates for commercial use.

A highly specific cDNA library from nematode-induced syncytia

Florian M.W. GRUNDLER, Petra VOSS and Piotr S. PUZIO

Institut für Phytopathologie, Universitaet Kiel, Hermann-Rodewald-Strasse 9, 24098 Kiel, Germany

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A new method for the isolation of specific RNA from nematode feeding structures (NFS) induced by cyst nematodes was developed. With the aid of a microcapillary the cytoplasm was extracted from NFS of *Heterodera schachtii* in roots of *Arabidopsis thaliana*. In order to determine RNA quality, total RNA was isolated and then hybridised with a 18S rDNA probe. The detection limit for the isolated RNA was determined by probing total RNA of 15, 25 and 50 NFS with 18S rDNA. The results indicated that an equivalent of 15 NFS is sufficient to produce distinct hybridisation signals. The cDNA of pyk20, a gene from *A. thaliana* which is highly active in NFS, was used as a probe. Gene expression could be monitored by Northern blotting and RT-PCR with isolated RNA equivalent to 25 to 50 and 3 to 5 NFS, respectively. Using a PCR based system a highly specific cDNA library was constructed which consisted of approximately 2.5 × 106 primary recombinants. This library opens a new dimension in the efforts to clone plant genes which are specifically regulated in NFS.

Use of the green fluorescent protein to study the localization of antagonistic bacteria in nematode-infested plants

Johannes HALLMANN [1], Andrea QUADT-HALLMANN [1], W.G. MILLER [2], Ste E. LINDOW [3] and Richard A. SIKORA [1]

[1] *Institut für Pflanzenkrankheiten, Nußallee 9, 53115 Bonn, Germany*
[2] *USDA-Food Health and Safety Unit, Albany, CA, USA*
[3] *Department of Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Plant-associated bacteria have been reported to be antagonistic to plant parasitic nematodes making them suitable candidates for biological control strategies. Modes of action include: competition for nutrients and space, production of metabolites with nematicidal activity and bacterial-mediated induction of plant defense mechanisms. In general, these antagonistic bacteria have been considered to be thorough colonizers of the rhizosphere, however, recent studies have shown that some isolates colonize the endorhiza. Wounds on the root surface caused by the nematode seem to play an important role in bacterial colonization and plant-nematode-bacteria interactions. They increase leakage of root exudates which stimulates microbial population growth in the rhizosphere, and also favours bacterial entry into the plant tissue. Whereas rhizosphere colonization by antagonistic bacteria has been studied intensively in the past, very little is known about the endophytic phase of colonization, especially in the presence of plant-parasitic nematodes. For the following studies we utilized the green fluorescent protein as a bacterial marker to detect, identify and localize the introduced antagonistic bacteria. The plasmid pGT-trp containing a trp promoter-GFP transcriptional fusion was introduced into the biocontrol strain *Rhizobium etli* G12 by mobilization, and studied in *Meloidogyne incognita*-infested potato and *Arabidopsis* plants. Bacterial colonization was evaluated using epifluorescent and confocal laser scanning microscopy. The plasmid pGT-trp was stable for more than 80 bacterial generations without selection and the green fluorescence it conferred allowed detection of single bacterial cells. The presentation will summarize the results on bacterial colonization with emphasis on nematode feeding sites.

A new species of cyst nematode (*Heterodera* sp.) on *Panicum coloratum* in Egypt, with observations on closely related species described on various grasses and rice in Florida and Louisiana in the United States

Zafar A. HANDOO [1] and I.K.A. IBRAHIM [2]

[1] *USDA, ARS, Nematology Laboratory, Beltsville, MD 20705-2350, USA*
[2] *Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In 1997, an undescribed cyst nematode (*Heterodera* sp.) closely related to *H. graminophila* Golden & Birchfield, 1972 and *H. leuceilyma* Di Edwardo & Perry, 1964 was found associated with Qasabagrass roots (*Panicum coloratum*) growing near date palm in Alexandria, Egypt. Soil samples around roots also yielded several juveniles of this species. It differs from *H. leuceilyma* in having second-stage juveniles (J2) with a shorter stylet (stylet length 23 (22.5-23.5) *vs* 26 (23-28 μm)) and in the shape of J2 stylet knobs, anchor-shaped *vs* prominently rounded. The new species differs from *H. graminophila* in that it has J2 with longer bodies and with longer, more hya-

line tail termini than those described from Louisiana and Florida populations: body length 516 (485-550) *vs* 430 (380-460 μm) in the Louisiana population on barnyard grass *(Echinochloa colonum*), and 391 (374-412 μm) in the Florida population on roots of *Panicum rigidulum*, hyaline tail terminus 40 (35-43) *vs* 32 (25-38 μm) in the Louisiana population and 27 (23-31 μm) in the Florida population. The new species also has bullae in the cysts, which are absent in *H. graminophila.* Also, the J2 stylet length in the Florida population of *H. graminophila* is shorter than that of the new species from Egypt 18.2 (17.6-18.6) *vs* 23 (22.5-23.5 μm). The male specimens previously reported in *H. graminophila* and *H. leuceilyma* were absent in the samples from Egypt. The known distribution in Alexandria, Egypt is restricted to the original site thus far. Because this species is limited in distribution, its economic importance in rangeland grasses and cultivated crops such as rice is not known.

Yield loss in strawberries caused by *Aphelenchoides blastophthorus*

Solveig HAUKELAND SALINAS and Kari BREKKE

Norwegian Institute of Crop Protection, Department of Entomology and Nematology, Hogskoleveien 7, 1432 Aas, Norway

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Aphelenchoides blastophthorus is ecto- and endoparasitic on leaf and bud tissues of scabious and some other plants; it also readily reproduces on fungi. *A. blastophthorus* was recognized for the first time as a problem in Norwegian strawberry production in 1972. In recent years this nematode appears to be increasingly important in strawberry production in some parts of the country. Symptoms, runner-production and yield was compared for the strawberry varieties: Honeoye, Inga, Jonsok, Korona and Zephyr. Certified plants were artificially infested with 100 nematodes per plant and planted outdoors in confined microplots. Planting took place at the end of May. Symptoms were not expressed in the first year; however, samples of runners demonstrated that nematodes were present. The following spring symptoms were recorded as distorted/darker green leaves, and decreased growth. In this experiment, damage was most severe on Honeoye plants whereas the Korona plants appeared less affected. As the season progressed, immediate symptoms became less clear and by July/August it was difficult to differentiate infested and non-infested plants. For all the varieties yield loss and the number of runners per plant was significantly reduced in infected plants compared to uninfected controls.

Biological control agents against soil borne plant diseases and nematodes

Rüdiger HAUSCHILD, Johannes HALLMANN and Richard SIKORA

Institut für Pflanzenkrankheiten, Phytomedizin in Bodenökosystemen, Universität Bonn, Nussallee 9, D-53115 Bonn, Germany

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A disease complex comprised of root-knot nematodes (*Meloidogyne* spp.) and the wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* causes severe economic losses in tomato production in the middle east and world wide. Control of this disease syndrome has been accomplished for decades only on the basis of methyl bromide soil fumigation which will officially be banned for use in the year 2001. Finding new, effective and environmentally safe alternative management systems therefore is a major challenge facing plant pathology. In our project we attempt to develop new approaches to control the disease complex on tomato by enhancement of antagonistic microorganisms. In a first phase, the impact of different rhizobacteria on reduction of *Fusarium* and *Meloidogyne* attack has been analysed and effective strains selected. Remarkable reduction of both pathogens by antagonistic bacteria will be presented. Further experiments are on the way to improve formulation and application techniques of the respective rhizobacteria.

The analysis of light reflected from the potato crop as a diagnostic assay for infection by potato cyst nematodes

William HEATH [1], Patrick HAYDOCK [1], Andrew WILCOX [1] and Kenneth EVANS [2]

[1] *Plant Nematology and Soil Pests Group, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK*
[2] *IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Potato cyst nematodes invade the roots of developing potato plants, damaging the root structure and reducing the uptake of nutrients by the plant. Current methods of determining PCN population densities are both expensive and time consuming, providing only estimates of the average population levels within fields without measurement of variability or means of identifying developing foci of infestation. Nematode infestation may alter both the structure and chemical content of leaves. As each element in a leaf reflects light at different wavelengths, correlation of a change in canopy reflectance with nematode infestation may provide a method of identifying foci of infestation. A hand-held spectroradiometer has been used to record canopy reflectance from potato plants infected with a range of PCN densities. Preliminary results indicate that PCN infestation can be detected by spectral analysis. Spectral readings from a field experiment correlated Normalised Derived Vegetation Index (NDVI) with root invasion (R^2 = .825, N = 138, cv. Estima) and was supported by a concurrent pot experiment (r^2 = .866, N = 285, cv. Pentland Dell). NDVI is a broad measure of plant condition and a further experiment is underway to pursue spectral response features specific to PCN infection. Further development of the analysis is envisaged using airborne remote sensing, leading to a comparison of ground and airborne readings with data recorded from a planned series of high resolution, infra-red environmental monitoring satellites.

Transformation with *Agrobacterium rhizogenes* induces nematode resistance in *Raphanus sativus* roots

Petra HEINEN [1], Inke NITZ [1], Piotr S. PUZIO [1], Wladyslaw GOLINOWSKI [2], Miroslaw SOBCZAK [2], Stephan OHL [3] and Florian M.W. GRUNDLER [1]

[1] *Institute of Phytopathology, Hermann-Rodewald Str. 9, 24118 Kiel, Germany*
[2] *Department of Botany, Warsaw Agricultural University, Rakowiecka 26/30, 02528 Warsaw, Poland*
[3] *ZENECA-MOGEN, Einsteinweg 97, 2333CB Leiden, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Raphanus sativus plants (cv. Siletina) susceptible to *Heterodera schachtii* were transformed either with wildtype *Agrobacterium rhizogenes* (WTHR), or with *A. rhizogenes* that carried the barstar gene fused to the promoter rolD (BSHR). In the wildtype roots a susceptible response with the formation of females was observed, while in hairy roots only males reached the adult stage whereas many juveniles stagnated in the J2 and J3 stage. This response also occurs in resistant plants (*e.g.* cv. Pegletta). No difference between WTHR and BSHR could be observed so the response appears to be based on the properties of hairy roots. The structure of uninfected wildtype roots (WT) and transformed roots (WTHR and BSHR) was identical, but syncytial elements in WTHR and BSHR hypertrophied only slightly. The endoplasmic reticulalum (ER) of young syncytia was found to be less abundant than in WT but no other striking differences occurred. Entering the J3 stage, the syncytial cytoplasm of WTHR and BBHR become heavily stained and granular. They contained only few ER membranes and other organelles while their cell wall did not thicken. Later the syncytia fully degenerated.

Expression of a nematode responsive promoter of *A. thaliana* in potato

Petra HEINEN [1], Piotr PUZIO [1], Stephan OHL [2] and Florian M.W. GRUNDLER [1]

[1] *Institut für Phytopathologie, Hermann-Rodewald Str. 9, 24118 Kiel, Germany*
[2] *ZENECA MOGEN, Einsteinweg 97, NL-2333 CB Leiden, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A promoter with high activity in nematode induced syncytia was tagged in *Arabidopsis thaliana*. Promoter::*gus* constructs of different length were examined for their expression in *A. thaliana* during development and after infection with *Heterodera schachtii* and *Meloidogyre incognita*, respectively. Based on these results promoter construct *ppyk20*-C was chosen for further applications. Potato plants cv. Desiree were transformed with a ppyk20-C::gus construct *via A. tumefaciens*. Regenerated potato plants were tested for gus expression in roots and shoots. In contrast to the expression in *Arabidopsis*, expression patterns in potato varied remarkably between different lines and between different plants within one line. The most striking result was a strong gus expression upon wounding in both root and shoot tissue. In addition to the histological determination of promoter activity, fluorimetric gus assays were performed with plant material of two transgenic potato lines. Responsiveness to treatment with plant hormones was shown. The promoter is currently tested for application in different anti-nematode strategies. Potato was transformed with *ppyk*20-C::barnase/rolD::barstar and genes with nematicidal effects. Preliminary results will be shown.

Extraction of free-living nematode stages from soil with zonal centrifugation

G. HENDRICKX

Agricultural Research Centre, Department of Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

When using conventional centrifugation for extraction of nematodes, the extracted volume is limited; maximum 50 ml of soil can be extracted. An apparatus was developed that is able to process 100 ml of soil. The extraction with the apparatus is based on the principle of zonal centrifugation and is fully automated. The machine is built as a table model and is 0.6 m high, 1 m wide and 0.6 m deep. All individual parts, as well as the control by a PLC (Programmable Logic Controller), are contained in this space. Water is added to 200 ml soil in a beaker until a volume of 2 l is reached. Of this soil suspension, 1 l is centrifugated. At the end of the process, nematodes are collected in a 100 ml beaker. The apparatus can also be used to extract nematodes from a suspension of blended roots. Extractions with the apparatus are reproducible, reliable and have a higher efficiency that the conventional centrifugation techniques.

Significant interceptions and other new or unusual plant-parasitic nematodes recorded in England 1996-1999

Sue HOCKLAND

Invertebrate Identification Team, Plant Health Group, Sand Hutton, Central Science Laboratory (CSL), York YO41 1LZ, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The Invertebrate Identification Team in the Plant Health Group, CSL, identifies plant-parasitic nematodes and other invertebrate pests for the Plant Health and Seeds Inspectorate, pest control consultants, farmers and growers. A selection of notable nematode identifications made on imported material is summarised here. The international trade of ornamental plants has continued to grow in the last few years, and comprises the most important route for entry of non-indigenous species. Amongst notable interceptions were *Aphelenchoides besseyi*, various *Helicotylenchus* spp. including *H. dihystera, Hirschmanniella mucronata, Tylenchorhynchus* cf. *annulatus, T.* cf. *coffeae*, *T. leviterminalis* and *Xiphinema* spp., all associated with penjing from China, *Helicotylenchus tunisiensis* on ornamental *Allium* from Uzbekistan, *Hoplotylus* cf. *montanus* from Bhutan, *Rotylenchulus* cf. *reniformis* on *Schefflera cana* from Cuba, and *Scutellonema bradys* on yams from South America. *Meloidogyne chitwoodi* has been intercepted in imported ware potatoes, but the pest remains absent from the UK. The occurrence of other, non-statutory, root-knot nematodes in imported potatoes, such as *M. javanica*, has stimulated research into improved diagnosis tools for this family.

Improving detection and identification of root-knot nematodes in potatoes by immunochemical techniques

Sue HOCKLAND, Chris DANKS, Sarah DUFF, John BANKS, Mark DELANEY, Louise MACLEOD and Sue CRACKNELL

Plant Health Group, Central Science Laboratory (CSL), Sand Hutton, York YO41 1LZ, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The European Plant Health Directive and phytosanitary regulations of many countries list several plant-parasitic nematodes as quarantine pests. Identification is routinely done using traditional microscope techniques to distinguish key morphological features. This has always presented particular difficulties because nematodes often have few distinguishing characters, and the variability of some of these makes positive identification difficult. At CSL, the use of immunological techniques as reliable, robust tools to assist in the detection and identification of plant-parasitic nematodes has been investigated. *Meloidogyne chitwoodi* and *M. hapla* were used as model species. An ELISA has been developed incorporating monoclonal antibodies prepared by immunisation of juvenile and female nematodes. This has enabled detection of nematodes in potato tubers and tomato root extracts at levels not previously detected by visual inspection, but it is not yet able to distinguish between species. Associated work on improving electrophoretic tools for diagnosis of *M. chitwoodi* will also be illustrated. This work is funded by MAFF (Plant Health Division).

