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Nematodes 5ICN Down Under

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Session One – Plenary Session

Chairs: Michael Hodda & David Chitwood

Is Nematology a Jigsaw, a Tapestry or a Strange Attractor?

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This paper is an attempt at an overview of the science of nematology: what are its characteristics, where it has been, where it is heading, and where it might go (which is not necessarily the same as where it is heading!). I will also discuss the relationships between nematology and other scientific disciplines, and what can be learned but also given back.

Nematology is an amazingly diverse field of study. I will argue that this complexity is one of its greatest strengths, along with the particular characteristics of the organisms themselves. To simplify my discussion, I will use the metaphor of a two-dimensional artwork to explain what I mean, with examples from parts of nematology that I am familiar with.

Is nematology a jigsaw of interlocking pieces, falling into place to form a larger picture? To some extent, missing pieces of a scientific puzzle can be identified, researched, and then slotted in to solve larger problems, for example in adding molecular evidence to evolutionary trees. This metaphor fits well in some circumstances, but not others.

Is nematology a tapestry of threads with different strands of science crossing and weaving together form a coherent image? To some extent, researchers can take from entirely different scientific disciplines to create new advances, for example in identifying resistance genes from analogues in free-living model nematodes, the knowledge and cultures of which were the result of basic studies of nutrition. This metaphor, too, fits in some cases, but not others.

If neither of these models fit all the time, is nematology instead a strange attractor, the densely patterned picture produced as a result of a certain type of dynamic mathematical system, where the individual results jump around in an apparently chaotic manner, but nevertheless overall form a beautiful, if abstract, picture. This sort of picture may not be to everyone's taste, but can nevertheless be the outcome of the underlying processes.

In many cases of nematological research, the last model may be the one closest to reality. The development of, and results from, *Caenorhabditis elegans* as a model organism, begun before the double helix was even known, is a case in point. If this is the model for much of nematology, there will be a few surprises in store, but equally it will point the way to new areas where there could be great advances. These advances may very well be initially in fundamental areas of science, and only later be translated into applied fields. Viewed in this way, nematology has a great deal to offer the broader scientific community.

Metagenomics, Big Science, and the Reformation of Nematology

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Metagenomics is the genomic analysis of an assemblage of organisms. Ultra-high throughput sequencing can now generate gigabase outputs in a single run. The day is approaching when an entire soil nematode community can be profiled *en mass* with unprecedented precision. The process, however, is expensive and without reference databases, the information obtained is limited.

A Practical Future for Nematology in the Real World

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SESSION TWO – ECOLOGY AND BIODIVERSITY OF SOIL NEMATODES IN SUSTAINABLE SOIL CONSERVATION

CONVENORS: GREGOR YEATES & NIGEL BELL

Nematode Assemblages and Soil Properties Are Closely Linked

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Nematode faunal composition is closely linked to the soil chemical and physical properties that define the microenvironments in which nematodes interact with each other and with other organisms. Nematodes play relevant functions in the soil food web, in which keystone species and interaction forces have a dominant role determining system stability. Nematode species and their interactions respond to environmental drivers, and such relationships result in reciprocal causal associations in which nematodes affect and reflect soil properties.

Relationships between nematodes and soil properties depend on soil management and scale of observation, both of which affect the interpretation of these relationships. Thus, it may be difficult to extract general patterns between nematode assemblages and soil properties, and such associations must be evaluated through case-specific inferences.

At a microcosm level, soil texture, soil nitrogen, microbial organic carbon, microbial diversity, bulk density and the presence of food sources and natural enemies play roles in determining nematode assemblage composition. For example, abundance of many bacterial feeders, such as *Acrobeloides* and *Mesorhabditis*, are often correlated with soil N measures, and some nematode species may be used as enrichment indicators. Other soil properties, such as soil texture, may be a limiting factor in fitness of plant-feeding nematodes like *Meloidogyne*. On the other hand, nematodes affect plant and microbial growth, carbon allocation in the rhizosphere, microbial diversity, organic matter decomposition and nitrogen mineralization. While some relationships appear at diverse scales, other common findings may reflect spurious correlations due to common spatial patterns in distribution variables.

At landscape level, longitude, elevation, temperature, pH, vegetation and land use may determine the composition of nematode assemblages. Introducing computational tools as GIS or geostatistical analysis into data analysis may help improve our understanding of nematode ecological relationships.

Nematode Diversity and Function in Dutch Sand Dunes

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Dutch coastal sand dunes are dominated by *Ammophila arenaria* (marram grass) that is planted to stabilize the sand surface. Yearly deposition of wind-blown sand from the beach enables a temporary escape from plant-feeding nematodes and pathogens in the lower sand layers, facilitating vigorous growth of the grass at the seaward side of the dunes.

Several endoparasitic and ectoparasitic plant-feeding nematodes are associated with various dune grass species. Some are generalist and some are specialist feeders, however, specificity is not always related to occurrence. For example, *Meloidogyne duytsi* multiplies on two related grass species, but in the field occurs with only one of them.

The cyst nematode *Heterodera arenaria* migrates towards new root layers that are formed on top of old root layers in response to sand burial. Part of the population remains behind, which is disadvantageous for their own fitness. However, it provides insurance for the population when, in extremely dry years, the emigrant nematodes fail to produce offspring.

Competition with other plant-feeding nematodes may regulate the density of a species that otherwise would be harmful to the plant. In a field experiment with *A. arenaria*, *M. maritima* added alone developed faster and to higher densities than concomitant addition of *Heterodera arenaria* and *Pratylenchus penetrans*. Moreover, addition alone resulted in lower plant biomass.

Interactions may lead to competition or facilitation, depending on host plant identity. We added *Tylenchorhynchus microphasmis* and *T. ventralis* to *A. arenaria* (good host for both) and *Carex arenaria* (good host for *T. microphasmis* only). Addition of *T. ventralis* did not affect multiplication of *T. microphasmis*. However, on *A. arenaria*, *T. ventralis* experienced interspecific competition, whereas on *C. arenaria*, *T. microphasmis* facilitated multiplication of *T. ventralis*. Therefore, including nematode interactions may lead to different conclusions on nematode population dynamics than focusing on each species alone.

Nematode Diversity under Commercial Banana Production

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Radopholus similis remains an economic constraint to sustainable banana production for many tropical countries. Nevertheless, *R. similis* is not an economic problem on some banana farms although the nematode is present. The mechanisms of why some soils become suppressive to *R. similis* may be uncovered with a better understanding of the soil ecology. Soil nematodes and their assemblages have been used as an indicator of soil ecological processes, which may help to develop more sustainable banana production systems. Investigations were made in commercial banana production at two different locations, Australia and Costa Rica.

A survey conducted in Australia comparing nematode assemblages under banana production with neighbouring forest or pasture soils found that banana production had a significantly lower nematode diversity (Shannon-Weiner index) and greater proportion of plant-parasitic nematodes relative to forest and pastures sites. Further studies in a replicated field experiment found diversity of soil nematodes was significantly lower when bare soil was maintained in the interplant space compared to vegetated areas. Furthermore, changes in soil labile C and soil NO_3-N levels were correlated with increasing nematode diversity. This finding was confirmed in a survey of 10 commercial banana farms.

Simultaneously, poor root growth and decreasing production was being experienced in banana plantations in Costa Rica. A survey of 21 commercial banana plantations found that the diversity of soil nematodes was at a maximum on farms with a soil pH of 6. Furthermore, when *in vitro* banana plantlets were grown in soil collected from the farms and inoculated with *R. similis*, fewer nematodes were recovered from the roots of plants grown in soil with a neutral pH.

To maintain diversity of soil organisms in banana production, the management of soil carbon, nitrogen, pH and mix of plant species was found to be important. However, investigations at regional levels were needed as different constraints to nematode diversity develop under different conditions.

Acknowledgements
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How Different or Similar are Nematode Communities in Paddy and Upland Rice Fields

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Paddy field (PF) is now attracting worldwide attention as a valuable land use for biodiversity conservation and as a land use with significant greenhouse gas emission, as well as being important for food production. Nematodes may play an important role in soil food-web and ecosystem function in paddy, as both microbial feeders and prey for soil and aquatic animals. Thus, we characterized nematode community structure in paddy before, in and after flooding, with adjacent upland rice fields (UF) as a reference site. It is generally accepted that in PF, bacteria dominate over fungi but decomposition is slow due to low oxygen concentration during flooding. We also expect that greater physicochemical changes due to flooding affect ecosystem status to maintain an r-selected nematode community. In this context, we analyzed community structures using multivariate and index approaches. For indices, we hypothesized that each of Maturity, combined Maturity, Structure, Channel and Enrichment Index would be lower in PF. In the flooded period, however, our hypothesis was confirmed only for Channel Index. Distinctive occurrence of *Rhabdolaimus, Tobrilus* and monhysterids, and greater occurrence of dorylaimids in PF seemed to be responsible for the deviation. Other than such differences, we found that total nematode density and plant feeder frequency were lower when flooded in paddy. Interestingly, some common taxa in UF and other terrestrial habitats, such as *Acrobeloides* and *Filenchus,* still occur under water in flooded paddy. We also discuss how nematode communities different between PF and UF, before and after flooding and discuss nematode community dynamics in PF.

A Perspective on Diversity within Nematode Feeding Groups across Ecosystems

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Recently there has been strong interest in nematode diversity and the use of nematodes as indicators of soil conditions. Key papers on feeding groups, a maturity index and structural indices reflected current understanding of nematode biology. Soil nematodes contribute to ecosystem services so potential build-up of populations, or their contribution to ecosystem processes, across regions and ecosystems are relevant. What do we really know, or can infer, about nematode biology on these scales? Plant nematologists consider the effects of many plant-feeding nematodes in a given field - whether their spatial heterogeneity was due to soil factors or plant traits. In some habitats plant-feeding nematodes are not abundant but, given that these have been demonstrated to increase 'leakage' of photosynthate into the soil microbial biomass, and thus enhance microbially-mediated nutrient cycling, with a paucity of these plant-feeders there may be a rather different soil biology - assuming mealy bugs, aphids and the like are not acting in their place. As a corollary, if the roots are strongly mycorrhizal a different functional set of nematodes may be active. Knowledge of fungal-feeding nematodes is developing. Some bacteria may be too large for particular bacterial-feeding nematode species to ingest, but, on a wider scale, why do the bacterial-feeding nematode assemblages in particular soils tend to be dominated by cephalobids, panagrolaimids, plectids or rhabditids? Such questions can only be tested in comparable ecosystems. In annual or cropping systems the decay of the annual crop is likely to overshadow other effects, although seasonal patterns may be useful. In forests litter processes may be strongly influenced by year-to-year climatic differences. Permanent grasslands or ecosystems on similar soils but with contrasting functional pathways (e.g., mycorrhizal versus non-mycorrhizal) with may provide the optimum ecosystem to investigate complementarity between, and diversity within, nematode feeding groups.

Session Three – Mutualistic/Phoretic Associations and Invertebrate Parasitic Nematodes

Convenors: Robin Giblin-Davis & Kerrie Davies

Entomophilic Nematodes for Predictions of Worldwide Nematode Species Diversity

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The phylum Nematoda is the poorest known of the major metazoan phyla with about 25,000 nominal species. Based upon current dogma, most nematode diversity is represented by species that are free-living in soil, marine, or freshwater ecosystems with a comparatively small fraction hypothesized to be parasites of animals or plants. However, when insects are seriously considered as hosts with the potential for spatial and temporal allopatry and resulting cladogenesis, there could be a large proportion of nematode species involved in highly specialized relationships with insects and other invertebrates. We use a clade-specific approach for identifying lucrative radiations of unidentified nematode species. Firstly, we associate suitable environmental factors (i.e., moisture, temperature and food availability) with an invertebrate lineage to see if nematode prospecting makes sense. Invertebrates in certain terrestrial environments may be less likely to have nematode associates than others because all of their life history occurs outside the physiological range of potential nematode associates. Secondly, we check for invertebrate lineages that have previously been reported with seemingly unique or host-specific nematode genera. Thirdly, we work with invertebrate groups that are easy to find in abundance and for which expertise is available for identification. The correct identification of hosts and proper vouchering is critical to reproducibility and understanding issues of host-specificity. This step can be accomplished by sequencing the invertebrate host when experts are not available. Finally, we rear out or collect invertebrates for dissections and are prepared to culture or sequence dauers or other nematode stages that may be recovered. We have recently tried this approach with termites for focal sampling of invertebrate-associated nematode diversity from the rainforests of Central America and temperate regions of North America. We have also tried it with wood-boring insects from temperate forests in Japan, and figs, bees, and weevils from around the world. We report on some of our unusual discoveries and discuss prospects for a better estimate and understanding of patterns of entomophilic nematode biodiversity.

Host Specificity, Speciation, and Coevolution in *Fergusobia* (Nematoda: Neotylenchidae) - *Fergusonina* (Diptera: Fergusoninidae) Galling Mutualists on Myrtaceae

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A unique tritrophic association between *Fergusobia* nematodes and *Fergusonina* flies forms galls on various myrtaceous hosts. Fly/nematode associations occur on *Eucalyptus* in Australasia, *Angophora, Corymbia, Leptospermum*, and *Melaleuca* in Australia, *Syzygium* in Australia and India, and *Metrosideros* in New Zealand. *Fergusobia* has a complex life cycle, involving a plant-parasitic generation followed by an insect-parasitic generation. Feeding activity by the plant-parasitic stages of the nematode apparently leads to formation of a gall in which the fly larvae feed. The adult female fly provides nutrition for the insect-parasitic stage of the nematode and ensures its dispersal. Nematodes developing with male flies apparently die when the fly larvae pupate. Because both organisms appear to benefit, the association between *Fergusonina* and *Fergusobia* can be described as a mutualism. Transmission of nematodes between flies is vertical (mother to offspring), suggesting cospeciation between flies and nematodes.

Galls, flies and nematodes were collected from myrtaceous plants from Australia and New Zealand. Molecular phylogenies for *Fergusobia* nematodes and *Fergusonina* flies were generated using multiple genes and standard techniques. Inferred trees were compared with each other, myrtaceous host phylogeny, gall form and fly morphology. The associations are highly host-plant specific, and each species of nematode is associated with a single species of fly. There is good phylogenetic congruence of the terminal, but not the deeper nodes of the compared nematode/ fly phylogenies. These results are consistent with a general model of cospeciation of nematodes and flies. Speciation by nematode/ fly associates appears to be accompanied by periodic host plant shifts; there is strong evidence of evolutionary conservation of plant host genus.