Cyst nematodes *Heterodera* spp. in cereal fields in Norway — preliminary results

Ricardo HOLGADO [1], Christer MAGNUSSON [1], Stig ANDERSSON [2] and Janet ROWE [3]

[1] *The Norwegian Crop Research Institute, Plant Protection Centre, Dep. of Entomology and Nematology, Høgskoleveien 7, 1432 Ås Norway*

[2] *Swedish University of Agricultural Sciences, Dep. of Plant Protection Sciences, P.O. Box 44, S-230 53 Alnarp, Sweden*

[3] *ICAR-Rothamsted Dep. of Entomology and Nematology, Harpenden Hertfordshire AL5, 2JQ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Several species of cyst nematodes in the genus *Heterodera* attack cereals. The most common, and maybe the most important species, is the cereal cyst nematode (CCN) *H. avenae*, which was first reported in Norway in 1925. The dominating cereals in Norway are barley, followed by oats and wheat. Field damage by CCN is shown as patches with uneven growth, which is most clearly seen in oats. In addition to cereals, several species of grass are hosts of CCN. Analysis of 218 samples from the period 1995-1998 demonstrates the genus *Heterodera* to be common in Norwegian cereal fields. *Heterodera* has been recorded from the southern part of Rogaland county, up to a position 65.5°N in the county of Nordland. The highest frequencies of occurrence were observed in oats and wheat. There is an increase in the damage in cereals in recent years. Earlier studies have demonstrated Ha 51 to be the dominating pathotype of CCN in Norway. Our studies confirm the presence of *H. filipjevi*, and indicate the possible presence of new *Heterodera* spp. The increasing damage may relate to the lack of resistance to Ha 51 in current cultivars, or change in the pathotype frequencies. The increased acreage of wheat, and susceptible cereals as precrops to oats and wheat could also contribute to the increased damage. The occurrence of species and pathotypes of cyst nematodes on cereals in Norway needs to be studied further, and current cultivars need to be screened for resistance against cyst nematodes. The development of effective nematode control strategies is a priority.

Identification of novel nematicidal compounds by microinjection into syncytia

Roland A. HOLZ [1], Linda VAN DER MEY [1], Marcel BREEDEVELD [1], Amanda CARLILE [2], Florian M.W. GRUNDLER [3] and Stephan A. OHL [1]

[1] *ZENECA MOGEN, P.O. Box 628, 2300 AP Leiden, The Netherlands*
[2] *ZENECA Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, UK*
[3] *Institut für Phytopathologie, Hermann-Rodewald Str. 9, 24118 Kiel, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Economically important plant-parasitic nematode genera, such as *Meloidogyne*, *Heterodera* and *Globodera*, are obligate feeders on plants. Attempts to culture them in the absence of their host plant have been largely unsuccessful so far. Screens for orally active nematicidal compounds have therefore mainly relied on bacterial feeders such as *C. elegans.* At ZENECA, we apply a method to microinject potentially nematicidal compounds into *Heterodera schachtii* feeding sites. Some compounds with known nematicidal activity, *e.g.* proteinase inhibitors, tested positive in this assay. Screening extracts from microbial organisms we have identified potentially novel sources of nematicidal activity. Their biochemical characterisation is in progress.

Antioxidant proteins of *Globodera rostochiensis*

John T. JONES [1], Lee ROBERTSON [2], Alison PRIOR [1], Dave KNOX [3] and Mark S. PHILLIPS [1]

[1] *Mycology, Bacteriology and Nematology Unit, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK*
[2] *Universidad Autonoma de Madrid, Departmento de Biologia, Laboratorio de fisiologia vegetal, Cantoblanco, 28049 Madrid, Spain*
[3] *Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Plant parasitic nematodes are likely to be exposed to reactive oxygen species generated by their own metabolism and by plant defence mechanisms but almost nothing is known about how they protect themselves from oxidative stress. We have cloned cDNAs encoding a variety of secreted antioxidant proteins of *G. rostochiensis* which may play a role in protecting these parasites from active oxygen species generated by their hosts. Genes identified to date include secreted forms of glutathione peroxidase, thioredoxin peroxidase and superoxide dismutase. We have biochemically analysed the proteins encoded by these genes in order to investigate their function. Our findings suggest that thioredoxin peroxidase, superoxide dismutase and a secreted fatty acid binding protein (GPSEC-2) may function in the host-parasite interaction but that other antioxidant proteins such as glutathione peroxidase have a different role.

ESTs from potato cyst nematodes — from sequences to functional analysis

John T. JONES [1], Vivian C. BLOK [1], Alison PRIOR [1], Mark S. PHILLIPS [1], Herman POPEIJUS [2], Erin BAKKER [2], Hans HELDER [2] and Geert SMANT [2]

[1] *Mycology, Bacteriology and Nematology Unit, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK*
[2] *Nematology Department, University of Wageningen, Wageningen, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Expressed sequence tag (EST) projects offer a rapid route to the discovery of novel genes. Genes expressed in a wide range of parasitic nematodes of medical or veterinary importance have been investigated using EST analysis but these techniques are only now being applied to plant parasitic nematodes. We have undertaken a small scale EST project using cDNA libraries made from the two species of potato cyst nematode *Globodera rostochiensis* and *G. pallida* in order to assess the utility of this approach and to identify cDNAs encoding abundantly expressed secreted proteins. 1000 sequences have been obtained from *G. rostochiensis* and 100 from *G. pallida.* A variety of genes has been characterised and approximately 10% of the cDNAs sequenced are apparently PCN specific. Secreted proteins have been identified including a PCN homologue of chorismate mutase, a cDNA recently cloned from the gland cells of *Meloidogyne javanica.* While larger scale projects of this type will undoubtedly be important in the future, our efforts are now focussed on analysing the function of the proteins encoded by the identified genes. Function is being analysed using large-scale approaches including differential screening and *in situ* hybridisation. More detailed studies on the most interesting genes are also underway.

The phloem-specific sucrose carrier AtSUC2 is activated in syncytia induced by the beet cyst nematode *Heterodera schachtii*

Katja JUERGENSEN [1], Joachim SCHOLZ-STARKE [2], Norbert SAUER [2], Paul HESS [3], Aart J.E. VAN BEL [3] and Florian M.W. GRUNDLER [1]

[1] *Institut fuer Phytopathologie, Universitaet Kiel, Hermann-Rodewald-Strasse 9, 24098 Kiel, Germany*
[2] *Lehrstuhl Botanik II, Universitaet Erlangen, Staudtstrasse 5, 91058 Erlangen, Germany*
[3] *Institut für Allgemeine Botanik und Pflanzenphysiologie, Senckenbergerstrasse 17, 35390 Giessen, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Syncytia are metabolically highly active cell complexes and act as strong sinks within the roots. As the syncytium is symplastically isolated from the surrounding root tissue the mechanism of phloem unloading appears to be apoplastic. Accordingly, the assimilate must be translocated *via* specific transport proteins. To identify such a transporter, transgenic *Arabidopsis thaliana* plants with promoters of several sugar transporters fused to the reporter gene β-glucuronidase (GUS) or GFP (green fluorescent protein) were infected with *Heterodera schachtii.* It could be shown that the promoter of the sucrose carrier AtSUC2 which is exclusively expressed in the phloem companion cells of uninfected plants is also active in syncytia. Two days after cyst nematode infection 80% of the induced syncytia showed GUS activity. The specific expression of the gene product SUC2 was confirmed by RT-PCR and immunolocalisation. We suggest the sucrose carrier SUC2 to be an important link in the assimilates translocation during the syncytium differentiation and nematode development.

Environmental factors affecting sexual differentiation in the entomopathogenic nematode *Heterorhabditis bacteriophora*

Hamutal KAHEL-RAIFER and Itamar GLAZER

Department of Nematology, ARO, Volcani Center, Bet Dagan, 50250, Israel

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The present study was aimed at determining the influence of various environmental factors on sex differentiation (SD) in the entomopathogenic nematode *Heterorhabditis bacteriophora* HP88 strain, under *in vivo* and *in vitro* culture conditions. Injection of individual nematodes into last instars of *Galleria mellonella* resulted in development of a similar number of females and hermaphrodites (35-40%) and 20-25% males. Increasing the number of nematodes that were injected into the insect did not change these proportions. In smaller insects (0.7-1.5 cm long) an increase in the proportion of hermaphrodites was recorded as compared with larger size cadavers (2.4-2.7 cm long). When individual hermaphrodites were placed on NGM, the proportion of hermaphrodites, females and male progeny was 63%, 31% and 6% respectively. Rearing on richer medium ("Dog-food" agar) resulted in reduction in the proportion of hermaphrodites. Nematodes introduced to the symbiotic bacterium obtained from other nematode strains (IS-5 and IS-33) developed similarly to the culture reared on the HP88 bacteria. Rearing the nematodes at a temperature range between 21°C to 30°C also did not have a significant effect on the sexual differentiation among nematodes that were cultured on NGM. The proportion of hermaphrodites increased as starvation period of hatching nematode juveniles lengthened (> 6 h). The data obtained in the present study strongly suggest that the main factor affecting sex differentiation in *H. bacteriophora* is the nutrition source. The practical and biological implications of the results are discussed.

Survival and infectivity of *Meloidogyne hapla, M. fallax* and *M. chitwoodi* at different temperatures

C.J. (Hans) KOK and Ate DE HEIJ

Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Hatching, survival and infectivity of three temperate *Meloidogyne* species was compared at 5, 10, 15, 20 and 25°C. Egg masses were incubated on hatching sieves and the hatched nematodes (J2) were counted over time. Batches of J2 were inoculated in containers with sand and stored at the different temperatures. On sampling, J2 were extracted for survival measurements. In other containers lettuce or tomato were planted as bioassay plants to determine the infectivity of J2. The energy reserves of J2 were determined with oil red O. At 5°C infective juveniles of all three species were found even after 1 year of incubation. At higher temperatures the survival time and infectivity was strongly reduced. The energy reserves of the juveniles was correlated to infectivity. At low temperatures hatching of *M. fallax* and *M. chitwoodi* was faster than that of *M. hapla*. This shows that *M. hapla* is less adapted to cold temperatures than *M. chitwoodi* or *M. fallax*. The high activity of *M. fallax* and *M. chitwoodi* at low temperatures indicates that delaying the time of sowing in spring could be effective against these nematodes. The dynamics of the hatching process do not agree with the linear degree-days model used for nematode-temperature interactions.

Identification and expression of transcription factors in *Meloidogyne incognita*-induced tomato giant cells

Hinanit KOLTAI, Jennifer E. SCHAFF, Murielle G. THIERY, Ann E. GREENE and David M. BIRD

Plant Nematode Genetics Group, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695, USA

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

We are interested in the cascade of host gene expression necessary for formation and function of giant cells. One gene expressed in *Lycopersicon esculentum* giant cells, defined by the clone DB#280 (*Le-phan*), encodes a Myb transcription factor that exhibits strongest identity to *PHANTASTICA* (*PHAN*), from *Antirrhinum majus. PHAN* is involved in the formation and maintenance of meristems, and based on experiments in maize and *Antirrhinum* may act as an epigenetic repressor of class-I knotted homeobox genes (*KNOX*) or *vice versa*. We examined the pattern of *Le-phan* expression in tomato in healthy and nematode-infected tissues, and found *Le-phan* transcripts to be spatially and temporally coincident with those of *Tkn2 KNOX* gene, in marked contrast to its expression pattern in simple leaf plants. We also found *KNOX* and *PHAN* to be co-expressed in giant cells, which might reinforce a general role for these genes in maintenance of meristems (implying that giant cells exhibit meristematic characteristics). Alternatively, *Le-phan* might play a more general role in the establishment of cell fate. Transgenic plant experiments currently being conducted in our lab will reveal the functional relationship of *PHAN* and *KNOX,* including the role of these genes in the induction and maintenance of the pathological state.

Root-knot nematodes, *Meloidogyne* spp., in Poland

Stefan KORNOBIS

Plant Protection Institute, Miczurina 20, 60-318 Poznań, Poland

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

At present, eight species of the *Meloidogyne* genus are known in Poland. *M. arenaria, M. javanica* and *M. incognita* occurr only in glasshouses, because in open fields they cannot survive the winter conditions. *M. ardenensis* was detected three times on birch roots. *M. kralli* occurrs sometimes on peaty meadows, on plants from the *Cyperacae* family. *M. duytsi* was found only once on sandy dunes in western part of Polish Baltic coast. *M. naasi* was found at low population densities in two sites in north-west part of Poland. The most common root-knot nematode species in Poland is *M. hapla.* It occurs both in open field and glasshouses. Polish populations of the species cannot develop on cereals and maize, but in the field they survive on dicotyledonous weeds. Within the populations there are three distinct morphological groups of the second stage juveniles. They significantly differ in body length and all related measurements.

Host suitability of some important arable and green manure crops to *Meloidogyne chitwoodi*

Gerard KORTHALS, Rien NIJBOER and Leendert MOLENDIJK

PAV: Applied research for Arable farming and Field production of Vegetables, P.O. Box 430, 8200 AK Lelystad, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In the south east of the Netherlands the root-knot nematodes *Meloidogyne fallax* and *M. chitwoodi* cause serious problems in arable farming and the production of field vegetables. From 1996 until spring 1998 a field trial was conducted on soil with a natural infestation of *M. chitwoodi*. During two years the most important arable crops, field vegetables and green manure crops were grown in plots of 6 × 6 m in at least four replications. The host status was determined. Each plot was sampled in March (*Pi*), October (*Pf*) and again after winter in the next March (*Pi* after). Potato proved to be a very good host followed by summer wheat, summer barley, and maize as moderate hosts. Sugar beet and hemp decreased the population strongly and can be considered as very poor hosts. The green manure crops Tagetes and English ryegrass declined the population as much as black fallow and are therefore non hosts. The crops oil radish, Italian ryegrass and white mustard are poor hosts while rye is a moderate host leaving final population densities on the same level as maize. Furthermore the results will be compared with our present knowledge on *Meloidogyne*. The newly developed information on host-status will be used to control *M. chitwoodi* within rotation schemes.

Survey of baited insect parasitic nematodes from the lowland regions of Switzerland

Igor KRAMER [1], Odile HIRSCHY [2] and Juerg M. GRUNDER [1]

[1] *Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, P.O. Box 185, CH-8820 Wädenswil, Switzerland*
[2] *University of Applied Sciences (HSW), CH-8820 Wädenswil, Switzerland*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

About 600 soil samples were baited for insect parasitic nematodes with the larvae of the greater wax moth *Galleria mellonella*. In 112 cases insect parasitic nematodes could be harvested from the *Galleria* cadaver. They belonged to the group of *Steinernema* (104 strains), *Heterorhabditis* (3) and other genera from Rhabditidae (5). Almost half of all *Steinernema* records were *S. intermedium*. Other species found, ranked in decreasing frequency, were *S. kraussei, S. affine, S. feltiae, S. bicornutum, S. carpocapsae* and a single record of a member of the *S. glaseri* group. Several specimens have still to be confirmed and could belong to *S.* species B, *S.* species E1 and other not yet described species of *Steinernema*. Two strains of *Heterorhabditis megidis* and one of *H. bacteriophora* could be isolated from sandy soils close to river banks in Switzerland. At least five additional species could be recorded for the first time in Switzerland. Species richness seems to be much higher in the lowlands than in the Swiss Alps (Steiner, 1998). Also improved determination keys and the consequent checking of the lateral field region of the infective juveniles could explain some of the gained species richness.

Ty3-*gypsy* group of LTR retrotransposons in plant parasitic nematodes

Amar KUMAR, Julia GROSKI, Ann HOLT, Jane WISHART-LINDSEY GRAY, Vivian C. BLOK and Mark S. PHILLIPS

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The genomic organisation of the Ty3-*gypsy* group retrotransposons in three different plant parasitic nematodes, namely *Globodera pallida, G. rostochiensis* and *Meloidogyne incognita* has been investigated using the partial sequences of the reverse transcriptase genes. A population of partial sequences of the reverse transcriptase genes was amplified using a pair of degenerated primers derived from the conserved domains of the gene. Sequence and phylogenetic analyses showed that the reverse transcriptase sequences among three nematode species are different but they are highly homogeneous within each individual species. Moreover, analyses of synonymous and nonsynonymous substitutions suggested that there is a high percentage of elements carrying functional RT domains in Ty3-*gypsy* populations. Southern hybridization analyses are being conducted to assess whether Ty3-*gypsy* elements are present in variable copy within geographically diverse races of each nematode species. This is the first report on the presence of LTR (long terminal repeat) retrotransposons in the plant parasitic nematodes. Additionally, these elements have the potential to be used as molecular markers for genetic diversity study within these plant parasitic nematodes.