Evolution of Mutualism, Phoresy, Parasitism and Amensalism in *Bursaphelenchus* Nematodes

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The genus *Bursaphelenchus* encompasses fungal and plant-parasitic nematodes that are mostly entomophilic. The relationships between *Bursaphelenchus* nematodes and their vector (carrier) insects are mostly considered to be commensal (phoresy: nematodes use insects for transport) or population mutualists (the plant-pathogenic nematodes kill more host plants to serve as oviposition resources for their vectors, or fungivorus nematodes reduce fungal enemy loads in the carriers' habitat and gain mobility to nutritive breeding sites). Recent advances in our understanding of the associations between *Bursaphelenchus* and their insect hosts challenge current dogma. For example, some *Bursaphelenchus* species have an 'insect-parasitic juvenile' and/or 'insect- parasitic adult' stage classifying them as parasites. Some species enter an insects' tracheal system obstructing and swelling it in excessive infestations that result in a reduction of the insect host's longevity (amensalism). Thus, carrier (vector) usage by *Bursaphelenchus* nematodes seems more divergent than originally expected. In the present study, we coded insect usage by *Bursaphelenchus* species on molecular phylogenetic trees to help infer the putative origins or patterns of ecological and symbiotic divergence. The genus *Bursaphelenchus* was separated into three major clades. The first clade contains only one species, the soil-dwelling bee associated *B. abruptus*, which is closer to a neighboring clade, and may be more appropriate for inclusion in a new monospecific genus. The second clade, which was not clearly resolved and comprises several subclades, contains many wood-boring Scolytidae- and Curculionidae-associated species. The third clade was well-defined with high bootstrap support, but is the most variable in biology and morphology, including species associating with wood-boring Scolytidae, Curculionidae, and Cerambycidae as well as soil dwelling beetles (Nitidulidae) and bees (Halictidae). Both insect amensalistic and parasitic relationships were observed in the second and third clades, but the nature of these associations was different and presumed to have evolved independently within each of the clades from commensal ancestors.

Phylogenetic Analysis of Parasitism of Invertebrates by Rhabditid Nematodes

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Aside from nematodes that use invertebrates as intermediate or paratenic hosts for vertebrate parasitism, there are several types of parasitic associations characterized by whether the nematodes inhabit the host's intestinal lumen or parasitize the host's coelom as adults or larvae. About a dozen higher taxa (superfamilies, infraorders or suborders) are represented among the nematodes parasitizing invertebrates as a single (definitive) host or killing an invertebrate host to consume its body contents once they are processed by symbiotic bacteria. Within the order Rhabditida (sensu De Ley and Blaxter, 2002), clades consisting entirely of nematode associates of invertebrates are relatively common suggesting that radiations of invertebrate-associated nematodes may have coevolved with certain terrestrial invertebrate lineages more readily than less derived clades of freeliving nematodes. Molecular phylogenetic analysis (SSU and D2D3 LSU rDNA) of nematode diversity in earthworms, terrestrial molluscs, insects and diplopod millipedes demonstrates that the colonization by nematodes of these invertebrates was not a singular evolutionary event, but happened at least two or three times. Taxa comprising the nematode associates of invertebrates cluster in both basal and terminal nodes of rhabditid phylogeny. In some cases, large radiations of invertebrate-associated nematodes are sister groups for a set of genera in the Rhabditomorpha or Cephalobomorpha. For example, angiostomatids of terrestrial molluscs cluster within Rhabditidae whereas drilonematids are inferred to occur within the Cephalobidae. Conversely, Thelastomatoidea (Oxyuromorpha) and Ransomnematoidea (Rhigonematomorpha) show a basal position in Rhabditida phylogeny. Unlike drilonematids, two species-rich families of the nematodes that are parasitic in earthworms (Ungellidae and Homungellidae) are basal to the Cephalobomorpha. Entomopathogenic nematodes form clades within three levels of rhabditid phylogeny – on the generic level (*Phasmarhabditis*), on the family level (Heterorhabditidae) and closer to the basal nodes (Steinernematidae). This latter family is inferred with a basal node for the remaining Strongyloidoidea (*Strongyloides*+*Alloionema*+*Rhabditophanes*).

Nematode Parasites of the Urban Cockroach *Periplaneta americana* from Córdoba City, Argentina

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The hindguts of one hundred and fifty cockroaches of *Periplaneta americana* (nymphs, females and males) were analyzed with the aim of evaluating the presence of parasitic nematodes. Insects were collected from households in Córdoba city (Argentina). One hundred percent of *P. americana* individuals was parasitized; 95% of cockroaches were infected by males and females of *Thelastoma* spp. and *Hammerschmidtiella* sp., whereas different juvenile stages were observed in the remaining 5%. Prevalence was 83% for the genus *Thelastoma* and 79% for *Hammerschmidtiella*; mean infection abundance was 2.3 and 3.6, respectively. Sixty six percent of cockroaches were simultaneously parasitized by both genera. The degree of infection did not depend on the sex of the host, neither was it correlated with the developmental stage of the insect (nymphs or adults). Further studies are needed to determine the nematodes' species identity and, at the same time, to elucidate local nematode species richness in *P. americana*.

Session Four – Resistance Breeding Against Sedentary Nematodes

Convenors: Phil Roberts & Forest Robinson

Breeding for Resistance to the Clover Root-knot Nematode and the Clover Cyst Nematode in White Clover

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New Zealand's pastoral industries produce animal products that are internationally competitive in spite of the country's distance from major markets. White clover is a key contributor to the system as it fixes nitrogen and provides high-quality feed. However, clover's potential is rarely reached due to abiotic (e.g., drought) and biotic factors. Chief among the latter are the clover root-knot nematode (*Meloidogyne trifoliophila*), clover cyst nematode (*Heterodera trifolii*) and lesion nematode (*Pratylenchus* spp.). Control options studied have used management and biological options but with most effort in resistance/tolerance. In a recurrent selection programme based in the greenhouse, resistance to *M. trifoliophila* and *H. trifolii* has been improved and resistant lines crossed to cultivars to improve field performance. Tolerance to root-infecting nematodes and other factors has been improved in a field-based programme in which the best-performing plants were selected and crossed. Resistance to *M. trifoliophila* and *H. trifolii* is under polygenic control: resistance genes for *H. trifolii* show evidence of dominance whereas resistance genes for *M. trifoliophila* show recessiveness. In grazed field trials, lines from both programs performed as well or better than cultivar controls. Counts of nematodes in roots showed that resistance reduced numbers but results varied between sites and years. This review paper summarises progress made in the white clover breeding programs and also examines options in genetic mapping and introgression as they affect resistance to *M. trifoliophila* and *H. trifolii*.

Resistance to *Rotylenchulus reniformis* in Cotton (*Gossypium hirsutum*)

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The reniform nematode (*Rotylenchulus reniformis*) is a major problem in cotton (*Gossypium hirsutum*) production and resistant cultivars are lacking. The development of cotton cultivars with resistance to reniform nematode appears possible but presents a significant research challenge, primarily for two reasons. First, the best sources of resistance occur within diploid species that are genetically incompatible with upland cotton. Second, without molecular markers, reniform nematode resistance can only be detected by lengthy nematode reproduction assays. Thus, it is absolutely essential to discover a molecular marker for each resistance source to provide seed companies with a tool for monitoring inheritance of resistance as they proceed through the cultivar development process. Simultaneous introgression of resistance from sources within several species is the wisest approach, because incomplete expression or incorporation of closely linked deleterious genes is possible in all cases, and in each case an investment of many years is required before success can be gauged by field testing of elite resistant breeding lines at multiple sites. Currently, researchers at more than a dozen USDA laboratories and state-supported universities in the United States have projects targeting introgression of reniform nematode resistance into agronomic upland cotton, from primitive tetraploid accessions of *Gossypium hirsutum* and *G. barbadense*, and diploid *G. arboreum* and *G. longicalyx*. This task will take many years to complete. However, significant progress has been made toward developing cultivars carrying resistance from each source, and the first resistant cultivars could appear within 3 years, with committed follow through by commercial planting seed companies.

Marker Assisted Transfer of Cereal Cyst Nematode Resistance from Bread Wheat into Durum Wheat

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Cereal cyst nematode (CCN) (*Heterodera avenea* Woll.) is a damaging disease of bread wheat, *Triticum aestivum* L. and durum wheat, *T. turgidum* L.. Breeding for the host-plant resistance is the most economically effective means of controlling the disease. The objective of this study was to transfer CCN resistance gene, *Cre1* from hexaploid to tetraploid durum wheat, so that *Cre1* can be used directly for the improvement of durum wheat. NB-LRR gene sequences from the *Cre3* locus were previously shown to identify homologues that were diagnostic for bread wheats carrying the *Cre1* gene. Molecular probes diagnostic for *Cre1* based on the *Cre3*-derived sequences were used to select tetraploid wheat derivatives from BC_1F_2 progenies of breadwheat x durum wheat cultivars while Dgas44, which is diagnostic for the presence of D genome was also used to select against progenies carrying D genome. Selected BC_1F_3 tetraploids with and without the *Cre1* diagnostic probe were then examined in a CCN bioassay in France and Australia for the levels of nematode reproduction. The results revealed successful transfer of CCN resistance gene, *Cre1*, from bread wheat to four durum wheat cultivars - Arstar, Cham1, Creso and Villemur and confirmed perfect association between the diagnostic marker and *Cre1* resistance in the tetraploid

Breeding for Resistance to Root-knot Nematodes in Vegetable Crops

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Most vegetable crops are hosts for one or more common root-knot nematodes (*Meloidogyne* spp.), and suffer yield loss from infection. Several important crops have natural sources of host resistance in the crop species or in wild relative species. Opportunities and challenges for breeding effective resistance into commercial varieties differ between crops based on the genetics and specificity of the resistance. Examples from breeding and analysis of resistance in carrot, bean crops, and tomato are used to describe the nature of resistance, its specificity with respect to species and populations of *Meloidogyne*, the process required for successful introgression of resistance genes, and challenges related to incidence and selection of nematode virulence. In carrot, major dominant gene resistance is available and is being bred into elite fresh market varieties via a combination of intense phenotypic selection and use of co-dominant flanking STS markers for indirect selection. In lima bean, three independent genes for resistance to root galling and nematode reproduction provide a broad-based resistance to *M. incognita* and *M. javanica*, but require a gene pyramiding approach in resistance breeding. In both cowpea and tomato, single major gene resistance used for many years in commercial varieties has been highly effective but has led to selection of virulent nematode populations able to break resistance. In these crops plants, second generation resistance genes are available that block virulent populations, but they require breeding into elite varieties.

The Potential of Resistance Gene Analogs (RGAs) of Wild Beet *(Beta procumbens)* for Breeding of Rapeseed *(Brassica napus)* Cyst Nematode Resistance

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Plant-parasitic nematodes cause >$100 billion annual losses to world agriculture, of which cyst nematodes and root-knot nematodes contribute over 80%. Oilseed rape (*Brassica napus*) is highly susceptible to the beet cyst nematode *Heterodera schachtii* (BCN) and no resistance in *Brassica* germplasm is available. To convert oilseed rape from a host into a resistant trap crop for BCN, attempts had been made worldwide to transfer resistance e.g. from radish (*Raphanus sativus*) and white mustard (*Sinapis alba*) resulting in resistant monosomic addition lines, but breeding BCN resistant oilseed rape still remains a great challenge. Here, we report the transfer of two BCN resistance genes isolated from wild beet (*B. procumbents*) into the oilseed rape genome and functional analysis of transgenes in respect to their conferring BCN resistance in oilseed rape. Transgenic plants expressing the gene $Hs1^{pro\text{-}1}$ and a RGA cZR-3 respectively were produced by *Agrobacterium*-mediated transformation. Pyramiding of two genes in the oilseed rape genome was realized by crossing of respective transgenic plants. Nematode resistance tests with transgenic plants expressing the trangenes were performed *in vitro* and *in vivo* as well. As a result, transgenic plants, independent of the transgenes, all showed significant reduction in the mean number of developed cysts per plant, compared to control plants. Strikingly, a clearly pyramiding effect of two genes expressing in oilseed rape on the inhibition of nematode development was given. More than 70% of transgenic plants expressing the double genes had less than 2 cysts, compared with 48% and 50% for plants expressing *cZR3* and $Hs1^{pro\text{-}1}$, respectively, and 2.5% for control plants. These results strongly demonstrate the potential of resistance genes and RGAs from wild beet for breeding BCN resistance in oilseed rape. A possible mechanism of the gene pyramiding effect is discussed.

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SESSION FIVE – CURRENT TRENDS IN NEMATODE PHYLOGENY, EVOLUTION AND CLASSIFICATION

CONVENORS: JAMES BALDWIN & LYNN CARTA

Molecular Phylogenetic Perspectives for Character Classification and Convergence: Framing Some Issues with Nematode Vulval Appendages and Telotylenchid Tail Termini

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Characters flagged as convergent based on newer molecular phylogenetic trees inform both practical identification and more esoteric classification. Nematode morphological characters such as lateral lines, bullae and laciniae are quite independent structures from those similarly named in other organisms like fish and insects. Within nematodes, naming and classifying similar morphological structures and refining them within independent phylogenetic frameworks often requires qualification, formal definition, or novelty in character names. Micrographs and drawings of vulval appendages were analyzed from the literature and considered within frameworks of an 18S phylogenetic tree and ecological associations. Three major vulval appendage classes based on composition, position and orientation included membranes, flaps, and epiptygmata. They were distributed across subsets of terminal and phylogenetically distant taxa that were almost exclusively commensal or parasitic. For instance epiptygmata were present within Aphelenchida, Tylenchida, Rhabditida and Enoplida. In another study, a more refined 18S molecular phylogeny based upon 18 taxa of plant-parasitic Telotylenchidae (Tylenchida) stunt nematodes and two relatives was constructed. Relatively thickened hyaline tail termini in Telotylenchidae were mapped on the tree, demonstrating a mosaic distribution among taxa with shorter hyaline tails. Phylogenetic similarity was demonstrated between *Trophurus minnesotensis*, with a long hyaline tail, and the relatively short-tailed *Quinisulcius acutus*. Overlapping measurements of hyaline tail termini support the notion that *Paratrophurus* could be synonymized with *Tylenchorhynchus*. Phylogenetic support was found for the presence of *Pratylenchoides ritteri* within the Merliniinae. Epiptygmata in these distinctly classified taxa may provide further support for the taxonomically upsetting notion of a possible new family for a *Pratylenchoides*. Expanded molecular phylogenetic trees with ultrastructural and possibly developmental comparisons are needed to refine these tail and vulval characters before using them to amend current classifications.

Evolution of Feeding Structures for Plant Parasitism: Addressing Molecular Phylogenetics' Challenge to Classical Morphological Evolution and Classification

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Molecular phylogenies often challenge traditional views that nevertheless seem to be based on plausible interpretations of morphological evolution. These include classical light microscope-based hypotheses of transformation from the muscular pharyngeal basal bulb of microbivores to the glandular basal bulb associated with plant parasitism (Tylenchomorpha). Congruent with this hypothesis is evolution from the open stoma of microbivores through several intermediates to a protrusible stylet of Tylenchomorpha. However, molecular-based phylogenetics, contradicting these hypotheses, instead point to a counterintuitive morphological transformation to plant parasitism evolving within microbivorous Cephalobomorpha. Whereas superficially this extreme divergence appears unlikely, TEM reconstruction in representatives of Rhabditomorpha, Cephalobomorpha, Tylenchomorpha, and outgroups demonstrates congruence of this transformation with evolution of the glandular basal bulb. We have also employed TEM including 3D computer reconstruction from serial micrographs to demonstrate new hypotheses of homology and a plausible hypothesis for stylet evolution that is congruent with molecular-based insight. The cuticle that forms the lining of the open channel stoma (Cephalobomorpha) and the stomatostylet (Tylenchomorpha) are both produced by a suite of distinctive hypodermal, arcade, and pharyngeal cells. Whereas these cells and their relative positions are conserved, details of their expression effect highly divergent phenotypic expression of feeding structures. In Tylenchomorpha, hypodermal cells (cheilostom) are positioned to form the lining, including the framework, vestibule, and vestibule extension, that guides the stylet. Arcade cells (gymnostom) are expressed between molts as reduced syncytia that form the cone and shaft of the stylet. The anteriormost pharyngeal radial muscle cells (stegostom) are homologs of stylet protractors and form stylet knobs. Examples we have studied underscore that more slowly evolving cellular patterns may underlie dramatic phenotypic change and, in fact, the more conserved underlying patterns may be most directly phylogentically informative. It is often molecular phylogenies that direct us to the most interesting and tractable questions of morphological evolution.