Distribution patterns of virus symptoms and their vectors *Longidorus arthensis* and *L. macrosoma* in two cherry orchards

Paul KUNZ and Juerg M. GRUNDER

Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture, P.O. Box 185, CH-8820 Wädenswil, Switzerland

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In the central part of Switzerland, close to Arth and in Horgen on the Lake of Zürich, two different cherry orchards were sampled intensively for the presence of the nematodes *Longidorus arthensis* and *L. macrosoma* and their virus CRV (Cherry Rosette Virus) and RRV (Rasperry Ringspot Virus), respectively. The analysis of a sequence with four aerial photographs from the last 18 years resulted in a calculation of the spreading of the virus symptoms. On average, the speed of spreading within the orchard in Arth was 1.9 m per year. The virus infestation in Horgen went always parallel with the proven presence of *L. arthensis*. In the orchard in Horgen it could be shown that the spreading of the nematode and the infestation with the virus always started on a quadrant of the tree that is exposed towards the focus of the nematode presence. *L. macrosoma* was found once in Arth but in Horgen this nematode is distributed regularly throughout the whole orchard. The analysis of the nematode distribution pattern vertically and horizontally did not give any evidence of competition between *L. arthensis* and *L. macrosoma*.

Marker-assisted selection (MAS) of the *Ma* genes for complete root-knot nematode (RKN) resistance in *Prunus* rootstocks

Anne-Claire LECOULS [1], Maria-Jose RUBIO-CABETAS [1], Elisabeth DIRLEWANGER [2], Henri DUVAL [3], Georges SALESSES [2] and Daniel ESMENJAUD [1]

[1] *INRA, Unité Santé Végétale et Environnement, Nematology Group, 06600 Antibes, France*
[2] *INRA, Unité de Recherches sur les Espèces Fruitières et la Vigne, 33883 Villenave d'Ornon, France*
[3] *INRA, Unité de Génétique et d'Amélioration des Fruits et Légumes, 84140 Montfavet, France*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The Myrobalan plum (*Prunus cerasifera*; *Prunophora* subgenus) is a rootstock species in which the clones P.2175, P.1079 and P.2980 are highly resistant (R) to all tested RKN species including *Meloidogyne arenaria* (MA), *M. incognita* (MI), *M. javanica* (MJ) and also an unclassified population *M.* sp. Florida (FL) that overcomes the resistance in other commonly used *Prunus* sources from the *Amygdalus* subgenus. All three clones carry a single major dominant gene, respectively designated *Ma1, Ma2* and *Ma3*, considered as allelic or closely linked, that confers a high and complete-spectrum resistance (including at least MA, MI, MJ, FL and *M. mayaguensis*). Bulked segregant analysis associated with the RAPD or AFLP techniques were performed to detect markers linked to the *Ma1* gene using segregating intraspecific progenies from P.2175 (*Ma1ma1*) crossed by several host (H) parents (*ma1ma1*). Four RAPD and three AFLP markers covering a total distance of approx. 27 cM around the gene were identified and two of them were converted into more reliable SCAR markers with the nearest (SCAL19) located at 3.7 cM from *Ma1*. SCAL19 has been evaluated for MAS in an European rootstock programme based on Myrobalan plum × *Amygdalus* (peach, almond or almond-peach) hybrids and proved to be usable reliably in particular to detect any of the *Ma* genes in segregating progenies involving the resistance sources mentioned above, such as the peach Nemared, the wild peach *Prunus davidiana* and the almond Alnem. In the Japanese plum (another species of the *Prunophora* subgenus, interfertile with Myrobalan plum), a new resistance gene (R_{jap}) has been evidenced from an intraspecific cross J13 (H) × J222 (R) and appears to confer a resistance similar to *Ma.* One marker elaborated from SCAL19 and located at 10-15 cM from this gene has been obtained and suggests that *Ma* and R_{jap} genes could be homologous.

Hoplolaimus sp. — an under-estimated parasite of banana roots in the Jordan Valley, Israel

Yuval LEVY [1], Yair ISRAELI [1], Daniel ORION [2] and Evgeni KOZODOY [3]

[1] *Jordan Valley Banana Experiment Station 15132*
[2] *Department of Nematology, ARO, The Volcani Center, Bet-Dagan 50250, Israel*
[3] *Plant Protection and Inspection Services, Bet-Dagan 50250, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In the past the spiral nematode *Helicotylenchus multicinctus* was identified and recognized as the main limiting factor to banana production in the Jordan Valley. One of the major problems was that the use of traditional planting material to establish a new orchard caused nematode reinfestation. In recent years, growers use tissue culture plants, with the advantage that they are nematode-free. It was therefore assumed that this new planting system would reduce the nematode population in banana plantations providing that a crop cycle is also applied. How-

ever this assumption has proved wrong. In many cases, planting tissue culture plants even after proper crop rotation resulted in a rapid build up of nematode populations causing root injury in the form of lesions and other visible symptoms of root damage. To investigate this phenomenon, a 3 year (1997-99) survey was conducted in the region of the Jordan Valley around the Sea of Galilee, that included 40 banana plots, with a total 480 samples. Nematodes were extracted from roots and soil, and root damage was assessed. This data clarified the distribution of the spiral nematode and its population dynamics. The results of the survey show that many of the new plantations planted with tissue culture were infested by *Hoplolaimus* sp., most of them located in the southern part of the Sea of Galilee. It was also found the *Helicotylenchus multicinctus* gradually disappeared from the soil after 2-3 years of crop rotation. It is concluded that the *Hoplolaimus* sp. is a polyphagous ectoparasite that can survive crop rotation systems, and a strategy to control it should be considered.

Environmental characterisation of three species of the genus *Aporcelaimellus* Heyns, 1965 (Nematoda: Dorylaimida) in southeastern Spain

Gracia LIÉBANAS [1], Raimundo REAL [2], Ana L. MÁRQUEZ [2] and Reyes PEÑA-SANTIAGO [1]

[1] *Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas s/n" 23071-Jaén, Spain*
[2] *Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, 29071-Málaga, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Three species of the genus *Aporcelaimellus* Heyns, 1965 have been collected in a recent study of dorylaims from the Sierra Mágina Natural Park (province of Jaén, southeastern Spain): *A. amylovorus* (Thorne & Swanger, 1936) Andrássy, 1959, *A. obtusicaudatus* (Bastian, 1875) Altherr, 1968 and *Aporcelaimellus* sp., the latter probably being an undescribed species. Their distribution has been studied and analysed. The species were collected in 30.0, 79.8 and 29.0%, respectively, of nearly 200 soil samples collected; two significantly different distributional patterns (corotypes) have been detected for *A. amylovorus* and *Aporcelaimellus* sp., whereas the same did not occur for *A. obtusicaudatus*. Species distribution with respect to 18 abiotic/biotic environmental factors was analysed using logistic regression to characterise the corotypes. Distribution of *A. amylovorus* is mainly influenced by $CaCO_3$ content ($y = 0.0280CaCO_3 - 1.5766$), whereas that of *Aporcelaimellus* sp. is influenced by altitude and clay content ($y = 0.0011\text{Altitude} + 0.0592\text{Clay} - 4.3643$).

How is the biodiversity of dorylaims influenced by altitude in a natural area from southeastern Spain?

Gracia LIÉBANAS [1], Ana L. MÁRQUEZ [2], Raimundo REAL [2] and Reyes PEÑA-SANTIAGO [1]

[1] *Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas s/n" 23071-Jaén, Spain*
[2] *Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, 29071-Málaga, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The distribution of the taxocoenosis constituted by dorylaimid nematodes has been studied in the Sierra Mágina Natural Park (province of Jaén, southeastern Spain). This study considers several environmental factors, altitude being one of them. Over nine thousand specimens of 138 species collected in 203 soil samples have been studied. Altitudinal range of the region is 600-2100 m. Kolmogorov-Smirnov test showed that altitude presents a normal distribution in the studied area. A univariate linear regression analysis was used to find out the relationship between species richness (y) and altitude (x); the following regression equation was obtained: $y = 0.0066x + 3.0749$. Thus, the number of dorylaimid species significantly increases with altitude in the geographical area considered, *i.e.*, an altitudinal distributional pattern is detected.

Protein MJE36: a stage- and genus-specific putative collagen gene product detected in *Meloidogyne javanica*

Jia LIU [1], Hinanit KOLTAI [1], Nor CHEJANOVSKY [2] and Yitzhak SPIEGEL [1]

[1] *Department of Nematology, A.R.O., The Volcani Center, Bet-Dagan 50-250, Israel*
[2] *Department of Entomology, A.R.O., The Volcani Center, Bet-Dagan 50-250, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

We have cloned and expressed a fusion protein containing a nonconserved 58-amino acid sequence encoded by a DNA sequence between the homology block A and the first (Gly-X-Y)n block from *Meloidogyne javanica* Mjcol-4 gene. Rabbit antiserum, which was raised against the fusion protein, detected a strong reacting protein band (Mw 36 kDa) on western blots of *M. javanica* eggs extracted by 2-mercaptoethanol (BME). This protein was designated as MJE36 and was found to be collagenase sensitive. MJE36 was not detected on western blots of *M. javanica* second-stage juveniles (J2) or adult females extracted by BME, which suggests that MJE36 expression is stage-specific. A band of the same molecular size was detected with *M. incognita* eggs extracted by BME but not detected with *Heterodera avenae*. These findings imply that the expression is also genus-specific. *M. javanica* eggs immunolabelling assays showed that the antiserum reacted only with the broken *M. javanica* eggs densely and irregularly, while no reacting bands were detected with purified *M. javanica* eggshells extracted by BME, suggesting that MJE36 was not in *M. javanica* eggshells.

The improvements of enthomopatogenic nematodes in pest control

Attila LUCSKAI and Miklos NADASY

Pannon University of Agricultural Sciences Georgikon Faculty, Department of Entomology, Deak F. str. 57, 8360 Keszthely, Hungary

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The entomopathogenic nematode procedure of control can be well fitted into the integrated plant protection programmes. Nematodes practically occur everywhere in the soils, but the density of the present population is not sufficient to restrict the increase of pests in agricultural crops. Only by multiplying them and bringing them back to their area of origin can the individual number of pests be efficiently decreased. To improve the effectiveness of entomopathogenic nematodes the following factors are important. Preliminary insect susceptibility tests and experiments are needed to know which nematode and how many should be used. Effectiveness is greatly influenced by the ecological conditions, so it is preferable to choose a period to spray nematodes in late afternoon to avoid UV radiation and warmth. These nematodes can be applied before the activity of soil dwelling insects, and for this insect monitoring is needed. Thus they can be employed as a preventive defence. The nematode preparations can be stored for a longer time when cooled, so it is advisable to use them rapidly. Of course, protection against pests cannot be solved by the mere use of entomopathogens, even if an entomopathogenic nematode suitable for all pests of a crop is found, since *e.g.* weather can be a powerful limiting factor.

Meloidogyne hapla on field crops in Norway

Christer MAGNUSSON and Bonsak HAMMERAAS

The Norwegian Crop Research Institute, Plant Protection Centre, Department of Entomology and Nematology, Høgskoleveien 7, NO-1432 Aas, Norway

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In recent years, the root knot nematode, *Meloidogyne hapla*, has been found to damage field grown vegetables in Norway. In a global perspective *M. hapla* is known to occur in areas with annual mean temperatures of + 6°C or higher, indicating that Norwegian coastal areas, as far north as the county of Nord-Trøndelag, would offer possibilities for nematode establishment. The development of *M. hapla* is known to require 554°C × days over a basal temperature of + 8°C. In Nord-Trøndelag temperature sums, at a soil depth of 10 cm, exceed 560°C × days, which would allow for the completion of one generation. Damage on carrots, onion, lettuce and turnip has so far been recorded in the southern counties of Vestfold, Aust-Agder and Rogaland. In these areas temperature sums reach 1000°C × days in many localities. This would almost allow for the development of two generations of *M. hapla*, and recurrent infections during the growing season are expected. *M. hapla* may cause severe losses of carrot crops. In one field infested with *M. hapla*, much of the carrot crop was lost, and the preceding lettuce crop suffered a 50% reduction in yield. Farmers need to take precautions to avoid *M. hapla* infestations.

Effect of *Meloidogyne incognita* on field performance of cassava (*Manihot esculenta* Crantz) genotypes

Nakato N. MAKUMBI-KIDZA [1], Paul R. SPEIJER[1,†] and Richard A. SIKORA [2]

[1] *International Institute of Tropical Agriculture, Eastern and Southern Africa Regional Centre, P.O. Box 7878, Kampala, Uganda*
[2] *Soil-Ecosystem Phytopathology and Nematology, Institut fuer Pflanzenkrankheiten, University of Bonn, Nussallee 9, 53115 Bonn, Germany*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In response to a devastating outbreak of cassava mosaic disease in the early 1990s, breeding programmes in East Africa have been concentrating on developing and releasing cassava mosaic genotypes tolerant to the disease. Root-knot nematode problems on cassava have been reported from a number of African countries, including Uganda. Therefore, the more recently developed cassava genotypes were also evaluated for resistance and or tolerance to root-knot nematodes. A field experiment was set up in which 25 genotypes were tested. Plots were divided into subplots of equal size. One plot was planted with clean plantlets, while the other was planted with infected plantlets (1000 infective juveniles per plant). The main plot treatments were genotypes. Root-knot nematodes reduced the total storage root yield by as much as 25%. This loss was mainly caused by a reduction in the number of storage roots formed, which was significant ($P \leqslant 0.05$) for MH95/0324, MH95/0349 and MH95/0196. The number of large storage roots was reduced ($P \leqslant 0.05$) for MH95/0324, MH95/0372, MH95/0161 and MH95/0067. The results show that a relatively low initial nematode pressure is able to cause significant production losses in cassava and that cassava response to *Meloidogyne incognita* (race 2) is genotype dependant.

Integrated control strategies for potato cyst nematodes

Stephen MINNIS [1], Patrick HAYDOCK [1] and Kenneth EVANS [2]

[1] *Plant Nematology and Soil Pests Group, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK*
[2] *IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In the UK, the most problematic pests of the potato crop are the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*. Populations of *G. rostochiensis* can be managed by the integrated use of nematicides (granular and fumigant), crop rotation and resistant cultivars. A field experiment was done to compare the use of the resistant cultivar Santé with 1,3-dichloropropene and the granular nematicide oxamyl at full and half-rates on an experimental site containing both species of PCN (mainly *G. rostochiensis*) at an initial population density of 189 eggs g^{-1} of soil. Root invasion studies showed significantly different levels of invasion between cultivars and between fumigation treatments. Nematode multiplication was significantly decreased with Santé compared to the susceptible variety Estima. 1,3-dichloropropene and oxamyl significantly increased speed of crop emergence and percentage ground cover. The combination of Santé, 1,3-dichloropropene and oxamyl at full-rate was the highest yielding treatment. In addition, the use of 1,3-dichloropropene increased the overall number of tubers and improved the tuber size distribution. The results of the experiment show that an integrated approach to nematode control on heavily infested sites can lead to significant decreases in nematode population levels and give economic yield benefits.