Phylogeny and Evolution of Rhabditid Nematodes

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A new, multigene molecular phylogeny resolves most relationships among major groups of rhabditid nematodes and provides a framework for studying character evolution. In this phylogeny, rhabditids are paraphyletic with regard to Strongylida and diplogastrids. The first branch is *Poikilolaimus* and there are three major clades: (1) 'Eurhabditis', which includes *Caenorhabditis* and its sister '*Protorhabditis* group' (which includes *Diploscapter*), the '*Rhabditis* group' (including *Oscheius*, strongylids, *Heterorhabditis*, and other clades) and *Choriorhabditis*; (2) *Rhabditoides inermis* + diplogastrids; and (3) 'Pleiorhabditis', which includes *Rhabditoides inermiformis*, *Rhabditoides regina*, *Pelodera*, and the '*Mesorhabditis* group' (which includes *Parasitorhabditis*). The phylogeny reveals that many characters have evolved convergently; e.g., reproductive mode, intron positions, developmental characters, and parts of male tail morphology. However, a number of morphological and developmental characters demonstrate high consistency with the molecular phylogeny, providing additional support for several clades. For example, a slit-shaped vulva is apomorphic for 'Eurhabditis'; the orientation of a cell division in the 2-cell embryo flipped 90° in the ancestor to the *Protorhabditis* group; species in the *Mesorhabditis* group share a posterior vulva and prodelphy; and a pharynx lacking a median bulb is derived and evolved only once in rhabditids. Several previously defined taxa correspond with monophyletic groups (e.g., *Oscheius*, *Caenorhabditis*, *Pelodera*, and *Poikilolaimus sensu* Sudhaus) or paraphyletic groups (e.g. *Protorhabditis* with respect to *Diploscapter* and *Prodontorhabditis*). Some other taxa are polyphyletic (e.g. *Pellioditis*, *Rhabditis,* and *Rhabditoides*). To support systematic research and studies in comparative biology and character evolution, we continue to build a representative living collection of rhabditid species (currently ~140). Current efforts are focused on finer phylogenetic resolution. Future goals are to develop a classification system, an electronic key, and a systematics database for rhabditids.

Turning Trees into Taxonomies

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What began with Plato and Aristotle (and arguably much earlier) and was ultimately canonized by Linné, culminated in a conflict that continues to this day, namely, what is the best way to classify life on earth? Ever since Darwin, the idea that all species can be depicted in a hierarchical tree of successively more inclusive groups has been a driving force behind the movement to produce taxonomies that are consistent with evolutionary history (phylogeny). Few quibble against the utility of such taxonomy. However, arguments over the best way to accomplish this goal have been most acrimonious. Key to the proposed solutions is the philosophical questions of whether natural groups exist independent of human observation, and how they should best be reflected in taxonomic systems. I discuss competing approaches in a historical context, and provide examples to demonstrate that Linné's 273 year-old system, with some modification, is sufficient to accommodate monophyletic classification.

Reconstruction Morphological Evolution on a Molecular Tylenchina Tree: Constraint Gonoduct Architecture *versus* Plasticity of Feeding Types

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Tylenchina are a morphologically and functionally diverse group of nematode species that range from free-living bacteriovores, over transitory grazing root-hair feeders to highly specialized plant-parasites with complex host associations. We performed phylogenetic analyses of small subunit rDNA sequences from 97 species including an analysis that account for the RNA secondary structure in the models of evolution. The present study confirms the sister relationship of the bacteriovore Cephalobidae with the predominantly plant-parasitic Tylenchomorpha. All analyses appoint the fungal-feeding Aphelenchidae and Aphelenchoididae as being polyphyletic but the morphology based hypothesis of their monophyly could not be significantly rejected. Within the Tylenchomorpha, the families that exclusively parasitize higher plants are joined in a single clade. However, only the monophyletic position of the (super)families Hoplolaimidae and Criconematoidea were supported; Anguinidae Tylenchidae, Belonolaimidae and Pratylenchidae appeared to be paraphyletic or polyphyletic. Parsimony and likelihood ancestral state reconstruction revealed that burrowing endoparasitism and sedentary endoparasitism each evolved respectively at least six and at least three times independently, mostly from migratory ectoparasitic ancestors. Only root-knot nematodes have evolved from burrowing endoparasitic nematodes. Traditional classifications are partially misled by this convergent evolution of feeding type and associated morphology. Contrastingly, mapping attributes of the gonoduct cellular architecture, including newly obtained data of 18 species belonging to the Aphelenchoidea, Criconematoidea, Anguinidae and Panagrolaimidae, revealed a broad congruence of the gonoduct characters and the molecular phylogenetic hypothesis. Yet, the proliferation of uterus cells has evolved multiple times, associated with derived endoparasitic feeding specialization and resulting reproduction mode. Ancestral state reconstruction further revealed that the gonoduct of the morphologically and ecologically dissimilar tylenchid and cephalobid nematodes evolved from a common ancestor.

Enhanced Soil Carbon: The Key to Improving Soil Health and Suppressing Nematode Pests

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Observations on sugarcane cropping soils in Australia have shown that conserving soil organic matter through practices such as residue retention and minimum tillage increases soil C levels (particularly the labile fraction that is oxidised by 33mM potassium permanganate). This results in an improvement in aggregate stability, rainfall infiltration rates, cation exchange capacity, amounts of potentially mineralisable N and a reduction in bulk density and surface crusting: all positively associated with soil and plant health. Suppressive mechanisms against plant-parasitic nematodes are also enhanced due to increased microbial activity. Experiments in microplots and the field have shown that *Pratylenchus zeae* and *Meloidogyne javanica* do not multiply as readily in soils that receive continual C inputs from a mulch layer of plant residue, are not disturbed by tillage, or are amended with high C/N residues. Numerous suppressive mechanisms are probably operating, but predatory fungi that obtain N from nematodes in low N environments appear to be involved. Results of experiments in other cropping systems also confirm the key role of organic matter in biological suppression of nematodes. In minimum till cereal cropping systems, *Pratylenchus thornei* reaches high population densities at depth but does not multiply readily in surface soils, where C levels are highest due to stubble retention and suppressive mechanisms are operating. In capsicum cropping systems, where biological activity is limited by low C inputs, excessive cultivation and reliance on soil fumigation, populations of *M. incognita* and the level of damage caused by the nematode can be reduced by a combination of organic amendments, minimum tillage and mulching with plant residues. Collectively, these results suggest that the key to improving sustainability and reducing losses from nematode pests is to introduce practices that reverse the decline in soil C that currently characterizes most cropping systems.

Changing Nematode Threats to Farming Systems in North Western Europe

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A tangible result of climate change in recent years has been the emergence of new nematode problems in both the arable and amenity sectors. Here we discuss some recent observations on potential nematode threats to agriculture in North Western Europe. Since its discovery in the year 2000 on a potato crop in the Netherlands, the root knot nematode *Meloidogyne minor* has become a major problem on soccer pitches and golf courses, particularly in Ireland and Britain. Sampling of coastal grassland has shown this new species to be much more common than first expected in Europe, and its spread into the amenity turf-grass sector appears to be a response to climate change and turf management practices. Early studies of the pest indicated that further damage to the European potato industry was likely in the future, and during 2007 *M. minor* was found causing damage to ryegrass pasture and potato crops in Ireland and the Netherlands. Investigations of these recent outbreaks have given a better indication of the potential threat from this new pest and here data are presented on the pathology of *M. minor* on potato and grassland and the possible factors driving its spread into the European potato sector. Additional trials have confirmed that most cultivated crops in northern Europe (except maize, *Zea mays*) to be hosts, together with many common weed species. Efficient management options may therefore be difficult due to the withdrawal of many nematicides in recent years.

The examination of grassland and turfgrass samples from the British Isles has indicated that there may also be growing problems from other ecto- and endo-parasitic nematodes including *Pratylenchus, Helicotylenchus* and *Pratylenchoides*. The role of these nematodes in a warming climate to the wider agricultural sector will also be discussed.

Cropping Systems for Sustainable Management of Sugar Beet Cyst Nematode, *Heterodera schachtii*

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Sugar beet cyst nematode (SBCN), *Heterodera schachtii*, on sugar beet is the most serious pests on sugar beet in Idaho, USA. Chemical strategies have been developed to manage the nematodes below the economic threshold level. Most of the chemical practices are commercially adapted by the growers but most of them are environmentally unsafe or some of them are economically nonviable. Considering the narrow host range of SBCN, cropping system patterns are best utilized for its effective management. Long term crop rotation studies were conducted with different crops in the rotation scheme. In this scheme, beet was included for every four years, three years, and two years. In the fall either the field was kept fallow or planted with a different variety of green manure crop which was incorporated after eight weeks. Non host crops such as corn, bean, wheat, onion or potato were included in the study. Among all possible combinations, onion or potato or bean planted as previous crop resulted in more reduction of nematode population followed by the significant increase in beet yield. There are several reasons attributed. One of the possible explanations is the irrigation schedule of these crops. More frequent irrigation in growing of beans, onion, potato cause significant reduction in SBCN population densities. Also the long rotation allowed to plant a green manure crops in the rotation scheme more often causes more reduction in SBCN population before beet is planted.

Options for Managing Nematode Pests in Dryland Cereal Cropping: Tales from Australia

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Cereal Cyst (*Heterodera avenae*, CCN) and Root Lesion (*Pratylenchus* spp., RLN) Nematodes are associated with loss of grain yield in south-eastern and western Australia. In these regions, rainfall is unreliable, cropping soils are inherently deficient in nutrients, soils are fragile with poor structure and little organic matter. Soilborne fungal pathogens are also common. This environment, coupled with economic constraints, poses a significant challenge to the management of nematode pests. Chemical control of these nematodes is not a profitable option, and no nematicide is currently registered for use on cereals in Australia.

The host range of CCN is narrow: effective management can be achieved through rotation with non-host crops (primarily legume and oilseed species), provided that grass weeds within and between cropping phases are adequately controlled. Development of cereals with resistance to CCN has offered a profitable method of managing the nematode in the dryland cereal cropping systems of Australia. Widespread adoption of well-adapted resistant cereal cultivars has resulted in significant decline in populations of, and losses due to, this nematode (particularly in south-eastern Australia) over the last 15 years. Cultivars with tolerance are also available, but there has been less emphasis on development of this trait. However, there are cultivars with both resistance and tolerance to CCN.

Several species of RLN occur in this region. In contrast to CCN, all have wide host ranges, so management through crop rotation is more difficult. However, evaluation of crop species and cultivars has provided information to assist with choice of crops and their sequence to reduce RLN populations and associated crop losses. Since crop species and cultivars differ in their capacity to host different species of RLN, and mixed populations can occur in the field, rotations must be tailored to the predominant species, and nematode population densities and species composition needs to be monitored. Crop nutrition, time of sowing and weed management are also important considerations in the management of RLN.

Cropping Systems for Sustainable Nematode Management of Tree and Vine Crops

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Over the last 15 years a major objective of our research has been to find alternatives to soil fumigation when replanting tree and vine crops. Our first task was to better characterize the various components of the replant problem. This was accomplished by monitoring plant growth in replant and non-replant settings following application of 150 potential remedies. Using this empirical approach while replanting grape, walnut, almond and stone fruits we characterized in 1999 the replant problem as having: a) rejection component, b) soil pest and disease component, c) soil physical and chemical component and d) a nutritional component. Among our test crops there are some non-fumigant alternatives effective against each of these components. We now have five examples from field settings where we have measured no significant or visible differences between our alternative and that achieved by fumigation whether the replants involved grape, almond or stone fruit. Our alternative is the trunk application of a systemic herbicide such as glyphosate after the last harvest followed by waiting one full year prior to replanting on a rootstock of very different parentage. Following grape, *Vitis vinifera,* we have been successful with *V. simpsonii* x Edna or *Vitis rotundifolia* parentage. Following peach, *Prunus persica* cv Nemaguard, our success has been with Hansen 536, a hybrid of Okinawa peach and almond. We now need commercial level field evaluations and greater availability of nematode resistance within rootstocks of different parentage. We refer to our fumigant alternative as: ‘Starve the soil ecosystem, switch rootstock parentage’.

SESSION SEVEN – CURRENT AND FUTURE TRENDS FOR INSECT CONTROL THROUGH EPN

CONVENORS: DAVID SHAPIRO-ILAN & PARWINDER GREWAL

Status and Future of Insect Control with Entomopathogenic Nematodes in Asia

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EPNs are widely used against insect pests in Korea, Japan, and China from the 1980s. Recently, India, Thailand, Vietnam and Turkey began to have an interest in EPNs. In Korea, native EPNs are extensively used against vegetable, ornamental, and turfgrass insect pests. In addition, newly-occurring insects in tea plantations, seedling beds, medicinal plant plantations, forest, propagation houses, greenhouses, and sustainable farms will be targets for developing effective methods. New strains of EPNs will also be continuously isolated from various habitats for effective control of native and introduced insects. In Japan, commercially-produced *Steinernema carpocapsae* and *S. glaseri* are used against turfgrass, orchard, and sweet potato insects. These are or will be selectively used against soil insects and sudden occurrences of insects. Commercialization of EPNs will be active for the time being in Japan. In China, invasive pests such as oriental fruit fly, Asiatic palm weevil and banana moth are good targets for EPNs. Local insects invading fruit and some other crops will be targets of EPNs. A combination of EPNs with insecticides will be attempted to augment efficacy. Insects of vegetable and aromatic plants are targets of *S. carpocapsae* in Thailand. Thai strains will be continuously isolated and used against the above pests with mass production. In Vietnam, black cutworm and armyworm were initially tested against Vietnamese EPN strains. However, turfgrass insects including billbug and white grub will be controlled using Korean EPNs in golf courses belonging to Korean owners. In India, Indian isolates of *S. thermophilum* and *S. riobrave* have received attention against vegetable, maize, and sugar cane insects. *S. thermophilum* and newly isolated Indian strains will be continuously evaluated against locally important insects.

Status and Future of Insect Control with Entomopathogenic Nematodes in Western Europe

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With accelerated development of insecticide resistance, particularly in greenhouse pests, the frequency of pesticide application was increased. As a consequence problems with residues in food produce surpassing the allowed levels occurred and consumers and retailers in Europe now demand almost residue-free vegetables and fruit. To meet the new standards, growers have increased the use of biological control agents, among them entomopathogenic nematodes, which have been the fastest growing market within the microbial segment. In greenhouse vegetables, thrips (*Frankliniella occidentalis*) are controlled with EPN by application through the drip irrigation and combination with predatory mites (particularly *Amblyseius swirskii*) and bugs on the canopy. White flies (*Bemisia tabaci*) are controlled by foliar application of EPN in combination with Biorend®, a chitosan polymer. Besides the use under controlled environmental conditions, EPN are more often used in outdoor crops, like codling moth (*Cydia pomonella*) and flat-headed rootborer (*Capnodis tenebrionis*). With growing demand for agricultural products from organic farming and increasing problems with insecticide efficacy and resistance development, the demand for EPN will constantly increase in vegetable and fruit production and in the traditional EPN markets in turf, mushrooms and ornamentals.