A new method to overcome underestimation of plant parasitic nematode populations

Leendert P.G. MOLENDIJK, Rien NIJBOER and Gerard W. KORTHALS

PAV: Applied research for Arable farming and Field production of Vegetables, P.O. Box 430, 8200 AK Lelystad, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Modern agriculture depends more and more upon a proper crop rotation or the cultivation of resistant varieties in order to control specific nematological problems. In contrast to the chemical way, this relies more heavily on a proper identification of the nematode species involved and an accurate estimation of the population densities. Although many new molecular tools have been developed to help the identification, there seems less interest in developing new extraction methods to improve the estimation of population densities. Within our work on different *Meloidogyne* species, it was observed that some populations increased during winter without crops. To investigate this phenomenon, a new extraction method has been developed by which soil is incubated to allow egg hatching. Several field studies were carried out and gave in total over 3000 samples to estimate the impact of this soil incubation method. From this large database it was possible to investigate the influence of sampling period, preceding crop and nematode species. The results clearly demonstrated that, depending on these factors, the most commonly used extraction methods may lead to dramatic underestimation and associated risks within today's advice to farmers. Therefore it is strongly recommended to use a proper period of soil incubation.

Molecular mechanism of resistance and virulence in the *Meloidogyne*-tomato interaction

Sergio MOLINARI

Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, Via Amendola 165/A, Bari 70126, Italy

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The *Meloidogyne*-tomato interaction has been extensively studied *in vivo* and *in vitro*. Different (a)virulent populations of *Meloidogyne* spp. collected in fields or selected in glasshouse were inoculated on a number of resistant tomato cultivars. Changes in antioxidant enzyme activities of roots have been monitored from 24 h after juvenile inoculation either in pots or in cultures of excised roots. Catalase and ascorbate peroxidase activities were found to be lower in infested roots in every incompatible interaction tested *in vivo* and *in vitro*. Isoelectrofocusing separations of root catalase isozymes showed that specific isozymes were completely inactivated due to nematode infestation. Nematode-induced inhibition of such H_2O_2-degrading enzymes causes an increase of oxidative and peroxidative activity in root cells which ultimately leads to cell death. The consequent formation of a large necrotic area surrounding the juvenile is responsible for the arrest of nematode feeding and reproduction. Normally avirulent populations can be selected for virulence by repeated inoculations on resistant tomato cultivars. Near-isogenic populations were tested for difference in electrophoretical patterns of antioxidant isozymes. More marked catalase and peroxidase bands suggested a higher activity in virulent *Meloidogyne* females. Furthermore, evidence is emerging of a superoxide dismutase (SOD) isozyme specifically found in virulent females.

A novel apparatus for extraction of nematodes from soil

Mishael MOR

Department of Nematology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet-Dagan, 50250, Israel

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Existing nematode extraction techniques are generally limited to certain nematode life stages (free-living or inert) and sizes. Most methods are laborious and consist of several phases. The objectives in developing the new method were to achieve extraction of a wide range of nematode forms in a single apparatus to simplify the extraction process and to enhance efficiency and convenience in operation. Unlike other elutriation techniques, the new method makes use of an up current of small and dense air bubbles in the suspension, rather than a water flow, for separating nematodes from soil particles and keeping them in suspension. The basic component of the apparatus is a graduated Perspex scaled cylinder (length, 50 cm; diameter, 10 cm; volume, 3 l) with water entering through a perforated stopper at the bottom, above which a stream of air bubbles is delivered through a Perplex tubule by means of a pump and flow-meter. At the upper end of the column, a plastic tube which serves as a cyst trap is attached; the other end of the tube is connected to a vacuum pump. The cylinder is mounted in a metal ring fixed to an axis which allows the apparatus to revolve 180°, thereby enabling formation of a well-mixed suspension of the soil in the water. This arrangement also facilitates *in situ* cleaning of the cylinder after completion of the extraction operation without the need to dismantle the apparatus for quantitative work. The facility is attached to a standard stainless sink (length, 120 cm; width, 40 cm) with a drain, serving two apparatuses. The apparatus is compact, simple and convenient to handle. It is relatively cheap and has a set of Perspex sieves (diameter, 15 cm). One person can comfortably operate several units simultaneously. The method is particularly suitable for separating large or heavy nematodes which normally sink quickly. Yields of nematode suspension are relatively free of debris or soil particles. Several years of experimentation with this method have shown that from a 200 g soil sample 70-90% of the nematodes are obtained, depending on soil type, nematode form, operating time, and number of repetitions. These experiments were conducted both with infested samples taken from the field and artificially infested soil samples previously infected in a laboratory with a wide range of nematode species. Cyst retrieval with a vacuum-sucked stream of water is 15% more efficient than that obtained with the Fenwick technique.

On the identity of *Heterodera latipons* Franklin

Mishael MOR [1] and Dieter STURHAN [2]

[1] *Department of Nematology, A.R.O., The Volcani Center, P.O. Box 6, Bet-Dagan 50250, Israel*
[2] *Biologische Bundesanstalt, Institut für Nematologie und Wirbeltierkunde, Toppheideweg 88, 48161 Münster, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The cereal cyst nematode, *Heterodera latipons*, was described from Gilat, Israel, in 1969 and has been recorded since then in many countries, mainly the Mediterranean and the Oriental regions. Comparative biochemical studies of cyst nematode populations of different geographical origin provided first evidence that the species previously considered as *H. latipons* is actually a mixture of species. Re-examination of *H. latipons* type specimens showed that even the type material consists of two morphological types, which are mainly distinguished by cyst characteristics, one type agreeing with that figured in the original description of *H. latipons*, the other resembling the related species *H. hordecalis* which is mainly known in northern and central Europe. The *H. "hordecalis"* type was

also found among populations identified as *H. latipons* in Libya, Iran and the Azores. It could be isolated even from the type locality Gilat recently, where it occurred together with *H. latipons* s. str. Populations of *H. latipons* s. str. and *H. "hordecalis"* showed differences in host range among species of grasses and varieties of several cereals. For example, *Phalaris minor* and *P. paradoxa* in Gilat serve as good hosts, whereas in a nearby locality they are non-hosts. There is evidence of an additional non-described species within the *H. latipons* species complex.

The effects of predatory nematodes for harmful insects (Melolonthidae, Noctiudae, Blattidae) in Hungary

Miklos NADASY [1], Gyula SARINGER [1], Attila LUCSKAI [1], Szilvia PEKAR [1], Andras FODOR [2], Csaba BUDAI [3] and Michael G. KLEIN [4]

[1] *Pannon University of Agricultural Sciences Georgikon Faculty, Department of Entomology, Deak F. str. 57, 8360 Keszthely, Hungary*
[2] *Eotvos University, Department of Genetic, Nuzeum krt. 4/A, 1088 Budapest, Hungary*
[3] *Csongrad County Plant Health and Soil Conservation Station, Biological Control and Quarantine Development Laboratory, P.O. Box 99, 6800 Hodmezovasarhely, Hungary*
[4] *Japanese Beetle Laboratory, O.A.R.D.C. Wooster, OHIO 44691, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The entomopathogenic nematodes are potential biological control agents against several insect pests. In the course of the experiments the following pests were subjected to examination: *Scotia segetum* Schiff, *Mammestra brassicae* L., *Melolontha melolontha* L., *Polyphilla fullo* L., *Blatta orientalis* L. and *Periplaneta americana* L. The entomopathogenic nematodes used in the experiments were: *Steinernema feltiae* Filipjev, *S. carpocapsae* Weiser, *S. glaseri* Steiner, *S. scapterisci* Nguyen and Smart, *S. riobrave* Cabaillas *et al.*, and *Heterorhabditis bacteriophora* Poinar. The experiments were carried out in laboratory culture pots at room temperature. The insect larvae were treated at two concentrations (100, 1000 and 10 000 infective juvenile (IJ)/ml) with 4 replications. Mortality of larvae was evaluated on 5th and 7th day after treatment, respectively. Against white grubs, the 100 and 1000 IJ/ml concentrations gave an average of 25.4% and nearly 100% mortality. The order of effectiveness against grubs was *H. bacteriophora* HH, *S. glaseri*, *S. riobravis* and *H. bacteriophora* AZ 32. Against Noctuidae larvae *H. bacteriophora* HH was the most effective, resulting in some 50% mortality. The mortality of cockroaches treated with 100 and 10 000 IJ/ml per dish was 74 and 100%, respectively, after 24 h.

Testing inference of phylogeographical patterns under species level in *Meloidogyne arenaria*

Alfonso NAVAS [1], Philippe CASTAGNONE-SERENO [2], Pilar FLORES-ROMERO [3] and Jesus BLAZQUEZ [1]

[1] *MNCN, CSIC, Madrid, Spain*
[2] *Lab. Biol. Invertebres, Antibes, France*
[3] *H. Ramón y Cajal, Madrid, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Forty eight isogenetic isolates have been used to describe the pattern of distribution of *M. arenaria* at population level in a restricted tobacco monoculture area. Previous genetic characterisation of the isolates resulted in six Multilocus Enzyme Electrophoresis (MLEE) variants. As Highly Polymorphic Tandem Repeat *loci* seem to be promising molecular markers, we have focused on application of mitochondrial VNTRs (Variable Number Tandem Repeats) as indicators of relatedness among isolates and MLEE variants. PCR amplification was performed using DNA from individual females centered in the 63 length bp mitochondrial VNTR. This *locus* showed high heterozygosity with eight different alleles according to the number of repeat copies (n5 to n12) in the isolates. Allele frequencies seem to have a random distribution at species level. However allele frequencies are not randomly distributed into MLEE variants showing, in addition, statistical significant differences among them. Nei's minimum and standard genetic distances (*D*m, *D*s) and stepwise weighted genetic distance (*D*sw) are calculated and compared. According to that and stepmatrices analysis, hypothetical phylogenetic relationships among MLEE variants have a high homoplasy while these relationships are more consistent among isolates within each MLEE variant. The phylogenies of isolates are geographically represented and epidemiological considerations are discussed.

Heat shock genes from the entomopathogenic nematode *Heterorhabditis bacteriophora* (HP88)

Yael NEVO-CASPI [1], Yafit ATIYA-NASAGI [1], Revital BRONSTEIN [1], Itamar GLAZER [2] and Daniel SEGAL [1]

[1] *Dept. Molecular Microbiology & Biotechnology, Tel-Aviv University, Israel*
[2] *Dept. Nematology, Volcani Center, ARO, Bet Dagan 50250, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Entomopathogenic nematodes, such as *Heterorhabditis bacteriophora*, are highly effective bioinsecticides against a wide range of pests. However, their sensitivity to environmental extremes, especially heat and desiccation, prevents them from achieving their full biocontrol potential. Heat tolerance is achieved in organisms by expressing high levels of heat shock proteins (*HSP*). Transgenic entomopathogenic nematodes expressing high levels of an *HSP* gene from *C. elegans* have been shown to display enhanced heat tolerance. We reasoned that releasing into the field transgenic nematodes overexpressing their *own* heat shock genes may meet less regulatory restrictions than transforming them with foreign *HSP* genes. For this purpose we have cloned from the commercial strain of *H. bacteriophora* (HP88) cDNAs and genomic fragments corresponding to two genes from the *HSP70* family. We found that these proteins are > 90% similar to Hsp70A and to Hsp70C of *C. elegans.* Northern analysis showed that the *HSP70A* and *HSP70C* genes are overexpressed (2- and 3-fold, respectively) following a 4-h heat shock at 33°C. We found that a natural non-commercial heat tolerant isolate of *Heterorhabditis* (IS5) overexpresses *HSP70A* and *HSP70C* 5- and 2.5-fold, respectively, with the same heat treatment. These genes should therefore be useful tools for generating transgenic entomopathogenic nematodes that will be heat tolerant.

Nematode-antagonistic trichothecenes from the fungus *Fusarium equiseti*

James K. NITAO [1], Susan L.F. MEYER [1], Walter C. SCHMIDT [2], James C. FETTINGER [3] and David J. CHITWOOD [1]

[1] *Nematology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Building 011A, Beltsville, MD 20705, USA*
[2] *Environmental Chemistry Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Building 011A, Beltsville, MD 20705, USA*
[3] *Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Culture filtrates of a strain of *Fusarium equiseti* isolated from eggs of the soybean cyst nematode *(Heterodera glycines*) inhibit hatching of the root-knot nematode *Meloidogyne incognita.* In this study, we isolated and identified two compounds with nematode-antagonistic activity from *F. equiseti via* bioassay-driven fractionation of the culture broth. The bioassay consisted of incubating surface-sterilized eggs with DMSO-solubilized fractions and determining the percentages of eggs that hatched and of juveniles that remained mobile. The fractionation process consisted of adsorption of compounds in the broth onto Amberlite XAD-16 resin, partition of the methanol eluate from the XAD-16 between ethyl acetate and 7% methanol in water, chromatography on silica columns with chloroform-methanol mixtures, and high performance liquid chromatography with a gradient of 40-90% acetonitrile in water on a C_{18} column. Compounds were identified with infrared spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, electron impact and chemical ionization mass spectrometry, and X-ray crystallography. Two active compounds were discovered to be responsible for the nematode-antagonistic activity: 4,15-diacetoxy-12,13-epoxy-3,7-dihydroxytrichothec-9-en-8-one (4,15-diacetylnivalenol) and 4,15-diacetoxy-12,13-epoxytrichothec-9-en-3-ol (diacetoxyscirpenol). This is the first published report of toxic activity of these two compounds to nematodes.

Nematicidal activity of essential oils and their components against the root-knot nematode *Meloidogyne javanica*

Yuji OKA [1], Sengul NACAR [2], Eli PUTIEVSKY [3], Uzi RAVID [3], Zohara YANIV [4] and Yitzhak SPIEGEL [1]

[1] *Department of Nematology, Agricultural Research Organization (ARO), Bet Dagan 50250, Israel*
[2] *Biology Department, Faculty of Science, KSU University, K. Maras, Turkey*
[3] *Department of Aromatic, Medicinal and Spice Crops, ARO, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel*
[4] *Department of Natural Resources, ARO, Bet Dagan 50250, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Nematicidal activity of essential oils extracted from 27 spices and aromatic plants were evaluated *in vitro* and in pot experiments. Twelve of the 27 essential oils immobilized more than 80% of juveniles of the root-knot nematode *Meloidogyne javanica* at a concentration of 1000 l/l. Most of these oils also inhibited nematode hatching at this concentration. Essential oils of *Carum carvi, Foenculum vulgare, Mentha rotundifolia,* and *Mentha spicata* showed the highest nematicidal activity among the tested oils *in vitro.* These oils and those from *Origanum vulgare*, *Origanum syriacum* and *Coridothymus capitatus* mixed in sandy soil at concentrations of 100 and 200 mg/kg reduced the root galling of cucumber seedlings in pot experiments. The main components of these essential oils were tested for their nematicidal activity. Carvacrol, *t*-anethole, thymol, and (+)-carvone were found to immobilize the juveniles and inhibit hatching at > 125 l/l *in vitro.* Most of these components, mixed in sandy soil at concentrations of 75 and 150 mg/kg, reduced root galling of cucumber seedlings. In 3 l pot experiments, nematicidal activity of the essential oils and their components was confirmed at 200 and 150 mg/kg, respectively. The results suggest that the essential oils and their main components may serve as nematicides.