Status and Future for Incorporation of Entomopathogenic Nematodes in Temperate Orchard IPM Systems

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Research and commercial application of entomopathogenic nematodes in temperate orchard systems has a long history. In the pursuit of commercial viability, there have been a number of success stories, but also quite a number of dead ends. In this presentation, we provide insight into the current status as well as new opportunities for entomopathogenic nematodes that are on the horizon. Some novel targets that are currently being researched include a variety of wood boring insects and weevil pests. Cropping systems that are currently targeted or may be targeted in the near future include (among others) almond, apple, cherry, citrus, peach, pear, pistachio, and pecan. The key will be incorporating the nematodes into feasible IPM strategies. Novel application methods and formulations that are being developed will facilitate incorporation and expanded use of nematodes as microbial control agents. Advances in biocontrol using entomopathogenic nematodes will also be enhanced through fundamental studies on the microbial ecology, epizootiology, and population dynamics of insect pathogens in orchard systems as well as basic studies on host-pathogen relationships.

Use of EPNs in South America: Present and Future

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In contrast to the situation in Europe and Northern America, the research on entomopathogenic nematodes (EPNs) and their application as biocontrol agents in South America is just beginning. Practical studies began during 1980s, with lab bioassays focusing on different soil pests. A new era for EPNs in South America started with a workshop in São Paulo, Brazil in 2000. Then in 2001 there was a National Course of Biological Control of Pests with Entomopathogenic Nematodes in Campos dos Goytacazes, Brazil, and in 2003 a Latin American Symposium on Entomopathogenic Fungi and Nematodes. Nowadays surveys and pathogenicity tests are being carried out in Brazil, Chile, Venezuela and Colombia, while studies on mass production, storing and formulation are being done in Brazil and Colombia. Other studies dealing with taxonomy, biology and classical genetics are also being carried out by different groups in Brazil. The lack of communication and integration is the major threat to the future of EPNs in South America. We need more international collaboration to continue learning and applying the acquired knowledge in our own conditions.

Genetic Improvement for Enhanced Efficacy of Entomopathogenic Nematodes

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Growing economic importance of entomopathogenic nematodes (EPNs) as biocontrol agents generates a strong demand for strains with the highest reproductive potential, storage stability, and field efficacy. Initiated over 20 years ago, research on genetic improvement of EPNs has employed a range of approaches, including strain hybridization, genetic selection and mutagenesis. Hybridization of geographically distant strains helped to widen genetic and phenotypic variation within the resulting populations, which could be used subsequently in selection processes. Attempts to improve both relatively well-defined traits (e.g. ability to disperse in the soil and host finding, infectivity at lower temperatures, or desiccation tolerance) as well as highly complex qualities (e.g. overall efficacy in controlling pest populations in particular environments) with the aid of artificial selection have been successfully conducted in several laboratories worldwide. Relatively little attention has been paid so far to mutagenesis as a source of new and valuable phenotypes in EPNs. Although the process of mutagenesis can produce a wide range of changes in individual genes, it is very unpredictable and requires proper screening for the mutant phenotypes. Moreover, not all nematode species respond equally to mutagens. Our earlier study on a series of mutagens applied to *Heterorhabditis bacteriophora* was discouraging, while the same agents produced many point mutations affecting morphology and/or behavior of infective juveniles in *Steinernema feltiae*. Of this material, mutations in at least two genes (*Sfrol-1*, *Sflon-1*) improved the nematode performance in soil penetration and infectivity.

Although all the tested methods revealed some potential for genetic improvement of EPNs, the populations obtained have had rather limited impact on the use of nematodes in agricultural practices, so far. Poor understanding of the genetic background responsible for the observed phenotypic changes, lengthy breeding procedures, and potential reversion of the improved characters to their wild type are most probable explanations for this situation.

Recent advances in research conducted at the genomic level, particularly of *H. bacteriophora* suggest, however, that hitherto missing genetic information as well as more precise and predictable methods may soon be available for further works on improvement of EPNs.

Session Eight – Resistance Breeding against Non-sedentary Nematodes

Convenors: Danny Coyne & John Thompson

Evaluating Wheat for Tolerance and Resistance to Root-lesion Nematodes

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Root-lesion nematodes are estimated to cost the Australian wheat industry AUD$260 million/year. *Pratylenchus thornei* is the dominant species in the north and *P. neglectus* in the south and west. We have tested wheat lines for tolerance to *P. thornei* on a dedicated 10-ha site of vertisolic soil near Jondaryan 170 km west of Brisbane. The site has been managed in a 4-year rotation of fallow-sorghum-wheat-wheat test plots, such that 2.5 ha are available each year with high *P. thornei* population and negligible other soil-borne wheat pathogens. About 2000 early-generation wheat lines are sown in unreplicated plots of 3 rows each 5 m long which are rated twice during the growing season for symptoms of nematode damage. Pre-release lines and varieties for the northern region are sown in plots of 7 rows by 8 m long in replicated experiments on two sowing dates. Yield of the lines/varieties determined from machine harvest is expressed as percentage of site mean yield (SMY) and averaged across trials and years. Tolerance indices derived from SMY have proved very predictive of varietal yield at independent sites infested with *P. thornei,* and are published annually in extension brochures. Resistance of wheat lines against *P. thornei* and *P. neglectus* has been assessed in replicated glasshouse experiments. The wheat lines are tested in pots of vertisolic soil inoculated with cultured nematodes and grown with optimum nutrition, water supply and temperature for nematode multiplication. After 16 weeks, nematodes are extracted from roots and soil from the bottom half of the pot and enumerated under a compound microscope. These methods have been used successfully to: (1) identify sources of resistance in wild relatives, landrace and synthetic hexaploid wheats, (2) characterise breeders' lines and varieties for resistance, (3) screen segregating progeny from backcross and topcross programs, and (4) characterise mapping populations for development of molecular markers to resistance genes.

Distribution, Virulence and Genetic Management of Root Lesion Nematodes in the Pacific Northwest USA

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Root lesion nematodes (RLNs) have been identified in more than 90% of dryland cropping fields in the Pacific Northwest (PNW; Oregon, Washington, Idaho) USA. *Pratylenchus neglectus* dominates but *P. thornei* and mixtures of both species occur commonly. Until recently, RLNs were only considered to cause significant yield loss in annual spring crops with traditional winter wheat-summer fallow rotations considered unsuitable for damaging RLN populations to develop. Recent research has shown that spring wheat and barley yields increased up to 98% and 15% respectively in response to aldicarb (4.2 kg a.i./ha) applied at planting in *P. neglectus* infested soil and 49% and 14% respectively in *P. thornei* infested soil. Yields of winter wheat and barley cultivars grown at the same locations also responded up to 21% and 27% respectively in *P. neglectus* infested soil and 15% and 20% respectively in *P. thornei* infested soil. Glasshouse resistance screening showed that all tested PNW wheat cultivars were susceptible to both RLNs. Barley cultivars were generally more resistant than wheat cultivars but still moderately susceptible to *P. neglectus* and moderately resistant to moderately susceptible to *P. thornei*. Crop species identified as resistant to *P. neglectus* and/or *P. thornei* and suitable for crop rotation are either not well adapted to PNW cropping systems or have marginal economic viability. Breeding cereal cultivars with tolerance and resistance to RLNs has proven an effective management strategy in similar international situations and has been undertaken collaboratively in the PNW. Characterisation of commercial cultivars and advanced breeding lines for tolerance and resistance has begun as has the introduction of elite sources of tolerance and resistance genes through a targeted crossing program. Molecular techniques are also being developed to identify RLNs to species from soil samples and resistance gene markers to assist breeding selection.

Identification of Multiple Resistance against Cereal Cyst (*Heterodera filipjevi*) and Root Lesion (*Pratylenchus thornei* and *P. neglectus*) Nematodes for International Bread Wheat Improvement

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Globally, Cereal Cyst (CCNs) and Root Lesion (RLNs) nematodes are economically important in wheat production systems. In Turkey, recent surveys have revealed widespread distribution of both nematodes which commonly occur together. Hence a clear strategy to identify resistance to both groups has been initiated under a joint Turkish/CIMMYT International wheat improvement program. More than 500 selected bread wheats from Turkish National and International (CIMMYT, CIMMYT/ICARDA/Turkey, other foreign sources) germplasm were screened in order to determine their resistance against CCNs *(Heterodera filipjevi)* and RLNs (*Pratylenchus thornei & P. neglectus*) using local Turkish isolates of the nematodes. Many of the lines screened were crosses made by breeders to pyramid sources of resistance from the global literature. Under greenhouse conditions with 7 replicates per line arranged as a Randomised Complete Block Design, an optimized screening methodology was used for both RLN and CCN. Results were evaluated 9 weeks later after nematode inoculation. Two partial resistant and susceptible lines were used for each nematode species as check lines. Plants were grown in the greenhouse at 26^{o}C with 16 hours light per day. Data were analyzed with ANOVA using quantitative numbers for each nematode species. Lines were then ranked according to the checks for partial resistance and susceptibility. In total 44 sources of resistance were identified – 22 spring and 20 winter. There were 4 sources of combined nematode resistance in spring wheats, 2 against CCN and *P. thornei*, and 2 against *P. thornei* and *P. neglectus*. Sources of resistance against individual nematode species have also been found including 15 sources for CCN (5 spring, 10 winter), 16 for *P. thornei* (9 spring, 7 winter) and 7 against *P. neglectus* (4 spring, 3 winter). Eleven of these lines are sisters. These results indicate the potential to identify nematode resistant germplasm for use in both International and National breeding programs.

First Report of Groundnut Genotypes Resistant to the Groundnut Pod Nematode *Ditylenchus africanus*

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The economically important, seed-borne, groundnut pod nematode *Ditylenchus africanus*, is omnipresent in the groundnut-producing areas of South Africa. Resistant genotypes could play an important role in reducing these parasites to levels below the damage threshold. Selected genotypes, including the cultivars Sellie (susceptible) and Kwarts (tolerant) were first screened for host suitability against this nematode in a microplot trial. Genotypes that showed resistance were subsequently tested again in a microplot and two field trials under natural infestations. In both microplot trials each seed was artificially inoculated with ± 3 000 *D. africanus* at planting. Significantly lower *D. africanus* numbers were present in pods of PC254K1, PC287K5 and CG7 compared to both reference cultivars. Differential levels of resistance that was identified in PC254K1 and PC287K5 was determined by inoculating each seed of both genotypes, Sellie and Kwarts with a range of initial populations (Pi's), viz. ± 0, 50, 250, 1 000, 2 500, 5 000, 10 000 and 20 000 nematodes. In this study PC254K1 and PC287K5 had reproduction factor (RF) values < 1 at all Pi levels, while Sellie and Kwarts showed RF > 1 except at the highest Pi. In terms of kernel quality PC254K1 produced choice grade kernels at Pi ≤ 1 000 and PC287K5 at Pi ≤ 10 000. Possible differences in reproduction and damage potential of *D. africanus* isolated from five different localities were evaluated on PC254K1 and Sellie in a greenhouse and microplot trial, with each seed having been inoculated at planting with ± 2 000 *D. africanus*. Although no significant differences existed among the five nematode populations in terms of reproduction and damage potential, PC254K1 persistently maintained significantly smaller nematode population levels than Sellie. Preliminary studies to identify molecular marker(s) associated with the resistance trait were done. Results showed that resistance to *D. africanus* is probably inherited by a single, recessive gene.

The seed-borne groundnut pod nematode *Ditylenchus africanus* is omnipresent in the groundnut-producing areas of South Africa and remains one of the most economically important pests of this crop. Resistant genotypes could play an important role to reduce *D. africanus* populations below damage threshold levels. Various evaluations of local germplasm, selected elite breeding lines as well as hybrids have been done previously. In this selected accessions were evaluated against this pathogen in microplots and resistant host responses were verified under natural infestations in the fields. The cultivars Sellie and Kwarts were used as susceptible and tolerant reference cultivars, respectively. In the microplot trials each seed was artificially inoculated with ± 3 000 *D. africanus* at planting. Significantly lower *D. africanus* numbers were observed in seeds and hulls of PC 254K1, PC287K5 and CG7 compared to both reference cultivars and the other accessions. Sustainability of resistance of PC254K1 and PC287K5 was evaluated in microplots. Each seed was artificially inoculated with initial population densities (Pi) of 0, 50, 250, 1 000, 2 500, 5 000, 10 000 or 20 000 *D. africanus* at planting. The reproduction factors (RF values) of PC254K1 and PC287K5 were ≤ 1 at all Pi's. For reference cultivars Sellie and Kwarts the RF remained ≥ 1 except at the highest Pi. PC254K1 produced choice grade kernels at Pi of up to 1 000, PC287K5 up to 10 000 and Kwarts only up to 250 nematodes. A search for molecular markers associated with the observed resistance of PC254K1 to *D. africanus* is currently in progress.

Evaluation of Banana Germplasm as Part of the International Musa Testing Program (IMTPIII) for Nematode Resistance

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Banana germplasm originated from a wide range of Musa material was selected to be tested for nematode resistance as part of an International *Musa* Testing Program (IMTPIII) for nematode resistance in PRO*MUSA*, a global network for *Musa* improvement. Green house test and a field test were executed. In total 19 genotypes were tested. In the green house, plants were infested with *Radopholus similis* (burrowing nematode) and evaluated for number of nematodes and root damage at week 6, 9 and 12 after inoculation. Grand Nain was used as the susceptible and Yangambi km5 as the resistant cultivar. Results showed that several genotypes including FHIA 1, FHIA 18, Paka and Kunnan were more resistant to *R. similis* than Yamgambi Km5. In the field trial *Meloidogyne* spp (root-knot nematode) and a low infestation of *R. similis* were present. After three crops (Plant crop, R1 and R2) it was obvious that *Meloidogyne* spp. was present on all genotypes with high numbers on Grand Nain, Bluggoe and Ducasse. *R. similis* was found on most genotypes but in lower numbers. However compared to Grand Nain, Kunnan, FHIA 1 and Paka showed some tolerance. Additionally Foconah and Selangor also showed some tolerance in the field trial. However, South African conditions are not optimal for banana germplasm and several genotypes did not adapt well therefore given very poor results. A combination of both green house and field trials executed in different climatic zones should however provide results that than can be used by breeders. Additionally some IITA efforts will be discussed to illustrate the development in resistant material in Africa.

Session Nine – Morphology and Development

Convenors: Wilfrida Decraemer & Vladimir Yushin

Morphology in a DNA Barcoding World

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In a changing DNA barcoding world we can question ourselves on the importance of morphological studies, in particular, morphology based on light microscopic observations, for identification and understanding relationships and functioning.

A case study will be presented based on a new genus found in bathyal oxygen minimum zone sediments off Peru. The new taxon closely resembles a known genus *Richtersia* in habitus and spiny ornamentations of body cuticle but differs in the structure of the buccal cavity. In the past, the morphology of the buccal cavity and its cuticular structures played an important role in determining relationships within the Chromadoroidea, more specifically with respect to the position of the Selachinematidae. The interpretation of buccal morphology and homology of its cuticular structures based upon light microscopy for unravelling relationships will be discussed. Animal-sediment interaction and morphological adaptations will be shortly addressed.

Male Reproductive System of Marine Nematodes: A Simple Tube?