Fungal eggs parasites of root-knot nematodes on vegetable crops in Spain

Cesar ORNAT [1], Soledad VERDEJO-LUCAS [1] and F. Javier SORRIBAS [2]

[1] *Institut de Recerca i Tecnologia Agroalimentàries, Crta. Cabrils s/n 08348-Cabrils, Barcelona, Spain*
[2] *Escola Superior d'Agricultura de Barcelona, Comte Urgell 187, 08036-Barcelona, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Surveys were conducted to determine fungal egg parasitism of root-knot nematodes in areas of intensive vegetable production of Barcelona (22 sites) and Almería (35 sites). Selected sites were currently infested by root-knot nematodes or had a history of nematode problems. The species of *Meloidogyne* identified in Almería were *M. javanica* (66%), and *M. incognita* (34%), and those in Barcelona were *M. javanica* (40), *M. incognita* (38%), and *M. arenaria* (22%). A diversity of fungal parasites was isolated from nematode eggs in 50 and 43% of the samples collected in Barcelona and Almería, respectively, and they infected *M. incognita* (11 isolates), *M. javanica* (15 isolates) and *M. arenaria* (2 isolates). These fungi included *Verticillium chlamidosporium* (2 isolates), *V. catenulatum* (1 isolate), *Gliocladium roseum* (2 isolates), *Acremonium strictum* (4 isolates), *Cylindrocarpon* spp. (2 isolates), *Fusarium oxysporum* (2 isolates), *Fusarium solani* (2 isolates), *Fusarium* spp. (6 isolates), two sterile mycelium, and five unidentified fungi. The adaptation of the fungi to climate conditions is suggested since *A. strictum* was only detected in the warmer southern province of Almería whereas *Verticillium* spp. were found in the cooler northern province of Barcelona.

DNA fingerprinting of bacterial communities of *Meloidogyne fallax* egg masses

Artemis PAPERT [1,2] and C.J. (Hans) KOK

[1] *Plant Research International P.O. Box 16, 6700 AA Wageningen, The Netherlands*
[2] *Present address: ICAPB, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The gelatinous matrix in which *Meloidogyne* eggs are embedded is considered to be a protection against physical stress on the eggs and against egg parasitic micro-organisms. The bacterial community of the gelatinous matrix may influence the protective action of the matrix, by being antagonistic either to the nematode eggs or to egg parasitic micro-organisms. The bacterial community of *Meloidogyne fallax* egg masses was analysed with Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified 16S DNA fragments. This technique yields a DNA-fingerprint of the major bacterial species in the community, including the non-culturable species. Samples were taken from egg masses and rhizoplane of potato, growing in pots with field soil from two experimental fields in the Netherlands. The DNA fingerprints show that the bacterial community of egg masses is different from the bacterial community of the rhizoplane. Furthermore, site-specific differences in the communities were found. Our conclusion is that the species composition of the bacterial community of the egg masses of *Meloidogyne fallax* is different from the composition of adjacent rhizoplane communities. This stresses the potential importance of the bacterial egg mass community as an ecological factor in the reproduction efficiency of the nematode and the biological control of *Meloidogyne.*

The growth dynamics of *Caenorhabditis elegans* and why nematodes moult

Mavji N. PATEL, Ricardo B.R. AZEVEDO and Armand M. LEROI

Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

There are 20 000 described species of nematode, and each has a cuticle which it sheds and regenerates several times before adulthood. Why they do so has long been obscure, since unlike arthropods, nematodes do not need to moult in order to grow. We measured the growth of *Caenorhabditis elegans* from egg hatch to adulthood at 20°C. Growth measurements were made at about 30 min intervals during each of the four larval stages. We found that during each larval stage, the nematodes increased in volume in a strictly linear fashion and the rate of growth only changed after each moult. If *C. elegans* were to grow to adulthood at the rate set by the first larval stage then it would take 173 hours against 43 hours that it actually takes. Our findings may provide an explanation for the existence of moults in nematodes.

Contribution to the knowledge of the genus *Takamangai* Yeates, 1967 in Spain (Nematoda: Dorylaimida)

Reyes PEÑA-SANTIAGO, Gracia LIÉBANAS, Joaquín ABOLAFIA and Pablo GUERRERO

Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas s/n" 23071-Jaén, Spain

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A nematological survey carried out mainly in natural areas of the Sierra Mágina Natural Park (province of Jaén, southeastern Spain) during the period 1996-1998 yielded interesting material belonging to the genus *Takamangai* Yeates, 1967 (syn. *Thonus* Thorne, 1974). Seven species have been distinguished: *T. eroshenkoi* Andrássy, 1991, *T. ettersbergensis* (de Man, 1885) Andrássy, 1991, *T. kaszabi* (Andrássy, 1959) Andrássy, 1991, *T. mediana* (Eroshenko, 1976) Andrássy, 1991, *T. cf. nothus* (Thorne & Swanger, 1936) Andrássy, 1991, and two unidentified (probably undescribed) species. Measurements, illustrations and maps of their distribution are provided. *T. eroshenkoi* and *T. ettersbergensis* are the most widely distributed species in the geographical area studied, having been recorded in 23.1 and 17.2% respectively of the near two hundred soil samples collected. *T. ettersbergensis, T. kaszabi* and *T. mediana* are recorded for the first time in the Iberian Peninsula.

An interesting belondirid species (Nematoda: Dorylaimida) from southeastern Spain

Reyes PEÑA-SANTIAGO [1] and Manuel PERALTA [2]

[1] *Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas s/n" 23071-Jaén, Spain*
[2] *I.E.S. "Auringis", Avenida de Andalucía s/n, 23005-Jaén, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Abundant material belonging to an undescribed belondirid nematode has been collected in several soil samples from natural habitats in southeastern Spain. It is characterized by having a thin cuticle; numerous lateral and ventral glandular bodies; lip region cap-like; circumoral refractive platelets present; odontostyle relatively short, robust and fusiform, wider than the cuticle at its level, and with wide aperture; odontophore rod-like; pharynx consisting of a slender and weakly muscular anterior part with a spindle-shaped, enlarged and valve containing region behind the odontophore base, and expanding gradually into the basal bulb which occupies about half of the total neck length and is surrounded by a thick dextrorse spiral sheath; female genital system didelphic-amphidelphic; tail rounded conoid; spicules and lateral guiding pieces dorylaimoid; and few ventromedian supplements situated in two pairs outside the spicules range. This material shows a combination of characters of Belondirinae and Dorylaimellinae, and could be a bridge between both subfamilies. The nature of the stylet (with robust fusiform odontostyle and odontophore without flanges) clearly resembles Belondirinae, but the lip region shape (cap-like and with perioral cuticularized platelets), the thin cuticle, and the abundance of lateral and ventral glandular bodies conform to a morphological pattern more similar to Dorylamellinae. However, stylet morphology is here regarded as having higher taxonomic weight, which is why it has been provisionally classified under Belondirinae.

Resistance and tolerance of *Rosa* spp. to *Pratylenchus penetrans*

Yunliang PENG [1], Jan DE MEUTTER [2], Godelieve GHEYSEN [2] and Maurice MOENS [1]

[1] *Agricultural Research Centre, Crop Protection Department, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium*
[2] *University of Gent, Laboratory of Genetics, K.L. Ledeganckstraat, 35, 9000 Gent, Belgium*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Pratylenchus penetrans is one of the most damaging nematodes of roses. To develop alternatives for chemical control, resistance and tolerance of *Rosa* spp. to *P. penetrans* were studied. Ten day-old seedlings of 21 rose rootstocks were inoculated with 250 *P. penetrans*. *R. virginiana* was found to be resistant and *R. canina cv*. Superba was very susceptible. When 70 day-old rooted *Rosa* spp. and cultivars were inoculated with 500 *P. penetrans*, differences were found in the nematode multiplication between the 140 tested accessions. For tolerance observation, 14 rose rootstocks were inoculated twice with 1000 *P. penetrans*. *R. rubiginosa*, was the most tolerant with the least reduction of the plant fresh weight. Multiplication of six *P. penetrans* populations was compared on 13 rose rootstocks. A French population (Lavaltrie) reproduced significantly less than the others. *R. virginiana* was resistant to all populations. Differences in pathogenicity to *R. dumetorum* 'Laxa' between the six populations were significant. An increase in guaiacol peroxidase activity was observed in infected roots of *R. virginiana*, whereas in roots of 'Laxa' and *R. canina* 'Pollmers' we observed higher KCN-inhibitable peroxidase-like activities 50 days after inoculation. Increased HO-production in the infected roots of 'Laxa' was found to be related to its susceptibility.

Life history and development of the mediterranean cereal cyst nematode (MCCN), *Heterodera latipons*, under field conditions

John PHILIS

Spyros Stavrinides Chemicals Ltd, Stassinou 28, Nicosia 1060, Cyprus

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Juveniles of *H. latipons* started to invade young roots of barley (*Hordeum vulgare* L. cv. Athenais) thirteen days after sowing, reaching peak soil populations at plant emergence. No juveniles were found in the soil after 80 days from sowing. Newly formed cysts were observed on the roots 68 days from sowing, most of them of small size (570×415 μm) while egg laying began 83 days from sowing, at a mean soil temperature of 14.5°C. Assuming that a basal temperature of 7°C is required for the development of the nematode then the accumulated heat, above 7°C, from nematode invasion to the 'cortex ruptured', 'appearance of cysts', 'formation of eggs' and 'embryonated eggs' developmental stages reached 130, 215, 277 and 386 day × °C or 21, 54, 70 and 98 days, respectively. The nematode completed only one generation during plant growth.

Improving foliar application technologies for entomopathogenic nematodes

Simon J. PIGGOTT [1], John S. CLAYTON [2], Roma L. GWYNN [3], Graham A. MATTHEWS [1], Clare SAMPSON [4] and Denis J. WRIGHT [1]

[1] *Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire, SL5 7PY, UK*
[2] *Micron Sprayers Limited, Three Mills, Bromyard, Herefordshire, HR7 4HU, UK*
[3] *MicroBio Limited, Unit 1, Harwood Road Industrial Estate, Littlehampton, West Sussex, BN17 7AU, UK*
[4] *Horticulture Research International, Stockbridge House, Cawood, Selby, North Yorkshire, YO8 0TZ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Control of cryptic pests such as leafminers (*Liriomyza* spp.) has proved difficult and a number of factors have been identified as reducing efficacy. The selection of the applicator and the formulation can be particularly critical, a water application through a hydraulic nozzle being the standard procedure for both field and protected crops. The present study describes the assessment of various formulations and applicators, including a prototype spinning disc, against *L. bryonae* on glasshouse tomato. Considerable differences in the density of infective juveniles (IJ) of *Steinernema feltiae* UK76 (Nemasys®) deposited on leaf surfaces were found, with hydraulic nozzles and a polymer-based formulation giving the greatest deposition rate (*ca* 32 IJs cm^{-2}). The density of nematodes deposited using the prototype spinning disc was one third less than with the above combination but was achieved with only half the applied nematodes. The nematode deposition density showed a positive correlation with the % mortality of *L. bryonae* larvae due to nematode infection, with average mortality of *ca* 57% being obtained with the hydraulic nozzle-polymer combination. The work is discussed in relation to further developments in spray application technology and the possible integration of entomopathogenic nematodes with other biological control agents.

Use of Vydate L (oxamyl) in drip irrigated crops in Spain: an example of an integrated solution for root-knot nematode control and foliar horticultural pests

Sebastian PONS, Paris MICHAELIDES and Stephen IRVING

DuPont Spain and DuPont de Nemours (France), S.A. European Research and Development Center, F-68740 Nambsheim, France

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The intensive horticulture under greenhouse or plastic in Spain produces high value crops in shorter cycle crops. The use of plastic covers, chemicals, drip irrigation and selected plant varieties allows the grower to obtain crops practically all year round. At the same time this situation benefits many pests like nematodes (*Meloidogyne* spp.), white flies, leafminers, etc. Oxamyl, the active ingredient of Vydate L, is registered in Spain for drip irrigation applications. Oxamyl has proved crop selective, systemic and active on nematodes and several foliar insects. Applied after transplanting on a regular basis during the crop cycle controls the nematode problem (nemostatic effect), and reduces significantly leafminer damage (*Liriomyza huidobrensis*). The application methodology of oxamyl has been shown to be important to obtain the best product performance. The type of application, by drip irrigation, which reduces enormously the risk of applicator exposure, and the selectivity to natural enemies and pollinators makes oxamyl fit in IPM programs.

Relative importance of plant-parasitic nematodes, the banana weevil and leaf diseases as *Musa* production constraints in Zanzibar

Khadija A. RAJAB [1,2], Paul R. SPEIJER[3,†] and Arnold VAN HUIS [2]

[1] *Plant Protection Division, P.O. Box 1062, Zanzibar, Tanzania*
[2] *Agricultural University Wageningen, P.O. Box 8031, 6700 EH Wageningen, The Netherlands*
[3] *International Institute of Tropical Agriculture, Eastern and Southern Africa Regional Centre, P.O. Box 7878, Kampala, Uganda*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Bananas and plantains are important staple food crops in Zanzibar, ranking second in production after cassava. A survey was conducted at the end of the rainy season May-July 1997, to establish the relative importance of pests and diseases affecting *Musa* production in the two islands of Zanzibar, Unguja and Pemba. A total of 12 representative sites with *Musa* cultivars of the genome groups AAA, AAB and ABB were selected. Pest incidence and severity were assessed and pest impact was measured by relating damage levels to plant growth parameters. In Unguja, bunch weights were low, almost half of those recorded in Pemba. The nematodes *Helicotylenchus multicinctus, Radopholus similis, Pratylenchus coffeae* and the leaf disease *Mycosphaerella fijiensis* were observed both at Unguja and Pemba. Banana weevil, *Cosmopolites sordidus*, damage was only observed in Unguja and appeared highly associated with *R. similis* and *P. coffeae* incidence. None of the biotic constraints in Unguja negatively affected bunch weight. It is most likely that, in Unguja, poor soil fertility (sandy, coral rag soils) and perhaps low rainfall, are the major production constraints. In Pemba, bunch weight was affected by root damage caused by the three nematode species, followed by leaf diseases.

Genetic polymorphism and relationships of *Meloidogyne* spp. from Brazil

Onivaldo RANDIG [1], Frédéric LEROY [1], Michel BONGIOVANNI [1], Regina GOMES CARNEIRO [2] and Philippe CASTAGNONE-SERENO [1]

[1] *INRA, Unité Santé Végétale et Environnement, BP2078, 06606 Antibes cedex, France*
[2] *EMBRAPA, Recursos Geneticos e Biotecnologia, CP02372, CEP70849-970, Brasilia, DF, Brasil*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The root-knot nematodes (RKN), *Meloidogyne* spp., are major pests of many important crops in Brazil. However, there is little knowledge about the genetic variability and the relationships of the main species, and no molecular data are currently available on RKN present in Brasil. In order to investigate the range of genetic polymorphism both between and within RKN species, a collection was built, including isolates from the major species (*M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla*), from some less damaging species (*M. exigua* and *M. paranaensis*), and also isolates presumably belonging to undescribed species. Genomic DNA was purified from each of the 18 representative isolates selected for analysis, and used as template in RAPD-PCR experiments. Amplified fragments were scored as 0-1 characters, and the whole data set was analysed using a parsimony method. The RAPD markers provided reliable polymorphisms at inter- and intraspecific levels, and the 18 *Meloidogyne* isolates used in this study could be clearly identified, based on the presence/absence of diagnostic bands. Clustering of isolates and relationships inferred from the phylogenetic analysis will be presented.

Identification and variability for virulence to Triticeae of CCN populations from mediterranean regions

Roger RIVOAL [1], Sylvie VALETTE [1], Makram BEL HADJ FRADJ [1], Aïssa MOKABLI [2], Jean-Pierre GAUTHIER [1], Joseph JAHIER [1] and Julie NICOL [3]

[1] *INRA, UMR Biologie des Organismes et des Populations appliquée à la Protection des Plantes (Bio3P), BP 29, 35653 Le Rheu cedex, France*
[2] *INA, Département de Zoologie Agricole, El-Harrach, DZ, Algeria*
[3] *CIMMYT International, Wheat Program, Lisboa 27, Apdo. Postal 6-641, 06600 Mexico*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Cereal cyst nematodes (CCN) belonging to the genus *Heterodera* compose a group of species of which the major are *H. avenae*, *H. latipons* and *H. filipjevi.* These species are presumed to cause damage to bread and durum wheats in circum-mediterranean countries. This work has two objectives: *i*) to characterise the species status of populations sampled in this area with molecular tools as restriction polymorphism of ribosomal DNA (PCR/CAPS); *ii*) to test the resistance efficiency of known or unknown genes and genotypes of cultivated and wild Triticeae to a wider range of CCN populations. Attention has been focused on the host reactions of a set of *Aegilops geniculata* accessions originating from different locations around the mediterranean sea. Populations recently sampled in bread and durum wheat fields from North Africa and West Asia have been confirmed to belong to either *H. avenae* or *H. latipons* upon their restriction patterns. Most of these *H. avenae* populations, compared to other populations from South Europe, Australia and India, differed significantly in their ability to reproduce (fitness) on susceptible hosts tested in miniaturised controlled conditions. These populations also exhibited variation in (a)virulence spectrum towards the known and unknown resistance genes or genotypes such as the *Ae. geniculata* accessions. No relationship has been shown between the (a)virulence status of the nematode populations and the geographical origin of the plant genotypes tested. Several of these genotypes present an indisputable interest for regional and/or global utilisation in resistance breeding programmes of bread and durum wheats.