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Extensive studies of spermatogenesis in many taxa of free-living marine nematodes now allows presentation of much original data on the ultrastructure of male reproductive system in a short review. The genital primordium of the first stage juveniles includes two primordial germ cells accompanied by two somatic gonad precursor cells. This close association of two cell lines persists in testes of adults. The somatic sells forms all the cells of the testis epithelium which encircle totally a cluster of developing germ cells. Three main parts may be detected easily in each testis: a distal tip cell (DTC), a testis main part, and a seminal vesicle. In the chromadorians the DTC is a unicellular gland which probably regulates conversion from mitotic to meiotic divisions of the germ cells; in the enoplids this gland is multicellular. In both cases these cells protrude long processes into the testis lumen to be in close contact with spermatogonia. The epithelium of the main part of testis is extremely thin; sometimes the testis wall reduces to a basal lamina. This weakness of the wall apparently favors a nutrient transfer from the intestine to the developing germinal cells which usually have great synthetic activity. The epithelium of the seminal-vesicle-encircling cluster of spermatozoa is thick and has distinct synthetic activity; the cytoplasm of the epithelial cells usually contains numerous secretory granules. The vas deferens of large enoplids is a real epithelial tube which at the distal end is characterized by a well developed muscular sphincter. The vas deferens of small nematodes is a long chain of glandular cells arranged in a single file without a distinct lumen. The vas deferens opens to a cloaca together with ducts of the unicellular ejaculatory glands. These are large glandular cells which bodies occupy a body cavity lateral to the precloacal part of intestine. The gross morphology of the male reproductive system in marine nematodes is based on a uniform histological pattern.

Evolution of the Embryonic Cell Lineages in Nematodes

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One of the unique features of the model organism *Caenorhabditis elegans* is its invariant development, where a stereotyped cell lineage generates a fixed number of cells with a fixed cell type. A complex mix of mechanisms is used in a very specific network of inductions to pattern this complex lineage. However, it remains unclear how these mechanisms evolved within the nematodes to give rise to the complex, invariant cell lineage of *C. elegans*. Therefore, we established two nearly complete embryonic cell lineages of two distantly related nematodes, *Rhabditophanes* sp. and *Halicephalobus gingivalis*, belonging to clade IV. A detailed cell-by-cell comparison with the *C. elegans* lineage reveals that the total fate distribution in these lineages is 85% identical, and that the fate distribution in AB follows a similar complex pattern. It is striking that despite the large evolutionary distance and the great genomic diversity between different nematode clades, there is a conservation of a '*C. elegans*' like polyclonal cell lineage with strong left-right asymmetry. We propose that an early symmetry breaking event in nematodes of clade IV-V is a major developmental constraint that shapes the subsequent asymmetric cell lineage in those nematodes. Preliminary studies of species in more basal clades Plectida, Chromadorida, Dorylaimia, reveal striking differences in the establishment of the bilateral symmetry which implies different mechanisms in the bilateral symmetry establishment.

Conserved and Divergent Aspects of Dauer Formation between *Pristionchus pacificus* and *Caenorhabditis elegans*

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Pristionchus pacificus shares with *Caenorhabditis elegans* many technical features and has been developed as a model system in evolutionary developmental biology. We have started a genetic and molecular analysis of dauer formation in *P. pacificus*. The dauer stage is important in the ecology of many nematodes and evolutionary alterations of dauer formation should play crucial roles in the adaptive radiation of nematodes. *P. pacificus* dauers have several features not common to *C. elegans*, such as prominent amphids, lack of alae, and the secretion of an oily substance on the body surface.

The control of dauer entry and exit is of utmost ecological importance because erroneous decisions lead to either less progeny or survival. Therefore, this control might be subject to substantial evolutionary modifications. To reveal if there are differences in dauer formation between *P. pacificus* and *C. elegans*, we tested if *P. pacificus* dauer formation is controlled by a pheromone. Extracts prepared from supernatants of *P. pacificus* liquid culture strongly induced dauer in *P. pacificus*. By contrast, *C. elegans* pheromone did not induce dauer formation in *P. pacificus*.

To identify the genes underlying the *P. pacificus* dauer formation, we screened for dauer formation abnormal mutants. We obtained more than 10 strains of dauer formation defective (daf-d) and dauer formation constitutive (daf-c) mutants. By mapping and cloning we identified mutations in *Ppa-daf-12* in some of the daf-d mutants, suggesting evolutionary conservation of the involvement of the nuclear hormone receptor in dauer formation. Furthermore, we found that dafachronic acid, a steroidal ligand for Cel-DAF-12, serves as ligand for Ppa-DAF-12. Thus, we identified the dafachronic acids-DAF-12 system as an evolutionarily conserved mechanism for dauer formation.

Dynamics of the Nematode Cuticle and the Structure of Marginal Zones

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The structure of the nematode cuticle, that generally (an example to the contrary is found in *Philometra obturans*) effectively protects the worm from chemical, physical and mechanical cues from the outside, is adequately described. However, we are not well informed of the dynamics of the cuticle during the growth of the worms. In *Ascaris* sp., in which the growth process has been studied, the L1 larva, some 250µm long, attains almost a size 1000-fold bigger as an adult only two months later. This is not only related to events during the moulting processes but in particular to events in between moults and to events during the growth phase of the adult worm. Another fascinating aspect is how the transition from the cuticle edge to underlying tissues is arranged structurally. As studied at a cellular level in the phasmid in *Hysterothylacium* sp., in the pharynx of *Anguillicoloides* sp., and in the rectal region of *C. elegans* these elaborate structural features become evident. We still know only little of the dynamics of the nematode cuticle although structural features exemplify the fact that we are dealing with an intricate organ which is the main reason for the evolutionary success of the Nematoda within the Ecdysozoa.

Session Ten – Sustainable and Organic Management through Biofumigation, Amendments and Suppressiveness

Convenors: Giovanna Curto & Richard Sikora

Impact of Green Leaf Application on the Management of Plant Parasitic Nematodes and its Effect on the Population of Predatory and Saprophytic Nematodes and Microflora in Soil

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Green leaves of a number of plants are well documented for nematode suppressant properties. Neem (*Azadirachta indica* A Juss.) and eupatorium (*Cromolaena odorata* L.) are two plants with potentially nematicidal properties. However, knowledge on the impact of these green leaves on the soil fauna is meagre. Hence an attempt was made to study the effect on the soil fauna and their consequent effect on the yield of okra and cowpea during rainy and summer seasons. Field experiments were laid out in a randomized block design. Chopped neem and eupatorium green leaves were applied 15 days prior to sowing the seeds at rates of 7.5 and 15 t per ha at a depth of 30cms. Nematodes were extracted by Cobb's sieving and sifting techniques and cleared by modified Baermann funnel method, while fungi, bacteria and actinomycetes were isolated by dilution plate method of Timonin using different media. The result showed that application of neem and eupatorium leaves at both doses reduced reniform nematode, *Rotylenchulus reniformis* in the root zone of okra and cowpea in soil both in summer (214 to 427 per 200g soil sample as against 727/200 g in untreated) and during the rainy period (324 to 646 per 200g as against 933 per 200 g in untreated). The population of predatory (two to three fold) and saprophytic nematodes (three fold) also increased significantly in the root zone of okra and cowpea. The rate of increase was more in eupatorium leaves when compared to neem leaves. The pre-sowing application of eupatorium leaves increased the population of fungi, bacteria and actinomycetes in the root zones of okra and cowpea whereas neem leaf at higher dose (15t/ha) reduced the multiplication of microbial population (except for the fungi) in the root zone of okra and cowpea in both the seasons. The stimulatory effect of eupatorium leaves in the multiplication of microbial flora and predatory and saprophytic nematode in our investigation is being reported for the first time. The pre-sowing application of neem and eupatorium at 15t ha resulted in significant improvement in plant growth characters which contributed the increase in yield of okra and cowpea in both the seasons. Thus application of green leaves suppressed the multiplication of plant parasitic nematodes by the nematicidal principles released during decomposition and it also indirectly managed the plant parasitic nematode population by stimulating the multiplication of predatory nematodes and beneficial microflora.

Cover Crops and Biofumigation for Managing *Pratylenchus spp.*

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Root lesion nematodes, *Pratylenchus* spp., are important pests in many crops worldwide both as primary pathogens and as collaborators in disease complexes. There are few crops with host resistance to *Pratylenchus* spp. and chemical control is expensive, so cultural tactics are crucial for sustainable production in infested fields. A wide range of cover crops has been tested for efficacy against *Pratylenchus* spp. Two of the most widely documented cover crops are forage pearl millet and marigold. Some cover crops are more effective, or only effective, against *Pratylenchus* spp. when incorporated as green manures for biofumigation. Our work demonstrates the potential of cover crops and biofumigation for managing *P. penetrans* and the early dying pathogen *Verticillium dahliae*. Forage pearl millet (*Pennesetum glucum*) as a cover crop and rapeseed (*Brassica rapa*) as a green manure were successful in suppressing *P. penetrans* and the potato early dying disease and increasing potato yield in field trials. Variability in the efficacy of these management tactics from field to field and year to year was high. Ancillary studies indicate factors that might explain, in part, inconsistent results in the field: the distribution of nematode populations among soil and root habitats, a relationship between nematode activity and the uptake of isothiocyanate compounds, and the role of microbial activity in the toxicity of decomposing residues to nematodes. There is a high rate of success in killing nematodes with crops and their decomposing residues in simplified laboratory systems. Duplicating that success in the field requires knowledge of the mechanisms responsible as well as an improved understanding of the temporal and spatial dynamics of nematodes in the field.

Amendments for the Suppression of *Radopholus similis* in Bananas in Australia

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Radopholus similis remains an economic constraint to sustainable banana production for many tropical countries. Nevertheless, *R. similis* may be suppressed by enhancing soil organisms that are antagonistic to plant-parasitic nematodes. A large range of amendments, with varying chemical compositions, are available, and may have varying effects on soil properties depending on application rates. To obtain a better understanding of how soil amendments may change the antagonistic potential of banana soils, it is necessary to understand changes in soil ecology following their application. Glasshouse experiments were conducted to determine the optimum types of amendments to suppress *R. similis* in bananas, followed later by a field experiment.

In a glasshouse experiment, banana plants were grown in field soil amended with nine different amendments, with known nutrient contents, at a rate equivalent to 40 t ha^{-1}. Banana plants were inoculated with 860 *R. similis* and grown in the soil amendment mix for 12-15 weeks. At harvest, there were significantly fewer *R. similis* in the roots of bananas grown in soil amended with lucerne hay, grass hay, banana residue or mill-mud relative to the untreated soil. These amendments had high carbon compositions.

In further glasshouse experiments, the suppression of *R. similis* in soil with additional high carbon amendments was confirmed. Additional nitrogen fertiliser had no significant effect on nematode suppression. However, the type of organic matter added to the soil significantly altered the level of suppression. With the addition of cellulose, suppression was significantly higher than in untreated soil or soil amended with lignin.

In a 2-year field experiment, mill-ash, compost, grass hay and mill-mud were compared to untreated soil. The grass hay treatment was able to decrease the number and proportion of plant-parasitic nematodes in the soil relative to untreated soil. Amendments high in carbon appear to be well suited to suppression of *R. similis* in bananas.

Acknowledgements
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Study of the Biofumigant and Suppressive Actions of Biocidal Plants, Defatted Seed Meals and Natural Compounds towards Root-knot and Cyst Nematodes, in Organic Farming

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In the last four years (2004-2007) a set of studies concerning the suppressive and/or biofumigant activity of plant selections towards *Meloidogyne incognita* and *Heterodera schachtii*, were carried out in Emilia-Romagna, Po Valley, Northern Italy. The immobilization and nematicidal effects caused by the hydrolysis products of several glucosinolates were checked in *in vitro* bioassays towards second stage juveniles, determining the LD_{50} after 24 and 48 hrs. The life cycle of either *M. incognita* or *H. schachtii* was studied successively in the roots of accessions belonging to Brassicaceae, Leguminosae and Graminaceae, in pot experiments in the greenhouse, with the aim of defining the best new varieties able to effectively interrupt or significantly slow the nematode life cycle. In an open field, some rotation schemes with either *E. sativa* cv. Nemat, or *R. sativus* cv. Boss or *B. juncea* sel. ISCI, as green manure intercrops, were evaluated in decreasing the *M. incognita* population in the soil, before either tomato (cv. Perfect Peel) or carrot (cv. Maestro F1) main crops.

In the same years, liquid formulations of natural compounds and pellet and liquid products based on *Brassica carinata* defatted seed meals, were both checked as amendment, fertilizing and biofumigant treatments. At the beginning, some semi-field trials were carried out, testing different doses of meal and pellet against *M. incognita*. Other trials were carried out on tomato crops in plastic greenhouse on sandy soil, with the aim of evaluating the *M. incognita* control by periodic drip applications of liquid plant extracts associated with either an intercrop, *E. sativa* cv. Nemat, or a pellet based on defatted seed meals of *B. carinata* applied in pre-transplant. Finally, the effectiveness of different cropping systems in organic farming were tested, from 2005 to 2007, in open fields infested by *M. incognita*. Eight combinations of crop rotation, biocidal intercropping and biofumigant treatment, were compared with the aim of verifying the level of nematode control.

Differences in Host Status of Biofumigant Cruciferous Crops for *Meloidogyne incognita* and *M. javanica*

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Cultivation of cruciferous cover crops, followed by incorporating green biomass into the soil (biofumigation) has been shown to reduce soil-borne pathogens and pests. Using this method to manage root-knot nematodes poses a risk, as many cruciferous crops are moderately good hosts for these nematodes. To avoid nematode build-up during cover crop cultivation, it is important to select poor- or non-host cover crop varieties. Previously, we evaluated the host range of 32 cruciferous varieties for an *M. incognita* race3 population in a pot-trial. The results indicated that there is a large variation in host-status between the cruciferous varieties. These results were confirmed in micro-plot trials, and also showed that selecting poor- or non-host varieties significantly reduced galling and root-knot nematode infestation in a following nematode-susceptible tomato crop, and resulted in tomato yields not different from a nematicide treatment (ca. 3x yield after fallow control). Recent results with the same 32 cruciferous varieties, but with a population of *M. javanica* show that the host status of the varieties is generally very similar for both root-knot nematode species (*M. incognita, M. javanica*).

SESSION ELEVEN – PARASATIC NEMATODES OF ANIMALS: RESISTANCE PROBLEMS AND THE NEED FOR NOVEL APPROACHES OF TREATMENT AND CONTROL IN THE GENOMIC ERA

CONVENOR: ROBIN GASSER

The Resistance Problem! Impact and Implications

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Anthelmintic resistance, the failure of antiparasitic drugs to control helminth parasites, is common in trichostrongyloid nematodes of sheep and cattle and the cyathostomins of horses. While the frequencies of putative resistance alleles have increased and shifts in dose response lines *in vitro* have been reported for human nematode parasites, resistance in these species is yet to be confirmed. Resistance in parasites of grazing animals is now so widespread globally that whole chemical classes are ineffective in some regions and non-chemical control is again being researched and advocated. Research into new chemistries and vaccines has been reinvigorated. So that control can be improved, the development of quick, cheap and accurate molecular tests for resistance is now a priority. Much of the research activity in this area is centred on sheep parasites such as *Haemonchus*, which has been chosen for its biological characteristics including its phylogenetic position close to *Caenorhabditis elegans* and, so benefiting from comparative and homology studies. Markers for benzimidaole resistance, based on tubulin polymorphisms are now well understood. Resistance mechanisms, and hence tests, for resistance to levamisole and the macrocyclic lactones (such as ivermectin) are not known. Candidate gene investigations have not been successful in identifying resistance alleles in field parasite populations but it is hoped that once the *Haemonchus* genome sequence is available and annotated functional genomics will deliver results for this parasite species. Not only will it help discover the origins, selection and spread of resistance genes it may also deliver molecular markers plus a deeper understanding of the genetics of nematode parasites. Ironically, the genetic diversity that is thought to contribute to the development of anthelmintic resistance may also complicate the use of genomic tools.

Human Hookworm Vaccines that Interrupt Blood-feeding

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Hookworms are gastrointestinal nematodes that infect almost one billion people in developing countries. The main clinical symptom of human hookworm infections is iron-deficiency anemia, as a direct consequence of the intestinal blood loss resulting from the parasite's feeding behaviour. Although treatment is available and currently used for the periodic removal of adult hookworms from patients, this approach has not effectively controlled hookworm in areas of rural poverty. Moreover, treated individuals remain susceptible to reinfection following exposure to third-stage infective hookworm larvae (L3) in the soil as early as 4-12 months following drug treatment. Therefore, a prophylactic vaccine against hookworm infection would provide an attractive additional tool for the public health control of this disease. The feasibility of developing a vaccine is based on the prior success of an attenuated larval vaccine against canine hookworm. Several laboratory and field studies have explored the development of a human anti-hookworm vaccine, describing potential protective mechanisms and identifying candidate antigens, one of which is now in clinical trials. Antigen discovery has focused on two distinct developmental stages of the parasite – (1) secreted proteins from the infective third stage larva (L3) and (2) intestinal proteases that digest haemoglobin from the adult worm. This presentation will focus on the biology of the blood-feeding process, and the selection and testing in pre-clinical efficacy studies of the major enzymes involved in the haemoglobin digestion pathway.

Gene Finding, Expression Profiling, and SNP Detection in *Ancylostoma caninum* using High Throughput Sequencing Technology and its Implications

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The impact of next-generation sequencing technology on genomics is multifold, and the transcriptomic data have multipurpose use. Hence, the transcriptomic data generated using massively parallel sequencing technology is a cost-effective way to comprehensively study different aspects of an organism's biology.

In this study we sampled 4 transcriptomes of the canine hookworm *Ancylostoma caninum* (infective L3, serum-stimulated L3, adult male and adult female) using both, conventional sanger (ABI) and massively parallel Roche (454) sequencing technology. The analysis of the 1.5 million 454 ESTs and comparisons to the ~90,000 ABI ESTs, indicate that the non-cloning based 454 sequence data: i) has greatly improved gene finding, mainly through representation of transcript expressed at a lower level; and ii) provide high coverage that enables both comprehensive identification of developmentally restricted transcripts, and identification of intra-specific genetic variations. We will report on the extended studies of the digital expression profiles that enabled us to deeper understand *A. caninum* at the molecular level.

This approach highlights the impact that next generation sequencing technology may have on genetics in general, and subsequently how genomics-based approaches can accelerate progress towards development of more efficient and sustainable control programs.

Using Molecular Techniques to Identify New Targets for Control across Species of Abomasal Parasitic Nematodes

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Infections with the abomasal parasites *Haemonchus contortus*, *Teladorsagia circumcincta* and *Ostertagia ostertagi* are a major cause of economic loss in the sheep and cattle industry. Billions of dollars are spent annually to treat animals with anthelmintics. However, the appearance of worm populations resistant against the three classes of anthelmintics currently used severely threatens the sustainable control of these parasites. Therefore, alternative control strategies, based on novel nematocidal compounds or vaccines, will be required in the future. The identification of such new drug and vaccine targets relies on a good understanding of the parasite biology and host-parasite interactions on a molecular level. The application and combination of genomics, transcriptomics and proteomics has in recent years given us better insights into processes such as hypobiosis, feeding and sexual differentiation. Quantitative information on gene expression during parasite development has been gathered and protein components released by the different life stages during the course of an infection have been identified. The results of these studies will be discussed in the light of target identification for these abomasal parasites.

Functional Genomics in Animal Parasites: Progress and Prospects

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The volume of DNA sequence data available for nematode parasites of animals has exploded in recent years: in all of the major taxonomic groups of parasites there are now two or more species with extensive expressed sequence tag (EST) collections, and genome sequencing projects are underway for at least one representative from each important parasite taxon. Some descriptive bioinformatic analyses and microarray experiments based on these EST collections have been published over the past 5 years, and it is almost certainly true that individual labs have made use of these data in order to identify their favourite gene(s) in their favourite parasite taxon. Sadly, however, there are few tools available to really test the function of genes *from* parasites *in* parasites. The three main approaches to date are reverse genetics via RNA interference, forward genetics via mutagenesis and 'knock-in' genetics via transgenesis. RNAi is controversial: there are reports of limited success and of consistent failures, so that the only conclusion that seems reasonable is that it is unlikely to prove to be a useful tool in animal parasites without significant modification, if at all. Transgenesis has been more successful in the few reported instances, but is so far restricted to a few species and has yet to prove its value as a means of testing gene function. Mutagenesis seems to have been largely overlooked, but there is some reason to believe that it could be applied in at least some species. The development of these tools in other organisms has usually followed from the adoption of a representative species as a model, followed by intense 'tool development' effort. There seems little prospect that these tools will be developed until animal parasitologists (and their funding bodies) accept the necessity of developing a parasite *Drosophila* or *C. elegans* and invest the time and energy that tool development requires.

SESSION TWELVE – ROOT-KNOT NEMATODE MANAGEMENT

CONVENOR: TANYA DAVARIAN

Root-knot Nematodes Infecting Plants in Some Eastern Parts of Iran

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Sedentary endoparasitic root-knot nematodes (*Meloidogyne* spp.) are economically important pathogens for crop plants in most parts of Iran and may cause severe damage or even total loss of the crop for susceptible hosts. During a survey conducted to determine the distribution of root-knot nematodes in eastern parts of Iran, roots of some infected plants were collected during spring and summer 2007. Females were extracted from roots, then morphology of perineal patterns were studied. *M. hapla* Chitwood, 1949, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949 were identified. *M. hapla* was extracted from apricot, carrot and potato roots. Apricot, box tree, carrot, cucumber, melon, parsley, pertulaca, pistachio, potato and tomato root samples contained *M. incognita*; *M. javanica* was extracted from carrot, cucumber, melon, sugerbeet and tomato roots. In 43% of cases, presence of *M. incognita* and *M. javanica* was observed in the same sample in carrot, cucumber, melon and tomato roots. These two species made significant damage up to 70% in quantity of some samples. In addition, tomato, a major crop in the whole area, was infected. Roots of different cultivars were observed and no completely resistant cultivars to *M. incognita* and *M. javanica* were found, although yield loss was more pronounced for *M. javanica* than for *M. incognita*. Most of the samples were cultured in soils with sandy or loamy texture.

Root-knot Nematode Problems Threaten Flower Production in Kenya

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The cut flower industry is one of Kenya's major export earners, generating billions of shillings per year. Kenya is the largest supplier of cut flowers to the European Union markets, accounting for 25 percent of all flower exports into the EU. Mobydick (*Asclepia* sp.) is one of the important cut flowers grown in Kenya for export. The country's flower export is on record as having grown by 41% in 2004. Mobydick is a relatively new cut flower in Kenya and its popularity has continued to grow among small-scale growers due to its high returns per unit area. The crop is therefore highly valued as a source of income for growers with limited farming land. However, mobydick production is currently hampered by heavy root-knot nematode (*Meloidogyne* spp.) infestations. About 95% of the mobydick producing farms in central highlands of Kenya have serious nematode infestations. In one of the farms surveyed, a 100% loss of yield was recorded. Growers are currently attempting to manage the problem using nematicides, some of which are very costly and cause considerable safety and environmental concerns. Consequently there is a need for using alternative and integrated approaches to manage the nematodes. Before these approaches can be successfully employed, it is necessary to understand the diversity and population biology of the root-knot nematode present. We are seeking to characterize root-knot nematode populations associated with mobydick production in Kenya in order to facilitate the development of effective rotations and introduction of suitable biological control agents for nematode management.

Biointensive Management of Root-knot Nematode in Coleus (Chinese Potato)

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Root and tuber crops are among the world's most important cash crops. The coleus or koorka (Chinese potato), *Solenostemon rotundifolius* Poir (Morton) is a short-duration, under-exploited tuber yielding vegetable and is mainly cultivated in the homesteads of South India. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood is a serious pest of coleus, causing damage to tubers both in field and storage. The attack of this nematode resulted in heavy damage of 92 per cent yield loss (Sosamma 1988). Populations of the nematode pest can be maintained below threshold levels and over-dependence on pesticides can be avoided by developing a suitable Integrated Nematode Management (INM) strategy using a combination of control methods and practices giving emphasis to biocontrol agents and other nonchemical control measures in the nursery and main field. A detailed study was undertaken to evolve an integrated management strategy in coleus at the College of Agriculture Vellayani. 2 X 2 m plots had an initial population of 230 to 250 nematode per 200 g soil sample in randomized block design using physical methods (hot water treatment, soil solarisation) biocontrol agents (*Paecilomyces lilacinus and Bacillus macerans*) and organic amendment (neem cake) in the nursery and main field. Among the nursery treatments studied, soil solarisation and application of either *P. lilacinus* or *B. macerans* were found effective and selected for further studies in Integrated Nematode Management (INM). Among the main field treatments, application of *P. lilacinus* (15g/m^2) in combination with either neem cake (100g/m^2) or *B.macerans* (15g/m^2) significantly reduced *M. incognita* population and improved the biometric characters, yield and quality parameters of *S. rotundifolius*. In integrated management, the selected treatments in the nursery and main field were evaluated using the resistant variety Sree Dhara. Based on the overall performance in reduction of nematode population and the improvement of biometric characters and yield of coleus tubers, integration of soil solarisation in nursery for 15 days with 150 gauge LDPE film and main field application of *P. lilacinus* (15 g /m^2) in combination with either neem cake (100 g/m^2) or *B. macerans* (15 g/m^2) were recommended as the best treatments in integrated nematode management strategy for *S. rotundifolius*. This strategy protected the crop against *M. incognita* and improved per ha yield to tune of 64.33 to 66.18 per cent. In addition, by reducing the nematode population in root (97.89 to 99.73 per cent), the quality parameters of tubers such as starch, sugar and crude fibre content were also maintained

Weeds Acting as Reservoir Hosts of Root-knot Nematodes and Implications for Nematode Management Practices

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The Genus *Meloidogyne* is known to have a wide range of hosts including crop plants, weeds and ornamental plants. A total of 43 weed hosts of root knot nematodes (RKN) were recorded during a survey of root knot nematode diversity and distribution in agricultural areas from Fiji. The pathogenicity of RKN on weeds was found to vary, depending on the species composition as the same weed species acted as good host for some populations while being a poor host for other populations. A total of 25 weed species were categorized as good RKN hosts, 18 as poor RKN hosts and 11as non-host to RKN. The presence of RKN on common weed hosts such as *Amaranthus viridis, Ageratum conyzoides, Vernonia cinerea, Physalis angulata, Coccinia grandis, Momordica charantia, and Cassia obtusifolia* has important implications for the effectiveness of cultural nematode management methods. The ability of RKN to infect and reproduce on weeds makes them good reservoir hosts. Knowledge about weed hosts of RKN is important for farmers and extension officers and should be considered to ensure the effectiveness of cultural control methods amidst concerns for reducing the use of chemical nematicides. Further research work on the interactions of RKN with weed species is necessary to improve our understanding about RKN pathogenicity and population dynamics.

Screening of Locally Available Organic Materials for the Mass Production of a Biological Agent, *Pochonia chlamydosporia* Used against Root-knot Nematodes

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Pochonia chlamydosporia has exhibited potential that needs to be exploited in the biological control of root-knot nematodes. Unfortunately, current methods for mass production of the fungus are not cost-effective in Eastern Africa. The objective of this study was to assess the suitability of locally available materials as substrates in the production of chlamydospores. *In vitro* experiments were conducted in which the organic substrates *Tagetes minuta* (mexican marigold) shoots, *Mucuna pruriens* (velvetbean) shoots, *Tithonia diversifolia* (shrub sunflower) shoots, *Crotalaria junceae* (sunnhemp) shoots, maize stover, cabbage leaves, maize cobs, bean straw, rice husks, sawdust, filter mud, chicken manure, cow manure and goat manure were tested, with rice as a control. The materials were ground, mixed with sand at 50%w.w, inoculated with the fungus and incubated at room temperature (18-23^{o}C) for 21 days before data on chlamydospore count and viability were collected. Chlamydospore counts from cultures of *M. pruriens* and maize cobs were 42 and 43 X 10^{6} .g^{-1} substrate, respectively, which was significantly different from a count of about 8 million in the rice grain standard used as a control. The viabilities of the chlamydspores were 78, 72, 70 and 55% in maize stover, *T. diversifolia* and *T. minuta* and rice, respectively. This study has clearly demonstrated that cheaper substrates such as crop residues and green manure plants can be used in the production of *P. chlamydosporia* inoculant instead of rice.

Session Thirteen – Survival, Adaptations and Tolerance of Nematodes in Extreme Environments

Convenors: David Wharton & Roland Perry

Desiccation Survival in an Antarctic Nematode: Molecular Analysis using Expressed Sequenced Tags

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Although water is essential for life, organisms like nematodes can survive exposure to extreme desiccation by entering into a state of suspended animation known as anhydrobiosis. Nematodes are the dominant soil animal in the Antarctic Dry Valleys and survive the extreme Antarctic environment in anhydrobiotic form. How well nematodes respond to desiccation and which genes are important in the response to desiccation are not well known. *Plectus murrayi*, which is tolerant to desiccation and freezing, represents an excellent model to dissect and characterize the molecular events during anhydrobiosis of nematodes. To study gene expression patterns upon desiccation, expressed sequenced tags (ESTs) obtained from cDNA library and subtractively hybridized library were analyzed and differentially expressed transcripts were identified. A total of 2,486 sequences were obtained and classified into different functional groups and assigned various biochemical pathways. A total of 79 transcripts showed differential expression following exposure to desiccation. Analysis of the ESTs in terms of functional processes, biochemical pathways, and expression profiling suggests differential expression of genes involved in a variety of functional areas, such as stress-related proteins, hormone signaling transduction, antioxidative response, transcriptional regulators, protein synthesis and destination, ion homeostasis, and metabolism. Changes in transcript abundance were validated for a selection of candidate genes using quantitative real-time PCR, which showed differential expression of stress and metabolism related transcripts and constitutive expression of few others. Results indicate that the response to desiccation in *P. antarcticus* is a complex, involving multiple molecular and metabolic pathways unique as well as common to other nematodes. This study presents the first genome-scale approach to characterize the desiccation-induced gene expression in an Antarctic nematode, providing a solid platform for further research on understanding the biological roles of these genes and their ecological significance.