Analysis of the sensory responses of J2s of *Globodera rostochiensis* using electrophysiological techniques

Richard N. ROLFE [1], John BARRETT [2] and Roland N. PERRY [1]

[1] *Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK*
[2] *Institute of Biological Science, University of Wales, Aberystwyth, SY23 3DA, Wales, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The electrophysiological technique for analysing sensory responses of live nematodes has been modified for work with second-stage juveniles (J2) of *G. rostochiensis*. To illustrate the potential of the technique, concentration-dependent responses of J2 were obtained to acetylcholine and the delay in response and adaptation were quantified. Significant increases in spike activity were obtained on stimulation of J2 with potato root diffusate (PRD), whereas non-host diffusate and female secretory-excretory products elicited no response. Comparative tests using a variety of semiochemicals provided some interesting contrasts. For example, significant increases in spike activity of J2 were obtained on stimulation with D-glutamic acid whereas J2 did not respond to the L isomer. Incubating J2 for 24 h in a monoclonal antibody showing specificity to amphidial secretions resulted in blocking of the normal response to PRD; the potential of the technique for quantifying perturbation of sensory responses and determining the rate of turnover of sense organ secretions will be discussed.

Use of the electropharyngeogram to analyse stylet activation

Richard N. ROLFE and Roland N. PERRY

Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The electropharyngeogram (EPG) technique was first used to analyse pharyngeal pumping of *Caenorhabditis elegans*. We have used the technique to record electrical activity of stylet protractor muscles of fourth-stage juveniles of *Ditylenchus dipsaci* and second-stage juveniles of *Globodera rostochiensis*. Individual nematodes were captured by the anterior end in a suction pipette electrode, from which the electrical activity was recorded. Stylet activity was initiated by exposure to the neurotransmitter serotonin and the changes in membrane electrical potential were recorded corresponding to the excitatory and repolarisation phases of the stylet protractor muscles. Data will be presented on the responses to a range of agonists and antagonists to illustrate the potential of this technique to evaluate stylet activity.

Root-knot nematode pathogenicity factor analysis at the protein level

Marie-Noëlle ROSSO [1], Neil LEDGER [1], Stéphanie JAUBERT [1], Arjen SCHOTS [2], Richard S. HUSSEY [3] and Pierre ABAD [1]

[1] *Institut National de la Recherche Agronomique, USVE, 123 Bd Francis Meilland, 06600 Antibes, France*
[2] *Laboratory of Nematology, Wageningen Agricultural University, 6709 PD Wageningen, The Netherlands*
[3] *Department of Plant Pathology, University of Georgia, Athens 30602-7274, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In order to identify and characterise *Meloidogyne* sp. pathogenicity factors two strategies based on protein analysis have been developed. The first strategy is indirect, and relies on monoclonal antibodies selected for specific binding to secreted proteins expressed in the oesophageal glands. Such antibodies are used for immunopurification of the secreted protein or for its inhibition *in planta.* In Antibes, we focus on inhibition of stylet secretions *in planta* by expression of single-chain antibodies in infested roots. A single-chain antibody named scFv 6D4 and directed to a stylet secretion from *Meloidogyne* was expressed in tobacco plants. The cytoplasmic expression of this scFv led to 40% reduction in the nematode progeny on transformed plants as compared to gus controls. Analysis of nematode development in the transformed roots and cytological observations will help understanding of the role of the 6D4-secreted antigen in parasitism. The second approach is the direct purification of stylet secretions after large scale growing of nematodes. 2D electrophoresis analysis allows internal sequencing of the isolated proteins and the design of degenerate oligonucleotides for RT-PCR. The amplified products are used for Southern-blot analysis and *in situ* hybridisation. The recently obtained results are discussed.

Consequences of errors due to subsampling and other laboratory procedures

Corrie H. SCHOMAKER and H. Thomas BEEN

Plant Research International, Postbox 16, 6700 AA, Wageningen, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In many nematological studies, in which soil sampling is an important step in data collection, it is common practice to collect a bulk sample but to investigate only a part, taken after mixing. Unfortunately, the rationale behind the choice of both bulk sample size and subsample size is seldom documented. To enable this choice a general theory is needed about the relation between nematode counts, sampling error and subsampling error and errors due to laboratory procedures (extraction, counting) on the total sampling error. For this purpose, fields infested with potato cyst nematodes were intensively sampled. Bulk samples were split into two or three subsamples. Subsamples of randomly chosen bulksamples were send to three different laboratories for elutriation. All other laboratory procedures including counting were done in one laboratory. Cysts were close to randomly distributed in the well-mixed samples resulting in variance between pairs or triples according to a binomial or trinomial distribution, respectively. The average coefficient of variation associated with elutriation ranged from 3.6 to 9.6% for the laboratories involved. However, the estimated 95% quantiles of the coefficients of variation depended on the number of cysts that were counted in the samples and ranged from 73 to 42% for laboratory 1, from 43 to 19% for laboratory 2 and from 10 to 3% for laboratory 3. The consequences of these laboratory errors for the discrimination between nematode densities, the detection probability of foci and the estimation of parameters in distribution pattern of nematodes, as well as methods to choose optimal sample sizes, are discussed.

Cloning and characterisation of *map-1*, a putative avirulence gene from the root-knot nematode *Meloidogyne incognita*

Jean-Philippe SEMBLAT, Antoine DALMASSO, Pierre ABAD and Philippe CASTAGNONE-SERENO

INRA, Unité Santé Végétale et Environnement, BP2078, 06606 Antibes cedex, France

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In tomato, the *Mi* resistance gene controls the three parthenogenetic root-knot nematode (RKN) species, *i.e.*, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. However, virulent populations able to reproduce on resistant plants have been isolated from many tomato-growing areas. To understand the molecular basis of (a)virulence in RKN, we selected three independent pairs of avirulent and *Mi*-virulent near-isogenic *M. incognita* lines, and a differential analysis of their genome was performed using AFLP molecular markers. Among the *ca* 25 000 amplified fragments, one appeared to be polymorphic between avirulent and virulent lines. When used as a probe in Southern-blot experiments, this fragment was confirmed to be differential at the genomic level. Screening of a cDNA library allowed the isolation of a full-length cDNA coding for a protein of 458 amino acids, with no significant homology in databases. The protein, named MAP-1, is characterized by the presence of a signal peptide, suggesting its secretion, and repetitive motives of 13 and 58 amino acids. Using antibodies, MAP-1 was localized around the amphids of second-stage juveniles, thus confirming it is secreted. The putative role of MAP-1 in the relationships of *M. incognita* with (resistant) tomatoes will be discussed.

Characterisation of *Meloidogyne javanica* surface coat

Edna SHARON [1], Yitzhak SPIEGEL [1] and Rosane CURTIS [2]

[1] *Department of Nematology, Agricultural Research Organization (ARO), Volcani Center, P.O. Box 6, Bet-Dagan, Israel*
[2] *Department of Entomology and Nematology, IACR-Rothhamsted, Harpenden, AL5 2JQ, Herts, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Several polyclonal and monoclonal antibodies, previously raised against *Meloidogyne incognita* race 1, second-stage juvenile (J2) surface-coat and excretory-secretory products, were tested for cross-reactivity with *M. javanica* live J2, using immunofluorescence. Three of six monoclonal antibodies (IACR-Misec 9F.5, 7E.1 and 3F.4) and only one (PC 373) of six polyclonal antibodies tested, reacted with *M. javanica* surface. A polyclonal antibody previously raised against *M. javanica* J2 (PC E2), was used as well. None of the antibodies which recognized the surface reacted with J2 excretion-secretion products *in vitro*, following stimulation with the neurotransmitter DMT. Immunolabeling of cryosections of the J2 showed that the antibodies PC E2 and 3F.4 labeled spots on the hypodermis, indicating the possible origin of these surface antigens. Antibodies were used for immunolocalization of the surface antigens *in planta* on cryosections of *M. javanica* infected tomato roots. PC E2 labeled the surface coat of parasitic stages and shedding of the surface was observed. Immunolabeling of *M. javanica* extracts, on Western blots with PC 373 and E2 revealed different labeling patterns. Bioassays were developed to test the effect of antibodies on nematode motility and invasion of *Arabidopsis thaliana* roots. All the antibodies tested changed the movement pattern of the J2 and inhibited root invasion.

Biological control of *Radopholus similis* through the 'biological-enhancement' of banana tissue culture derived plantlets with mutualistic fungal endophytes

Richard A. SIKORA[1,†], Luis POCASANGRE [1], Bjoern NIERE [1] and Paul R. SPEIJER [2]

[1] *Soil-Ecosystem Phytopathology and Nematology, Institut fuer Pflanzenkrankheiten, Universität Bonn, Nussallee 9, D-53115 Bonn, Germany*
[2] *Nematologist, Biological Control Program, ESARC, International Institute of Tropical Agriculture, Kampala, Uganda*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A large diversity of fungi have been detected in healthy roots and corms of a wide spectrum of banana cultivars from Africa, Asia and South America. These fungi have been shown to be non-pathogenic, causing no visible damage to the plant tissue even at high inoculum level. Research has clearly demonstrated that many of these non-pathogenic fungal isolates grow endophytically at high densities within banana root tissue. Furthermore, investigations have shown that some isolates have strong biocontrol capabilities when applied externally to tissue culture derived plantlets. The isolates reduced significantly *R. similis* densities below threshold levels in both the roots and rhizosphere. Isolates have the ability to colonize the cortical tissue extensively after inoculation to the plantlets in the form of spore suspensions. Significant levels of biocontrol were obtained on widely different *Musa* genomes representing both African Highland as well as commercial dessert cultivars. The results of these studies will be presented and the concepts of 'biological-enhancement' of tissue culture planting material for practical field use will be outlined and discussed.

Nematode taxonomists at the beginning of the third Millennium

N. SMOL

Nematology Course, Department of Biology, Ghent University, Gent, Belgium

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

In some western countries the number of nematode taxonomists is decreasing. An overview of nematologists world-wide, with special reference to taxonomists, will be presented to conclude if this trend is present in other parts of the world. The following items will be discussed: number of taxonomists per country, per continent; group of nematodes studied, context of taxonomy, tools used for identification; experts helping in identification, future perspectives, needs, funding agencies, international contacts, participation in symposia and/or workshops, *etc.* The results will be expressed in a north-south ratio.

Relative importance of plant-parasitic nematodes, banana weevil and leaf diseases as production constraints to plantain (*Musa* spp, AAB group) in Southern Nigeria

Paul R. SPEIJER[1,†], M. Omolara ROTIMI [1,2] and Dirk DE WAELE [2]

[1] *International Institute of Tropical Agriculture (IITA), Eastern and Southern Africa Regional Centre (ESARC), P.O. Box 7878, Kampala, Uganda*
[2] *Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Plantain production in West and Central Africa accounts for 50% of the world output. Nigeria is considered the largest producer. However, various biotic constraints, including plant-parasitic nematodes, the banana weevil and leaf diseases, affect Nigeria's plantain production. To establish a ranking among these constraints, a survey was conducted by the International Institute of Tropical Agriculture (IITA) in the plantain growing regions of Southern Nigeria. The predominant nematode species found in roots of plantain was *Helicotylenchus multicinctus*, occurring at all 69 sites. *Hoplolaimus pararobustus*, *Pratylenchus coffeae* and *Radopholus similis* were found in 64, 50 and 46% of the sites, while J2 stages of the genus *Meloidogyne* occurred at 68% of the sites. Weevil damage was observed in 71% of the sites. Black Leaf Streak disease was observed at all sites. Factors, derived from a Principal Component Analyses of damage observations for nematodes, banana weevil and leaf diseases, were related to plantain growth. The results suggest that the nematode species *P. coffeae*, followed by *R. similis* are the major biotic production constraints to plantain in Nigeria. Higher losses are anticipated from these species than from the banana weevil or leaf diseases.

Cadusafos: a most effective nematicide

Charles A. STAETZ

FMC Research Center, P.O. Box 8, Princeton, NJ 08543, USA

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Cadusafos, Rugby®, is a very effective compound with activity against a wide range of nematodes and also against many soil-dwelling insect pests. Cadusafos is currently utilized on a wide range of crops, including bananas, vegetables, citrus, tobacco, ornamentals, potatoes and spice plants, grown around the world under different soil, climatic and crop grouping conditions. The mode of action of cadusafos on nematodes as well as its impact on insects and plants will be presented. In addition, other topics such as soil movement in different soil types and the performance of several formulations under different field conditions will be reviewed. Lastly, the impact of resistance and the phenomenon of accelerated microbial degradation upon cadusafos will be discussed.

Phasmids in Tylenchidae and Anguinidae?

Dieter STURHAN [1] and Karin KIONTKE [2]

[1] *Biologische Bundesanstalt, Institut für Nematologie und Wirbeltierkunde, Toppheideweg 88, 48161 Münster, Germany*
[2] *Freie Universität Berlin, Institut für Biologie/Zoologie, Königin-Luise-Str. 1-3, 14195 Berlin, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Phasmid-like structures located in the postmedian region of the body in a dorsosublateral position have been reported for many members of Tylenchidae, several members of Anguinidae and for *Aphelenchus avenae* in Aphelenchidae. Our recent light-microscopical studies revealed the presence of such structures also in several *Eutylenchus* species in Tylenchidae, in *Halenchus* and *Pseudhalenchus* species in Anguinidae and in *Paraphelenchus* species in Aphelenchidae, but nothing could be detected in, *e.g.*, *Psilenchus hilarulus, Macrotrophurus arbusticola, Pleurotylenchus sachsi, Boleodorus* spp., members of the families Dolichodoridae, Belonolaimidae, Pratylenchidae, Hoplolaimidae, Heteroderidae and Aphelenchoididae, most of which have phasmids in a caudal position. Structures in a similar dorsosublateral position were, however, commonly observed in many members of Rhabditidae, Cephalobidae, Panagrolaimidae, Diplogasteridae and other families of Rhabditida, where they are called posterior deirids. We observed such structures also in males and juveniles of many *Steinernema* species. In these taxa, in addition to posterior deirids, phasmids are present in a caudal position. The 'phasmid-like structures' in postmedian portion of the body in Tylenchidae are likely to be homologous with the posterior deirids in Rhabditida rather than being homologous with phasmids. This is indicated by their presence in taxa with phasmids in the tail region and by the obvious absence of an external pore. True phasmids are obviously absent in Anguinidae and most genera of Tylenchidae.