Gene Induction by Desiccation Stress in Anhydrobiotic Nematodes Reveals Parallels with Drought Tolerance Mechanisms in Plants

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Many nematode species have become adapted to withstand extreme desiccation by entering into a state of suspended animation known as anhydrobiosis. Some nematodes can undergo anhydrobiosis at all stages of their life cycle, while many plant and animal parasitic nematodes have anhydrobiotic eggs or infective stages. Some taxa can survive immediate and prolonged exposure to rapid dehydration. However the majority of anhydrobiotic nematodes are slow dehydration strategists requiring preconditioning to moderate reductions in relative humidity to induce the biochemical changes necessary to survive in an anhydrobiotic state. We have isolated expressed sequence tags (ESTs) that are up-regulated in response to desiccation in *Aphelenchus avenae* (a slow dehydration strategist anhydrobiote) and in the infective stages of the insect parasitic nematode *Steinernema carpocapsae* (a desiccation tolerant non-anhydrobiotic nematode). Both datasets contained sequences which encode putative signalling molecules, transcription factors, antioxidants, molecular chaperones, C-type lectin sequences, sequences encoding membrane associated proteins and several distinct LEA sequences. LEAs (= late embryogenesis abundant) are hydrophilic proteins which occur abundantly in plants and whose expression is associated with the onset of desiccation tolerance, cold hardiness and seed maturation. Our EST datasets also contain novel sequences which encode putative hydrophilic and natively unfolded proteins. It is likely that these novel and putative proteins play an important role in desiccation tolerance, possibly by carrying out analogous roles in nematodes to those carried out by the other LEA protein classes in plants. We have recently sequenced 10,000 ESTs from the fast desiccation strategist nematode *Panagrolaimus superbus*. We are currently carrying out a bioinformatics analysis on this dataset. In our presentation we will provide a summary of the available information on the signaling pathways and molecular adaptations utilized by anhydrobiotic and desiccation tolerant nematodes.

Expressed Genome of *Heterorhabditis bacteriophora* Reveals Interesting Longevity and Stress Tolerance Genes in Entomopathogenic Nematodes

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Understanding molecular mechanisms of survival is extremely important for enhancing the utility of entomopathogenic nematodes that are used in biological control of insects. The entomopathogenic nematode *Heterorhabditis bacteriophora* is a cosmopolitan species which is found worldwide in diverse habitats. Our recent studies have revealed correlation between the infective juvenile longevity and environmental stress tolerance in natural populations of *H. bacteriophora*. In this species, we have also shown that both the heat and cold shock responses are correlated with trehalose metabolism. In anticipation of the availability of the genome sequence of *H. bacteriophora* TTO1, we obtained and compared expressed sequence tags (ESTs) to the ESTs and proteins of the free-living nematodes *Caenorhabditis elegans* and *C. briggsae* using BLAST algorithms. A total of 27,380 ESTs were generated and analyzed. We identified 334 ESTs encoding 29 aging and stress-related genes that include components in insulin/IGF-1, JNK, and TOR signaling pathways, which shed light on the aging mechanisms of *H. bacteriophora*. We found major differences in expressed genes between *H. bacteriophora* and *C. elegans* with respect to the lipid metabolism. We also identified 7 genes in RNA interference pathways, two of which are missing in the draft genome of *Brugia malayi*. So far, we failed to identify dicer- or Argonaut-encoding ESTs. However, as demonstrated in recently published work, RNAi pathway seems to be functional in *H. bacteriophora*. We expect to identify more components as more ESTs and the complete genome sequence become available.

An Antarctic Nematode that Survives Intracellular Freezing

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Panagrolaimus davidi is a free-living Antarctic nematode that lives associated with moss and algal growth in terrestrial coastal ecosystems of Victoria Land that are seasonally free of snow and ice. Liquid water is available during part of the summer and the nematode is exposed to an extremely variable thermal and hydric environment. *P. davidi* is the only organism known to survive extensive intracellular freezing. However, depending on the freezing conditions, this nematode may also lose water to the surrounding ice and survive by cryoprotective dehydration. We have confirmed that *P. davidi* can survive intracellular ice formation and shown that this ability is critically dependent upon the nutritional status of the nematode. We have compared the structure of, and pattern of ice formation in, fed and starved nematodes using light and electron microscope techniques. Results indicate a loss of control over the pattern of ice formation in the starved nematodes. An ice-active protein appears to be involved in controlling the formation and stability of ice in the nematode. Attempts to purify the protein by binding to ice were not successful. This, and other results, indicate that this protein is not an antifreeze protein but belongs to a novel group of ice-active proteins, called recrystallization-inhibiting proteins. We hope to purify and sequence this protein and determine the mechanism by which it interferes with recrystallization.

Survival of Plant-parasitic Nematodes in the Absence of a Host Crop

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Plant-parasitic nematodes have evolved various strategies to survive adverse conditions when host crops are unavailable. This talk will explore the various survival strategies of plant-parasitic nematodes to illustrate the adaptations necessary for survival outside the host plant. The ability of species, such as *Ditylenchus dipsaci* and *Anguina tritici*, to survive extreme desiccation is well documented and is associated with morphological and behavioural adaptations of a specific stage in the life cycle. The survival of cyst and root-knot nematodes as unhatched juveniles depends on the protection of the eggshell, perivitelline fluid and cyst wall or gelatinous matrix. In all these instances, the nematode is in a dormant state, either in diapause or quiescence. Soil dwelling stages of plant-parasitic nematodes have to survive the intercrop period, often in adverse environmental conditions. Work on *P. penetrans* and *M. chitwoodi* shows that survival of the two species in the intercrop period shows some similarities but also some remarkable differences. Like *P. penetrans*, surviving stages of *M. chitwoodi* are mainly detected in the root fraction. However, the survival dynamics of stages in the soil differ. Unlike *P. penetrans*, whose distribution after harvest remains nearly constant, the density of *M. chitwoodi* varies with the soil layer and also depends on the previous crop. Our data showed no evidence of *M. chitwoodi* moving to deeper layers during colder spells. Soil samples taken soon after harvest give highest detection for *M. chitwoodi.* As the time after harvest increases, the numbers of juveniles in the soil decrease and detection becomes more difficult. The possibility that these differences in survival behaviour of *P. penetrans* and *M. chitwoodi* may, in part, be linked to different patterns of egg laying will be discussed.

SESSION FOURTEEN – PLENARY SESSION: COMMONALITIES AND DIFFERENCES IN NEMATODE ISSUES ACROSS THE GLOBE

CONVENORS: JOHN WEBSTER & ROSA MANZANILLA-LOPEZ

Global Issues in Nematode Ecology and Management

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The many factors affecting nematode ecology and management change in response to environmental and economic pressures. Some current drivers include global climate change and mitigation measures, fossil fuel depletion, global trade agreements, land tenure and land use practices, and environmental hazards of pesticide usage. There are profound ecological consequences and responses to the interaction of the drivers of change; their integral influence results in change in geographic ranges of crop production, perhaps with concomitant movement of associated nematode species. Crop genotypes are exposed to habitats with novel nematode communities. Global trade agreements result in changes in regions of food production, with enormous influence on local and regional economies, and in concerns regarding pesticide use, residue testing and food safety. Emerging pesticide and environmental regulations, and fuel costs, dictate crop management based on soil and environmental stewardship as well as on maximization of highest quality production. On leased land, soil stewardship conserves and improves soil health for the benefit of future tenants. Land availability and educational programs may reduce slash and burn approaches in subsistence agriculture. In all forms of land tenure, functional complementarity and functional continuity become management goals in developing pest-suppressive soils. Highly diverse, mixed cropping systems provide a consistent food supply at the local level and mitigate climate change effects while generating ecosystem diversity and regulating pest populations. The legacy of crop management decisions is retained in the soil. In some cases, management practices will be developed that are less intrusive and disturbing so that services of the soil food web will increase in importance and significance. In other cases, novel strategies will be required which may be very disruptive to the ecosystem. New challenges in nematode management are emerging and there will be greater opportunities to design and develop crop management systems that have sound ecological bases.

Identification and Characterization of *Meloidogyne* spp. from Coffee Using Morphological, Biochemical, and Molecular Approaches

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The identification of 17 described species of *Meloidogyne* on coffee is a difficult task even for well qualified taxonomists because of the morphological similarity of the different species. Characterization of these *Meloidogyne* species is based primarily on morphological features of females, males and the second-stage juveniles. The esterase phenotypes (Est) were considered until now the most pratical marker for species identification, but they were available for only 12 of the species: *M. incognita* (Est I1 and I2), *M. exigua* (Est E1, E2), *M. coffeicola* (Est C2), *M. javanica* (Est J3*), M. hapla* (Est H1), *M. arenaria* (Est A2), *M. paranaensis* (Est P1=F1and Est P2)*, M. arabicida* (Est Ar2), *M. mayaguensis* (Est M2), *M. izalcoensis* (Est S4=I4) and *M. inornata* (Est I3). *M. konaensis* was reported as presenting three different esterase phenotypes, but only the isolate with Est F1 (=P1) parasitized coffee. Unfortunately, there are no enzymatic markers to identify: *M. africana*, *M. decalineata*, *M. kikuiensis*, *M. megadora* and *M. .oteifae* and these species were not available for study from international collections. In this presentation, brief descriptions of 17 *Meloidogyne* spp., parasitizing coffee in the Americas, Africa and Asia and comments about their intraspecific variability, will be presented. The multiplex PCR (Scar primers) allows unambiguous differentiation of the three main species in Brazil (*M. exigua, M. incognita* and *M. paranaensis*), alone or in mixture, and its potential for application as a routine diagnostic procedure was confirmed in field surveys. Enzymatic and molecular markers should be developed for all *Meloidogyne* spp. that parasitize coffee.

Similarities and Differences in Nematode Problems and Management Strategies as Revealed by a World-wide Questionnaire

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The last two decades' research on plant-parasitic nematodes has been confronted with interesting challenges. Molecular techniques have been developed and introduced, environmental and food safety concerns have raised questions as to the ubiquitous use of nematicides, and biological control has become more important. New threats have arisen from global trade and climate change. Results from a world-wide questionnaire has provided up-to date information on the impact of plant-parasitic nematodes, the control measures currently used to control them and the status of research on plant-parasitic nematodes. In total, 285 nematologists and plant pathologists from 52 countries contributed. Most responses came from Europe (24.6%) followed by Asia (21.9%), North America (21.1%), Africa (17.5%), South America (10.5%) and Oceania (4.4%).

Meloidogyne incognita, *M. javanica* and *Ditylenchus dipsaci* were found to be most prevalent. In Asia *Pratylenchus neglectus* was most reported, in Africa *Radopholus similis* and in Europe *Globodera rostochiensis*. On a global scale, chemical treatments are still the most widely used control strategy. However, in Europe the use of nematicides is significantly less compared with that in other continents due to the strict regulations and a total ban on the use of several compounds. Although most participating countries have a diagnostic service available, preventative soil sampling is not commonly used to avoid nematode problems. It is linked to improved awareness of plant-parasitic nematodes by growers and farmers. Molecular tools are widely used in developed countries in research but not yet for diagnostic services. In developing countries identification of nematodes is often limited to the species level, and molecular identification is absent. The use of biological control agents is still very limited throughout the world, most likely due to the absence of reliable results with the few commercial products available. The use of resistance is mainly focused on potato and soybean cyst nematodes and root-knot nematodes. This leaves many challenges for nematology where also genetic modification should be considered.

Based on the collected data on economic losses an estimate of the global impact of plant-parasitic nematodes will be made.

SESSION FIFTEEN – FOREST NEMATOLOGY AND PINE WILT DISEASE

CONVENORS: KAZUYOSHI FUTAI & CHRISTER MAGNUSSON

Bursaphelenchus xylophilus Surface Proteins: Stage-specific Changes and Characterization

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The surface coat (SC) proteins of plant-parasitic nematodes are likely to play a key role in plant-nematode interactions. It has been demonstrated that an important feature of the SC protein is its lability. In the present study, several kinds of lectins were tested for their binding characteristics to the SC proteins of the pine wood nematode, *Bursaphelenchus xylophilus*, to detect the stage- and isolate-specific changes of SC proteins. Furthermore, the characterizations of SC proteins were conducted by using some molecular techniques. The results demonstrated the stage-specific differences in binding characteristics of a lectin, wheat germ agglutinin (WGA). WGA binding was observed only to the outer surfaces of third-stage propagative juveniles and to the egg shells. Such bindings were detected at higher frequency in virulent isolates than in avirulent isolates. A greater variety of lectins bound to eggs than to other stages. In contrast, no lectin bound to the outer cuticles of dispersal stage juveniles. For characterization, the SC proteins were extracted and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE analysis detected various SC proteins and some of them differed among the stages of *B. xylophilus*. Since the most SC proteins were proved to be glycosylated, these glycosylations were investigated in detail by lectin blot analysis. In this presentation, we will also present other characteristics of the SC proteins of the nematode and discuss a possible role of these developmental changes in SC proteins in pine-*B. xylophilus* interactions.

The Norwegian Surveillance System for Pine Wood Nematode

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Surveillance of quarantine pests is important for national regulation strategies. The detection in 1999 of the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, in Portugal triggered surveys in many European countries. In 2000-2006 the Norwegian survey for PWN has been concentrated to 10 circular observation areas (zone sites) with 50 km radius centred in points of exposure to risk materials. Since clear symptoms from PWN infection of standing trees are not expected in Norway, detached wood and lying trees of *Pinus sylvestris* showing signs of *Monochamus* activity were the main objects sampled. Cordless drilling machines were used to obtain 3165 samples of wood shavings from 446 1-2 year old logging sites. Nematodes were extracted by Baermann funnels. Eighty-five percent of the samples were positive for nematodes. The order Rhabditida was the most frequent (71%) followed by Aphelenchida 44%, Tylenchida 21% and Dorylaimida 0.4%. PWN was not detected, but *B. mucronatus* was present in 0.35 % of the samples, occurring exclusively in wood of pine and particularly in cutting wastes. The probability (p) for a find of *B. mucronatus* varied from 0,0028 in the county of Hedmark and 0,0196 in Møre and Romsdal. Assuming a p-level for a find of PWN of 1/10 of the level recorded for *B. mucronatus* and requiring a 95 % level of confidence (ε), the number of samples (n) needed for a find of PWN can be calculated for each region according to: $n = \ln \varepsilon / \ln (1-p)$. The sampling of our permanent observation areas then would require total sample sizes of 1500- 11000 depending on region. Hence, we suggest that more than 24 000 samples should be taken nation wide and recommend an increase in the Norwegian surveillance activity. Early recognition of PWN infestations is fundamental for successful contingency and eradication.

Observations on the Occurrence and Relationship of Nematodes in Australian Conifers

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Australia has large plantations of the exotic conifer *Pinus radiata*. This species is highly susceptible to *Bursaphelenchus xylophilus*, which is not found in Australia. Potentially pathogenic nematodes were isolated from several dead *Pinus* trees in Williamstown, Heidelberg and other suburbs of Melbourne, Victoria in 2000-2002. A survey of the above-ground nematode fauna of *Pinus* and native conifers in south-eastern Australia was made. A total of 1140 samples from *P. radiata*, 50 from *P. pinaster* and 40 from *Callitris preissii* were examined. No nematodes were found in wood or young shoots of conifers. By contrast, nematodes were common in the bark samples.

Extracted nematodes were classified morphologically into five groups, including: aphelenchida (plant, fungal and lichen feeders), rhabditids and areolaimids (bacterial feeding), *Macrolaimus* spp. (saprophagus), tylenchids (plant feeding), and dorylaimids (bacterial and algal feeders). Aphelenchids are the most common trophic group. About seven different species of aphelenchoidoids were identified from all samples from the *Pinus* and *Callitris* plantations and five species of aphelenchoidoids were identified from diseased trees.