Studies on the interaction and *Pasteuria penetrans* and *Glomus* sp. as bio-management agents against *Meloidogyne incognita* on tomato

Miguel TALAVERA and Takayuki MIZUKUBO

Crop Nematode Lab. National Agriculture Research Center, Kannondai 3-1-1, Tsukuba, Ibaraki, 305-8666 Japan

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The effects of the triple interaction *M. incognita* × *P. penetrans* × *Glomus* sp. on tomato growth, nematode reproduction, bacterial infectivity and mycorrhizal colonisation of roots were tested in a greenhouse-pot experiment. Tomato shoot weight and length were reduced in all treatments when *M. incognita* was present, but an improvement in plant growth could be observed when tomato plants were treated jointly with *Glomus* sp. and *P. penetrans*. Final densities of *M. incognita* J2 in soil were reduced by 42.0% in mycorrhizal plants, by 43.3% after *P. penetrans* application and by 60.7% when both treatments were applied together. Infestation by *M. incognita* did not affect root colonisation by *Glomus* sp. when mycorrhizal inoculum was established two weeks before nematode inoculum. No significant effects of the mycorrhizal treatment on *P. penetrans* attachment on *M. incognita* juveniles or number of females infected were observed.

Radopholus similis: a possible trigger of the declining production spiral of highland cooking bananas (*Musa* AAA) in Uganda

Herbert A.L. TALWANA [1], Paul R. SPEIJER[1,†] and Dirk DE WAELE [2]

[1] *International Institute of Tropical Agriculture (IITA), East and Southern Africa Regional Centre (ESARC), Namulonge, P.O. Box 7878 Kampala, Uganda*
[2] *Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, 3001 Heverlee, Belgium*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Two nematode species *Radopholus similis* and *Pratylenchus goodeyi* co-exist on highland banana (*Musa* spp., AAA type) in Central and Southern Uganda. The relative importance of these two species to banana production decline was investigated in Mbarara (South Western Uganda, altitude 1430 m) by sampling 2-y old individual mats differing in nematode species profiles. Small suckers were detached from plants at harvest and the nematode populations in the roots were estimated. Suckers from mats with *R. similis* were smaller in girth, had a reduced root system, and supported lower populations of *P. goodeyi* compared to those suckers detached from mats without *R. similis*. In addition, bunch weight was 30% lower and the number of standing leaves was lower from plants where *R. similis* was present compared to those where it was absent. Soil temperature recorded in between adjacent mats and close to the mats was higher where *R. similis* was present than where it was not. The increase in soil temperature induced by *Radopholus similis* may trigger the unrecoverable production decline observed in East African highland bananas. High soil temperature favours *R. similis* reproduction and accelerates soil organic matter decomposition.

Characterisation of resistance to root-knot nematodes in pepper (*Capsicum* spp.)

Judy A. THIES [1] and Richard L. FERY [1]

[1] *U.S. Vegetable Laboratory, USDA, ARS, 2875 Savannah Highway, Charleston, SC 29414, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Root-knot nematodes (*Meloidogyne* spp.) are major pests of peppers worldwide. The *N* gene conditions *M. incognita* resistance in the recently released bell pepper (*C. annuum*) cultivars Charleston Belle and Carolina Wonder, and an allele of the *N* gene controls the resistance in the Scotch Bonnet (*C. chinense*) cultigens PA-353, PA-398, and PA-426. Peppers are often grown in hot climates where several *Meloidogyne* spp. occur. Therefore, we characterized the heat stability and specificity of resistance in these *M. incognita*-resistant *C. annuum* and *C. chinense* genotypes. All five genotypes exhibited a partial loss of resistance to *M. incognita* at 28 and 32°C compared to 24°C. However, at 32°C, reproduction on resistant cultivars was only 20% of that of susceptible cultivars and root gall indices were within the moderately resistant range. At ambient greenhouse temperatures (26 ± 3°C), these *M. incognita*-resistant genotypes exhibited resistance to *M. arenaria* races 1 and 2 and *M. javanica*, but not *M. hapla*. Although a partial loss of resistance to *M. incognita* occurred at high temperatures, resistant pepper cultivars should be a useful component of cropping systems designed to manage multiple species of root-knot nematodes.

Morphometrics of three species of *Hirschmanniella* (Nemata: Pratylenchinae) parasitising rice, with remarks on the genus

Alberto TROCCOLI

Istituto di Nematologia Agraria C.N.R., via Amendola, 165/A, 70126 Bari, Italy

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The genus *Hirschmanniella* Luc & Goodey, 1964 contains mainly tropical species, most of which are recorded as parasites of rice (*Oryza sativa* L.). They are easily distinguishable among members of the family by their very long and slender body, massive cephalic sclerotization, and elongate oesophageal gland lobe, but their identification at species level is rather difficult, owing to a strong resemblance to each other, and the habit of feeding preferably on the same host. The finding of three populations of *Hirschmanniella* from different geographic origins (identified as *H. gracilis* from Greece, *H. oryzae* from China and *H. spinicaudata* from Venezuela, all of them commonly occurring on rice crops) allowed an accurate comparative study of their morphology, with remarks on intraspecific variability for key characters within the genus. Morphometry of the three species is presented, with particular emphasis on cephalic region, stylet morphometry, shape of female and male tail, cuticular annulation, and other characters with diagnostic value, making identification easier. A new geographic record is also given for one of the species (*H. gracilis*), which was found for the first time in a natural habitat of a northwestern province of Greece.

Measures to control TRV transmission by trichodorid nematodes in ornamental bulb crops

Anne Sophie VAN BRUGGEN [1], Frans C. ZOON [2], Cees J. ASJES [1], Ate DE HEIJ [2] and F. Astrid DE BOER [1]

[1] *Bulb Research Centre, P.O. Box 85, 2160 AB Lisse, The Netherlands*
[2] *Plant Research International, P.O. Box 16, 6700AA Wageningen, The Netherlands*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Tobacco rattle virus (TRV) may infect several ornamental bulb crops. TRV is transmitted by trichodorid nematodes. In previous research *Paratrichodorus teres* appeared to be the most important vector species in bulb crops in the Netherlands. Effective cultural measures were developed to control virus transmitted by *P. teres*. They include cultivation of fodder radish (*Raphanus sativus*) during summer prior to a gladiolus or tulip crop. *Trichodorus similis* is another important vector. In this study, we investigated whether cultivation of fodder radish reduces TRV transmission by this species as well. In a pot experiment the effect of several crops on the multiplication of *T. similis* and the associated TRV serotype was studied. A selection of these crops is being tested in a field experiment with a natural infection of viruliferous *T. similis*. The infection potential of the soil (IPS) after culture of the test crops was determined by a Most Probable Number (MPN) method. Cultivation of fodder radish resulted in a reduction of the IPS, whereas other crops had no effect. This preliminary result indicates that a culture of fodder radish may be a feasible control measure for various trichodorid-TRV disease complexes.

Hatching behaviour of the Northern root-knot nematode *Meloidogyne hapla*

Jan VAN DE HAAR

RZ Research BV, P.O. Box 2, NL-9123 ZR, Metslawier, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Large-scale screening of plants for nematode resistance using monoxenic tests requires the aseptic production of huge amounts of juveniles. The Northern root-knot nematode *M. hapla* was reared in tissue culture containers. At 20°C the highest amounts of juveniles were yielded after an incubation period of 10 weeks after inoculation. After the liberation of the eggs from egg-masses by using hypochloride, the hatching usually stops after 3 weeks, resulting in a relatively low percentage of hatching. Primarily the immature eggs do not hatch. Incubation of untreated egg-masses prolongs the hatching period and increases the final numbers of juveniles. The well-known application of low salinity treatment (0.3 M NaCl) prohibited the hatching of free eggs, but did not adversely affect the embryonic development of these eggs. It not only seemed to synchronise the hatch after transferring the eggs to water, but also increased the number of hatched eggs up to the level of that from egg-masses. These observations do not support the hypothesis that hypochloride has a direct negative effect on the hatching of eggs. Probably the composition of the matrix within the egg-mass facilitates the development of the young eggs. It seemed to be possible to imitate these conditions by low salinity.

Pathogenic variation in *Meloidogyne chitwoodi* on *Solanum bulbocastanum*

J.G. (Hans) VAN DER BEEK and Leo M. POLEIJ

Plant Research International, Wageningen University and Research Centre, P.O. Box 16, NL-6700 AA Wageningen, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Resistance in potato to the quarantine root-knot nematode *Meloidogyne chitwoodi* is one of the most promising and environmentally safe control measures for this pest organism. Adequate resistance must be effective to the widest possible genetic range of the parasite. Testing of 18 nematode isolates to 6 genotypes of *S. bulbocastanum* revealed 4 pathotypes and 3 resistance factors. Experiments with virulent selections of these isolates, derived from *S. bulbocastanum* genotypes, showed that at least some isolates were mixtures of pathotypes. Breeding programmes in potato for resistance to *M. chitwoodi* are hampered by this genetic variation in pathogenicity.

Resistance of hybrid citrus rootstocks to the citrus nematode

Soledad VERDEJO-LUCAS [1], F. Javier SORRIBAS [2], Cesar ORNAT [1], Magda GALEANO [1], Juan B. FORNER [3] and Antonio ALCAIDE [3]

[1] *Institut de Recerca i Tecnologia Agroalimentàries, Crta. Cabrils s/n 08348-Cabrils, Barcelona, Spain*
[2] *Escola Superior d'Agricultura de Barcelona, Comte Urgell, 187. 08036 Barcelona, Spain*
[3] *Instituto Valenciano de Investigaciones Agrarias, Apartado oficial. 46113 Moncada, Valencia, Spain*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Tylenchulus semipenetrans is the most important plant-parasitic nematode that affects citrus orchards in Spain. The use of nematode resistant rootstocks can be an effective, economic and environmentally safe control management. The response of 66 citrus hybrid rootstocks to a Mediterranean biotype of *T. semipenetrans* was determined in greenhouse tests. One-year old trees were inoculated with approximately 1×10^4 eggs + juveniles per plant, and nematode infectivity (females/g root) and reproductive potential (eggs + juveniles/g root) were assessed 6 months after infestation. Sour orange and *Poncirus trifoliata* Rubidoux were included as susceptible and resistant standards to the citrus nematode, respectively. Fifteen selections of Cleopatra mandarin × *P. trifoliata*, three of *Citrus volkameriana* × *P. trifoliata*, and one of King mandarin × *P. trifoliata* were resistant to the citrus nematode. The rootstocks with Troyer citrange in their parentage supported nematode reproduction but showed different levels of susceptibility. The resistance response of some selected citrus hybrids to increasing inoculum pressure has been confirmed in microplots. Some of these hybrids possess other characteristics that are of interest for citrus production in Spain and elsewhere.

Parasite specific genes from *Meloidogyne artiellia*

Pasqua VERONICO [1], John T. JONES [2], Francesca DE LUCA [3], Mauro DI VITO [3], Maria Rosaria CORTESE [1], Franco LAMBERTI [3] and Carla DE GIORGI [1]

[1] *Dipartimento di Biochimica e Biologia Molecolare, University of Bari, Bari, Italy*
[2] *Scottish Crop Research Institute, Invergowrie, Dundee, Scotland, UK*
[3] *Istituto di Nematologia Agraria, CNR, Bari, Italy*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

We have characterised three *Meloidogyne artiellia* genes, two of which may be specific to plant parasitic nematodes. The first gene shows similarity to chitin synthases from *Caenorhabditis elegans* and fungi. The *M. artiellia* chitin synthase is present as a single copy in the genome and is expressed in unhatched nematodes. It seems likely that nematode chitin synthases are involved in production of the eggshell chitin layer. We are examining the temporal and spatial expression patterns of the *C. elegans* and *M. artiellia* genes in more detail. The function of the other genes isolated is unclear. One gene is similar to bacterial genes involved in capsular polyglutamate biosynthesis. This gene has no homologs in the *C. elegans* genome or in the large animal parasite EST dataset. It is expressed in all *M. artiellia* stages examined. The third gene is small and is transcribed in all life cycle stages tested. No homologs of this gene are present in any of the databases, although proteins with similar Mr and predicted pI are present in *C. elegans*.

Belgian nematologists prepare for battle against potato cyst nematodes

Nicole VIAENE [1] and Tina MAHIEU [1]

[1] *Agricultural Research Centre, Department of Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Since the detection of *Globodera rostochiensis* in Belgium in 1946, few studies have been conducted on the spread of potato cyst nematodes (PCN). Some farmers use crop rotation and resistant varieties for the management of PCN, but lack information about the degree of field infestation and the pathotype present in their field. In a recent survey in the north of Belgium, PCN was found in 8.6% of 1320 fields that were sampled by taking 60 soil cores per hectare (about 2 kg of soil). Species identification of cysts of 60 fields showed that 56 were infested with *G. rostochiensis*, three contained *G. pallida* and one field was infested with both species. The survey will be expanded to other regions. A test for choice of cultivars, performed in closed containers, was optimised for pathotypes of *G. rostochiensis* and made available for farmers. The test will be adapted for *G. pallida.* Detection of cysts in fields needs improvement. Several infested fields were sampled intensively to measure the nematode distribution. The data will be used to simulate a typical distribution pattern for Belgian fields and develop an adequate sampling scheme.

Diagnostic value of morphological characters in some genera of Actinolaimidae (Nematoda: Dorylaimida) with notes on the phylogeny of the family

Maria Teresa VINCIGUERRA and Mirella CLAUSI

Dipartimento di Biologia Animale, Università di Catania, via Androne 81, 95124 Catania, Italy

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The family Actinolaimidae, among Dorylaimida, is characterised by having four large onchia surrounding the odontostyle. Many genera have been described as belonging to this family, mainly differing from each other by one or very few morphological characters. Still, some genera of this family differ from all the others in several characters shared by all of them: a peculiar structure of pharynx, more slender odontostyle, higher lip region and a cuticle longitudinally ridged. This clearly monophyletic group of genera is attributed by some authors to the subfamily Brittonematinae. Recently the identity of some of these genera has been discussed in various papers by different authors and the diagnostic value of some morphological characters has been questioned. Our study, conducted both by light and scanning electron microscopy, reviews the variability and the consequent diagnostic value of those characters and allows a better definition of the genera of Brittonematinae considered valid. The main evolutionary trends of the family and their phylogenetic implications are also discussed.

Parasitism on roots and rhizomes of anthurium (*Anthurium andraenum*) by *Radopholus similis* in Madeira

Nicola VOVLAS [1], Isabel M. DE O. ABRANTES [2], Alberto TROCCOLI [1], Margarita PESTANA [3] and M. Susana DE A. SANTOS [2]

[1] *Istituto di Nematologia Agraria, C.N.R., Via Amendola 165/A, 70126 Bari, Italy*
[2] *Departamento de Zoologia, Universidade de Coimbra, Coimbra, Portugal*
[3] *Laboratorio agricola da Madeira, Dir. Serv. Investigação Agricola, PT-9135 Camacha, Madeira, Portugal*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Extensive sample collections were conducted in many anthurium growing areas of Madeira, Portugal, since the first report of occurrence of the burrowing nematode *Radopholus similis* (Cobb, 1893), Thorne, 1949. The survey was conducted on anthurium plants (*Anthurium andraeanum* Linden and Andre) imported and grown in artificial substrates in shade-houses to determine the level of parasitism, population dynamics, parasitic habits, and comparative morphology of the detected population with those from other hosts and origins. *Radopholus similis* was found parasitising roots (28-52 specimens/g of root tissues) and sometimes the rhizome, with induction of necrotic lesions. Infected plants showed signs of losing vigour, with slightly yellowed leaves, and undersized, distorted flowers. Histological analysis of root and rhizome tissues showed that the burrowing nematode is an exclusively cortical feeder when it parasitises roots, but feeds commonly on meristematic peripheral tissues of rhizomes. Occasionally, vascular elements can be damaged by its feeding activity. Newly laid and embryonated eggs, juvenile stages, mature females and males were recovered from necrotic tissues; the males/females ratio, in well established populations, varied from 1:20 to 1:50. Accordingly, this nematode reproduced efficiently in the anthurium rhizome. Morphometric and morphological characters of one population of *R. similis* are described, illustrated and compared with populations from other hosts and other geographic origins and carrot-disk cultures. The damage by this phytoparasitic species to flower cultivation and production is discussed.

PCR identification of single individuals of the dagger nematodes, *Xiphinema index*, *X. diversicaudatum* and *X. vuittenezi* using specific primers from ribosomal genes

Xirong WANG [1], Chantal CASTAGNONE [2], Roger VOISIN [2], Pierre ABAD [2] and Daniel ESMENJAUD [2]

[1] *Nematology Laboratory, South China Agricultural University, GuangZhou, P.R.C.*
[2] *INRA-USVE, Nematology group, B.P. 2078, 06606 Antibes, France*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The species *Xiphinema index* (XI), *X. diversicaudatum* (XD) and *X. vuittenezi* (XV) are often sympatric in Northern European vineyards. For grapevine, *X. index* and *X. diversicaudatum* are the vectors of grapevine fanleaf virus (GFLV) and arabis mosaic virus (AMV), respectively, the first one being the economically most important nepoviruses worldwide. *Xiphinema vuittenezi* has been suspected as the putative vector of grapevine chrome mosaic virus (GCMV) in Central and Eastern Europe. All three species may be mixed in the field and are difficult to identify reliably when only single or few adult or juvenile individuals are available. With the objective of developing a diagnostic method answering this concern, an approx. 1 kb ITS region spanning the 18s and 5.8s ribosomal genes

was sequenced in one population of each species, using two conserved primers. Internal sense primers specific to each species were designed. A single amplification product was obtained for each species (*i.e.* approx. 330 bp for XI, 810 bp for XD and 600 bp for XV). Then a reliable identification was obtained from single individuals (adult or juveniles) for each species, illustrated by *i*) the presence and *ii*) the expected size of the diagnostic fragment. The study is in progress to confirm the specificity of the primers in other populations of the three *Xiphinema* species and the possibility to use them in a multiplex test for mixed species.

Comparison of various combinations of ethylene dibromide and metham sodium with methyl bromide for control of weeds and nematodes in tobacco seedbeds

Jennifer WAY and Zibusiso SIBANDA

Tobacco Research Board, P.O. Box 1909, Harare, Zimbabwe

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The efficacy of the standard recommendation of methyl bromide applied at 50 g/m^2 for nematode and weed control in tobacco seedbeds was compared with metham sodium at 50 ml/m^2 alone, and in combination with ethylene dibromide (EDB) 41% at 35, 28 and 21 ml/m^2. Similar comparisons were made keeping the rate of EDB 41% constant at 35 ml/m^2 while varying rates of metham sodium from 15, 25 to and 35 ml/m^2. All treatments resulted in better and similar germination of tobacco compared to the untreated control. There were lower numbers of broad leafed weeds in all treated plots but only the methyl bromide treatment gave good control of grasses. Although the pressure of nematodes in the experiments was very low, metham sodium on its own was not able to provide satisfactory control. A suitable combination of metham sodium and EDB, which provides good nematode and weed control, could be a good candidate for the replacement of methyl bromide for seedbed fumigation in tobacco production in Zimbabwe.

Development of diagnostics for distinguishing *Meloidogyne chitwoodi* and *M. fallax* from other root-knot nematodes

Jane WISHART, Vivian C. BLOK, John T. JONES and Mark S. PHILLIPS

Department of Mycology, Bacteriology and Nematology, Scottish Crop Research Institute, Invergowrie, DD2 5DA Scotland, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Meloidogyne chitwoodi and *M. fallax* have recently become quarantine organisms in the European community. They are thought to have been introduced into The Netherlands from the United States, probably by infested tubers, ornamental or root-stock material. Initially, these nematodes were misidentified as *M. hapla* but now two species are recognized. They are serious pathogens of several European crops particularly potato, but also infect alfalfa, the cereals, maize, beet, carrot *etc.*, and because of this wide host range they are difficult to control. We have developed a sensitive PCR-based diagnostic which can be used to distinguish single juvenile or female nematodes of *M. chitwoodi* and *M. fallax* from other *Meloidogyne* spp. (*M. hapla*, *M. incognita*, *M. javanica*, *M. arenaria* and *M. mayaguensis*), following a simple procedure involving disruption of the nematode in water. We have also produced antisera to these nematodes which can be used to detect infected plant material.

Material balance of ^{14}C-oxamyl after the translocation in the plant and the consequence for root-knot nematode control strategies

Albert E. WISSING [1,2], Richard A. SIKORA [2] and Stephen N. IRVING [1]

[1] *Du Pont de Nemours (France) S. A., European Research Development Center, 24, Rue des Moulin, F-68740 Nambsheim, France*
[2] *Institut für Pflanzenkrankheiten, Phytopathology in Soil-Ecosystems & Nematology, Universitaet Bonn, Nussallee 9, D-53115 Bonn, Germany*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Oxamyl (Vydate®) has unique plant systemic properties. It controls effectively most nematode species and a wide range of sucking and piercing insects as well as mites on many field crops, vegetables, fruits and ornamentals. To effectively integrate oxamyl into a given pest management programme, the systemic action, which includes both upward acropetal and downward basipetal translocation of active material in the plant, needs to be quantified in order to optimise efficacy and minimise environmental impact. This study with radiolabelled ^{14}C-oxamyl shows significant uptake of oxamyl by the plant prior to any degradation. Up to 81% of the soil applied oxamyl was taken up by the plant within 10 days. The results show further that the crop itself exerts a major role in preventing leaching of oxamyl out of the rooting zone into the sub-soil water. The multiple application *via* a drip irrigation system permits the utilisation of smaller amounts of oxamyl. This prolongs the time of uptake by the plant and thereby reduces the chance of ground water contamination.

Planting depth and nematicide incorporation depth affect control of the potato cyst nematode and yield of potatoes

Simon WOODS and Patrick HAYDOCK

Plant Nematology and Soil Pests Group, Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A field experiment was done at Harper Adams to investigate the effect of nematicide incorporation and seed tuber planting depth on the yield of the potato cultivar Estima and the population control of the potato cyst nematode *Globodera rostochiensis*. Nematicide was applied at commercial field rate and incorporated to three depths: shallow, which involved the broadcast of the nematicide on to the soil surface prior to planting, medium incorporation to 20 cm and a deep incorporation down to 35 cm. Potatoes were mechanically planted to three depths: shallow (approx. 10 cm), medium (approx. 15 cm) and deep (approx. 25 cm). Results showed that the medium depth nematicide incorporation, when tubers were planted at a shallow or medium depth, reduced root invasion compared with the other treatments. Medium depth nematicide incorporation also gave consistently larger ware yields and better nematode control than the other incorporation methods, which were not significantly different from the control. However, yield and *Pf/Pi* ratios were not significantly affected by planting depth.

Incorporating granular nematicides into potato seedbeds: the current UK situation

Simon WOODS and Patrick HAYDOCK

Plant Nematology and Soil Pests Group, Crop and Environment Research Center, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The use of granular nematicides for managing infestations of potato cyst nematodes remains financially attractive to UK growers as part of an integrated management strategy. Nematicide granules were traditionally broadcast onto the soil surface and rotavated or harrowed to a depth of 15 cm. Potatoes would then be planted 5-10 cm deep and ridging up would take place after the crop had emerged. This method followed the industry recommendations and growers still using this approach are unlikely to have problems with nematicide efficacy. The adoption of potato beds has changed the way nematicides are incorporated into the seedbed. Soil is cultivated to a greater depth (over 30 cm) and planting/ridging is done in one pass with tubers often planted at depths greater than 20 cm. Many of the cultivation practices will not place the nematicide granules and seed tubers together in the soil. This has consequences for nematicide efficacy. The machinery combinations that can be potentially used in the field for incorporating nematicide granules and the consequences of these techniques for crop growth and nematode control are discussed.

Interactions between plant parasitic nematodes and the banana weevil on small banana plants

Nathalie WUYTS [1], Carine DOCHEZ [2], Paul R. SPEIJER[2,†] and Dirk DE WAELE [1]

[1] *Laboratory of Tropical Crop Improvement, Catholic University of Leuven (KUL), Kardinaal Mercierlaan 92, 3001 Heverlee, Belgium*
[2] *International Institute of Tropical Agriculture (IITA) — Eastern and Southern African Regional Centre (ESARC), P.O. Box 7878, Kampala, Uganda*
[†] *Deceased*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Cooking and beer bananas are primary staples in Eastern Africa. Production is threatened by a pest complex consisting of the banana weevil, *Cosmopolites sordidus,* plant-parasitic nematodes and leaf diseases. Surveys conducted in East Africa suggest an interaction between the banana weevil and nematodes, whereby nematode-infested plants suffer a higher attack by weevils. To investigate this interaction, 4-week old plants of the commonly grown cultivars, Mbwazirume (*Musa* AAA, highland cooking banana type) and Pisang Awak (*Musa* ABB, brewing banana type), were infested with *Radopholus similis* alone, *Pratylenchus goodeyi* alone, or not infested. Four weeks after inoculation, the plants were infested with the banana weevil or not (control). Presence of the banana weevil significantly increased percentage coefficient of infestation (PCI) and peripheral damage, particularly in *R. similis* infested plants. Moreover, the banana weevil had a strong preference for plants infested by *R. similis*, when compared to *P. goodeyi* infested or control plants. On the other hand, penetration of weevil larvae into the corm is less for *R. similis* infested plants and higher for *P. goodeyi* infested plants, when compared to the control.

Adaptation of a population dynamics model for prediction of potato cyst nematode distribution within fields

Jingyi YANG [1,2], Joseph PERRY [2], Patrick HAYDOCK [1] and Kenneth EVANS [2]

[1] *Plant Nematology and Soil Pests Group, Crop and Environment Research Center, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK*
[2] *IACR-Rothamsted, Harpenden, Herts, AL5 2JQ, UK*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The population dynamics equation of Jones and Perry has been used for the development of a model that will permit simulations to be made of within-field changes in population densities of potato cyst nematodes. Detailed information from one field has allowed us to estimate the most appropriate values for that field of the input parameters required by the model. Analyses of the sensitivity of the model to these parameters are presented. A convenient input screen has been designed to allow input parameter values to be specified for input of either average field population densities or datasets of within-field distributions, and examples of the latter are given. Other purposes for which the model can be used, such as modelling nematode population changes through rotations or following nematicide treatments, are also illustrated.

Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays

Carolien ZIJLSTRA [1], Dorine T.H.M. DONKERS-VENNE [1] and Mireille FARGETTE [2]

[1] *Plant Research International, P.O. Box 16, NL-6700 AA Wageningen, The Netherlands*
[2] *CBGP (Centre de Biologie et de Gestion des Populations) — IRD (ex-ORSTOM), BP 5045, 34032 Montpellier Cedex 1, France*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Three randomly amplified polymorphic DNA (RAPD) markers, species specific to the root-knot nematode species *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, were identified. After sequencing these RAPD-PCR products, longer primers of 18 to 23 nucleotides were designed to complement the terminal DNA sequences of the DNA fragments. This resulted in three pairs of species specific primers that were used to amplify the sequence characterised amplified regions (SCARs). The developed sets of SCAR primers were successfully used in straightforward, fast and reliable PCR assays to identify *M. incognita*, *M. javanica* and *M. arenaria*. The length variant SCAR markers can be amplified from DNA from egg masses, second stage juveniles and females. This species identification technique is therefore independent of the nematode life cycle stage. Moreover, the SCAR-PCR assay was successfully applied using DNA extracts from infested plant material. The method has potential to be optimised for routine practical diagnostic tests facilitating the control of these economically important pest organisms.

Isolation and characterisation of glycogen synthase gene from the insect parasitic nematode *Steinernema feltiae* IS6

Tali ZITMAN GAL [1], Ahron SOLOMON [2], Itamar GLAZER [1] and Hinanit KOLTAI [1]

[1] *Department of Nematology, ARO, The Volcani Center, Bet Dagan 50250, Israel*
[2] *Department of Animal Sciences, Faculty of Agriculture of the Hebrew University of Jerusalem, Rehovot 76100, Israel*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

Steinernema feltiae is used as a biological control agent. *S. feltiae* can partially tolerate desiccation by entering a shallow dormancy. A new, higher desiccation tolerant *S. feltiae* strain, IS-6, was recently isolated from the Negev desert in Israel. We are interested in the metabolic changes that enable the high desiccation tolerance of IS-6 strain. By using differential display we identified novel individual genes whose transcript level is modified upon induction of anhydrobiosis. One of the genes is glycogen synthase, the rate-limiting enzyme in the synthesis of glycogen. We isolated the entire open reading frame of IS6 glycogen synthase (*Sf*-gsy-1) and found it to potentially encode a 694-residue protein. *Sf*-gsy-1 mRNA is trans-spliced by the nematode trans-spliced leader SL-1. Southern blot analysis suggested that *Sf*-gsy-1 is a single copy gene. We also established the changes in the steady state level of *Sf*-gsy-1 transcripts upon dehydration, and observed a significant decline after 24 h of desiccation stress. The reduction in the steady state level of glycogen synthase transcripts correlated with reduction in glycogen and increase in trehalose levels. Understanding the genetic and biochemical net that results in high desiccation tolerance may contribute to the improvement of nematodes as biological control agents.

Quantitative detection of tobacco rattle virus (TRV) in trichodorid vector nematodes by Taqman RT-PCR

Frans C. ZOON, Paul C. MARIS, Orhan BICAKCI and Cor D. SCHOEN

Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

A molecular assay was developed to detect tobacco rattle virus (TRV) in trichodorid vector nematodes. It is based on selective amplification of cDNA synthesized from viral RNA, and detection of the PCR product by a fluorescent Taqman-probe. The target RNA-sequence is a part of the RNA1 genome common in TRV isolates studied so far. TRV was detected in plant samples infected with different TRV serotypes, in individual viruliferous *Paratrichodorus teres* and in nematode fauna extracted from soils containing various trichodorid species. An estimation of the number of TRV-particles in individual nematodes could be made by using real time measurement of fluorescence during the PCR and dilution series of RNA extracts from purified TRV. The range found was consistent with estimations based on the size and virus packing of the nematode oesophagus. The method provides a sensitive tool for quantitative studies on virus transmission. The possible use of this quantitative RT-PCR for quick measurement of the TRV infection potential in soil samples is under study.

Image Digitisation in Nematology — Digital Databases for Research, Teaching, and Extension, NemaPix, Vol. 2 (1999)

Ulrich ZUNKE [1] and Jonathan D. EISENBACK [2]

[1] *University of Hamburg, Faculty of Biology, Plant Protection, Institute of Applied Botany, D-20355 Hamburg, Germany*
[2] *Virginia Tech., Dept. PPWS, Blacksburg, VA 24060, USA*

Presented at the 25th International Nematology Symposium, Herzliya, Israel, 2-7 April 2000

The rapid development of image digitization for storage and display on personal computers offers to greatly enhance entomological research and teaching through the development of digital databases. One example of digitised images stored electronically on a CD-ROM is *A Journal of Nematological Images* edited by J.D. Eisenback & U. Zunke. NemaPix, Vol. 1 was published in 1997/98. Similar to Vol. 1 NemaPix, Vol. 2 (Dec. 1999) is now published. There are 1064 images from 26 contributors worldwide stored on this 2nd Volume on CD-ROM for use within research, teaching, and advisory services. The photographs and micrographs are easily searched and accessed for ease of use. The pictures are stable and not subject to degradation such as fading, scratching, staining, *etc.*, that are common with photographic images. The images are useful for observation directly on the computer screen or they can be printed on paper, overhead transparencies, or even photographic film. The images can be used for presentations with a digital projector or overhead projector panel connected directly to a computer or powerbook. The equipment and steps for the development and use of digitised images are presented in a diagram. On Vol. 2 there are 11 short digitized videos, a slideshow (200 Images for Presentation *etc.*) and references to books which are already out of print (*e.g.*, Cobb). For more information please contact: e-mail zunke@aol.com or jon@ut.eud or homepages: http://dreamwater.com/biz.mactode or http://www.zunke-photography.com.