Molecularly, six isolates of Australian Aphelenchoidoidea, *viz*., *Laimaphelenchus preissii* from native coniferous *C. preissii* trees, *L. australis* from the common pine plantation trees of *P. radiata* and *P. pinaster* and *L. heidelbergi* and two morphospecies of *Aphelenchoides* (H1 and K1) and *Cryptaphelenchus* sp. (K2) from diseased *P. radiata* trees, were studied using light microscopy, scanning electron microscopy and phylogenetic analyses of nearly full length sequences of SSU, D2/D3 expansion segments of LSU rDNA and a fragment of cytochrome oxidase subunit I (COI). Bayesian phylogenetic analyses of SSU, LSU and COI of the six nematode species revealed that none of these Australian aphelenchoidoids was inferred to be closely related to *Bursaphelenchus*. The selected isolates of *Aphelenchoides* and *Laimaphelenchus* used in this study were paraphyletic in all molecular analyses. *Cryptaphelenchus* sp. (K2) was inferred to be sister to *Seinura* with SSU sequences.

Molecular Characterization of *Bursaphelenchus* (Nematoda: Parasitaphelenchidae) spp. Distributed in Korea Based on ITS and D2D3 in rDNA Sequence Analysis

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Pine wood nematode (PWN), *Bursaphelenchus xylophilus*, is a causal organism in inducing pine wilt disease (PWD) in many varieties of pine trees. PWD was first introduced to Korea in 1988, but the damage increased dramatically since 2000. Recently PWD was newly reported in Korean pine trees (*Pinus koraiensis*) and is considered one of the most important forest diseases in Korea. Fifteen isolates of *B. xylophilus*, 3 isolates of *B. mucronatus*, and 2 unidentified *Bursaphelenchus spp*. were collected from different geographical locations and hosts in Korea, and were characterized by ITS and D2D3 rDNA sequence analysis. Template DNA was prepared by DNA extraction from a single female nematode. ITS and D2D3 regions were amplified by PCR and followed by cloning and sequence. As a result, all the sequences of ITS and D2D3 from *B. xyophilus* isolates were identical and there is no intraspecific variation. However, 2 genotypes of *B. mucronatus* were found, one from *P. thunbergii* was East Asia type and the other from *P. koraiensis* was European type. The data of two unknown species of *Busaphelenchus* sp.(A) and *Bursaphelenchus* sp.(B) were closely related to *B. tusciae* and *B. lini*, respectively, which was also supported by morphological characterization. The amplified size of ITS and D2D3 for all isolates were 950bp and 750bp, respectively. The only exception was for *Busrsaphelenchus* sp.(B). because it has 1.2 kb in ITS size. ITS-RFLP data also discriminated between different species and genotypes by using 5 enzymes of Hinf I, Alu I, Msp I, Hae III, Rsa I. *Bursaphelenchus conicaudatus* was used as the control species for this experiment, and informative nucleotide sequences of *Bursaphelenchus* were downloaded from GenBank in NCBI. All DNA sequence data were aligned by using Clustal W program and molecular phylogenetic analysis was performed by PAUP*4.0.

Asymptomatic Carrier Trees in Pine Wilt Disease: From an Ecological Viewpoint

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The pine wood nematode (PWN; *Bursaphelenchus xylophilus*), the causal pathogen of pine wilt disease, does not always kill the host pine due to the host-pathogen compatibility and several biotic/abiotic factors including environmental conditions. The present study focused on the asymptomatic host trees which show few, if any, symptoms even after infection with the PWN, to enlighten their ecological importance.

Inoculation tests using susceptible host pine seedlings and an avirulent isolate of PWN demonstrated that PWN could survive inside asymptomatic host seedlings without causing any symptoms for long time. Secondly, as a possible positive effect of such latent infections, it was investigated whether or not induced resistance due to PWN-infection could efficiently protect the host. As a result induced resistance seemed to be effective only in delaying the disease progress, but not in protecting the host for long periods.

To detect PWN inside host plant, a new method using molecular techniques was developed and applied to intensive diagnosis in natural field sites. The results showed that many pine trees in the field had been already infected even though some of them completely lacked any typical symptoms such as dysfunction in resin secretion. Also, a mass emission of volatile compounds, which should attract the vector beetles, from an asymptomatically infected pine tree was detected in the natural pine stands. Thus, asymptomatic trees should be of ecological importance since they may play a role as attractant of the beetles, PWN reservoir, and are therefore an important factor in recurrence of pine wilt.

SESSION SIXTEEN – PLANT-PARASITIC NEMATODE GENOMES: FROM EXPLORATION TO EXPLOITATION

CONVENORS: PIERRE ABAD & MAURICE MOENS

Sequencing and Analysis of the *Meloidogyne hapla* Genome: A Parasite Shares its Secrets

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Worldwide, *Meloidogyne* spp. are the most damaging and economically significant plant-parasitic nematodes. We selected *M. hapla* for complete genome sequencing based on its small genome (54 Mbp) and established genetic system (sexually reproducing diploid). We have completed the primary genome sequence of *Meloidogyne hapla* VW9 from multiple libraries, including 3, 6, and 8 kb shotgun libraries and 40 kb fosmid ends. Our sequence represents a 10.4X draft of the genome and covers >97% of the *M. hapla* genome in 1,523 scaffolds. We are merging a genetic (linkage) map with the genome sequence, using co-dominant markers from a VW9 x VW8 (avirulent) cross. We placed 100% of the sequenced markers on one or more scaffolds, and every marker examined has aligned with the sequence. Extensive automatic and manual annotation has revealed that *M. hapla* has significantly fewer genes than the free-living nematode *C. elegans* although they do share many genes in common. This difference may reflect a diminished need for the parasite to carry as full a gene complement as free-living species, due to dependence on the host to provide essential resources. We are interested in what functions *M. hapla* may have dispensed with (and, of course, what new functions this obligate parasite might have). Analysis further reveals areas of microsynteny between *M. hapla* and *C. elegans*, although substantial rearrangements have occurred. Analysis of the genome to date reveals suites of putative parasitism genes clustered in several areas, numerous horizontal gene transfer candidates, and strong coverage of the dauer pathway. The broader implications are substantial for developmental biology, evolution and phylogenetics. The completion of the *M. incognita* (a more recently evolved RKN) genome by Abad, et. al., coupled with other animal-parasitic and free-living nematode genomes, provides a unique platform for comparative genomics among the Animalia.

The *Meloidogyne incognita* Genome Sheds Light on Plant Parasitism in Metazoan

Abad, P. & International Sequencing and Annotation Consortium

UMR INRA 1301-UNSA-CNRS 6243 - Interactions Biotiques et Santé Végétale, Sophia Antipolis, France

Despite the plethora of information available for the free living nematode *Caenorhabditis elegans* and its sister species *C. briggsae*, very little is known about the other members of this diverse phylum. This is particularly notable for parasitic nematodes, which constitute half of the earth's nematodes, and which have remained largely unexplored. Recently, the genome sequence of the human filarial nematode parasite *Brugia malayi* has revealed significant differences with *Caenorhabditis* spp., and underlined the need to obtain additional genome data from representative species to investigate the outstanding diversity of the phylum[1]. The Southern root-knot nematode *Meloidogyne incognita* is a mitotic parthenogenetic parasite able to infect the roots of almost all cultivated plants, which possibly renders this species the most damaging crop pathogen in the world [2]. Here, I will present data obtained by a consortium of laboratories involved in the annotation of the genome sequence of this nematode. The main points of our studies are as follows:

- The spectacular presence of an extensive set of plant cell wall-degrading enzymes in this nematode, which has no equivalent in any animal studied to date. This suite of enzymes is likely to modify and subvert the host environment to support nematode growth. As *M. incognita* can infect the model plant *Arabidopsis thaliana*, our data make it a key model system for the understanding of adaptations to phytoparasitism by metazoans.

- The striking similarity of some pathogenicity genes to bacterial homologues, suggesting that these genes were acquired by multiple horizontal gene transfer (HGT) events. While inter-species HGT has been a major component of evolution in prokaryotes and some protozoa, HGT into metazoan genomes is relatively rare.

- Most of the genome is present in pairs of homologous but divergent segments, suggesting that this species is evolving in the absence of sex towards effective haploidy. These observations are consistent with a strictly mitotic parthenogenetic reproductive mode which can permit homologous chromosomes to diverge considerably, as hypothesised for bdelloid rotifers[3-4] Such genetic plasticity could explain the explain the extremely wide host range and geographic distribution of this polyphagous nematode.

- Finally, the comparative analysis with the free-living nematode *C. elegans* and the human parasite *B. malayi* reveals profound genomic diversity within this phylum, helping to account for the unmatched evolutionary success of these metazoans.

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4. Pouchkina-Stantcheva, N.N., et al., *Functional divergence of former alleles in an ancient asexual invertebrate.* **Science**, 2007. **318**(5848): p. 268-71.

Radopholus similis: Exploring the Transcriptome and RNAi Applications

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Radopholus similis is an important pest on fruit crops in the tropics, but only few molecular studies have been performed on this nematode. Unravelling the transcriptome of this migratory plant-parasitic nematode can provide insight in the parasitic process and lead to more efficient control measures. A total of 7007 ESTs from a mixed stage population representing approximately 3200 genes were analysed. The mean G+C content of the nucleotides at the third codon position was calculated to be as high as 64.8%, the highest for nematodes reported to date. Remarkably, no evidence was found for the presence of spliced leader sequences commonly occurring in nematodes, despite the use of various approaches.

Interestingly, tags most likely derived from *Wolbachia* were found, providing a molecular indication for the presence of this endosymbiont in *R. similis*. Several ESTs were found with significant similarity to nematode mitochondrial genes. An analysis of the mitochondrial genome of *R. similis* is in progress.

The parasitic life style of this nematode is reflected in the presence of tags derived from genes with a putative role in parasitism, such as genes coding for cell wall degrading enzymes, and proteins involved in detoxification of reactive oxygen species and host recognition. In addition, several unigenes had homology to parasitism genes with unknown function of other parasitic nematode species. We have selected endoglucanases and several housekeeping genes as target for RNAi. In vitro RNAi treatment of *R. similis* with dsRNA for an endoglucanase gene resulted in significantly lower infectivity of the nematodes. Constructs have been made for plant transformation and the generation of transgenic banana is in progress.

EST Analysis of *Bursaphelenchus xylophilus* and Functional Analysis of Parasitism Genes in this Species

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The pine wood nematode *Bursaphelenchus xylophilus* is the causal agent of pine wilt which causes extensive forest death in Japan and East Asia. *B. xylophilus* has recently been found for the first time in the EU, in Portugal, and strict controls are in place to prevent spread of the pathogen. *B. xylophilus* is also a scientifically interesting pathogen as it has the almost unique feature of being able to parasitise woody parts of trees. In addition, the lifestyle and taxonomic position of *B. xylophilus* offer opportunities for comparative genomics studies with *C. elegans* and with other plant parasitic nematodes including cyst and root knot nematodes. Previous molecular studies on *B. xylophilus* were, until recently, very limited and focused mainly on development of tools for diagnostics and detection of the pathogen. However, the application of expressed sequence tag (EST) analysis has greatly expanded the molecular information available on this parasite. Over 15,000 ESTs have been produced as a resource for studies on biology of *B. xylophilus*. The function of selected pathogenicity genes has been analysed by several studies and accumulated data has revealed that horizontal gene transfer (HGT) from both fungi and bacteria has played an important role in ability of nematode to feed on both fungi and plants. A series of pathogenicity genes including those encoding several cell wall degrading enzymes such as cellulase, pectate lyase and beta-1,3-glucanase have been characterised, and this work is continuing with studies on an expansin. Attempts to apply RNAi to this nematode are also underway in order to investigate gene function in more detail. The EST work and other relevant studies could be a precursor to a larger genomics project.

Completion of a *Pasteuria* Genome Sequence: A Heuristic Approach to its Common Thread

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Recent attempts to sequence the *Pasteuria penetrans* genome using traditional Sanger approaches proved unsuccessful because it was difficult to produce good libraries from miniscule amounts of DNA isolated from endospores. The majority of sequences obtained were from DOP amplified libraries which were biased. Instead, we have used a non-biased limited amplification step that eliminated the cloning step and using 454 sequencing technology, and have successfully sequenced the genome of *P. penetrans*. Annotation of the genome is currently being undertaken which will provide a step-change in research on this bacterium. The use of comparative genomics will assist in discovering new knowledge regarding the interaction of this obligate bacterial parasite with its nematode host. What sort of insights might we therefore expect?

The mapping of *Pasteuria* sequences from our previous genome survey onto completed genomes of *Bacillus* spp., and using software such as ARTEMIS, has provided insights into both attachment and culturing. For example collagen-like sequences only present in animal parasitic *Bacillus* spp. are responsible for the production of hair-like collagen filaments present on the surface of the exposporium, and the number of G-x-y repeats relates to the length of the hair-like fibril. TEM studies of endospores have revealed two types of similar hair-like fibrils. Genes in *Pasteuria* have been identified that encode for collagen-like fibrils which we believe are involved in attachment.

Similarly, comparative genomics can be applied to other genes of interest and, combined with biochemical knowledge have provided insights into *in vitro* culturing. Thus, the sporulation pathway in *Bacillus* is well researched and the switch between vegetative growth and sporulation is governed by a phosphorelay pathway. Cation concentration is important for this pathway to function and the presence of copper appears to inhibit sporulation. The removal of copper from vegetatively growing *Pasteuria* cultures aided the cultures to sporulate.

SESSION SEVENTEEN – NEMATODES IN TROPICAL HORTICULTURE

CONVENORS: PATRICK QUÉNÉHERVÉ & DAVABAI

Plant-parasitic Nematodes Affecting Banana and Plantain in Africa: A Shifting Focus?

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Plant-parasitic nematodes are a key cause of banana and plantain (*Musa* spp.) crop loss in Africa. Severely-affected plants can result in total loss of bunches. The problem is exacerbated by the continued use, by farmers, of nematode-infected planting material. The use of healthy planting material thereofore, provides a practical first step towards nematode management in Africa. However, the use of nematode-resistant cultivars will provide a highly complementary option for nematode management. *Radopholus similis* has long since been the focus of resistance breeding in *Musa* spp., and remains the accepted key nematode pest of *Musa* spp. across the globe. In Africa, however, recent surveys have observed an increasing incidence of *Pratylenchus coffeae*, and now regarded as the most important species in some cases. Other species, such as *Meloidogyne* spp. and *Helicotylenchus multicinctus*, are also involved in nematode community complexes across the continent. In higher, cooler locations, *Pratylenchus goodeyi* is often a key nematode species, but has lately been found occurring in hot, coastal areas. The distribution of nematodes and their relative importance on banana remains scant for many areas of Africa. Furthermore, the recent use of molecular techniques is raising questions as to the validity of some species identification. If breeding programmes are to target the key nematode species, the evidence indicates that greater emphasis should be placed on *P. coffeae.* Additional efforts should focus on accurate diagnosis. Resistance against one species, for example, does not necessarily confer resistance against another. Moreover, strains or pathotypes of the same species can also react differently to resistance. Addressing the nematode problem initially depends on improved use of healthy planting material. Meanwhile, the use of resistance needs to be accurately targeted in relation to key needs, based on good quality and accurate diagnostics, as a sound basis for *Musa* nematode management in Africa.

Modelling Nematode Populations in Horticultural Systems

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To date, nematode dynamic models have been very simple, driven only by few parameters without accounting for host quality or environment characteristics. However, these approaches provided only a basic description of nematode population dynamics, and a few mechanistic insights into the relation between the nematode, host and the environment. Recently, more specific models were developed for a wide range of plant-parasitic nematodes and horticultural systems (*Pratylenchus penetrans* in rotations; potato cyst nematode and *Meloidogyne incognita* on potato systems; *Radopholus similis*, *Pratylenchus coffeae* and *Helicotylenchus multicinctus* on banana based systems). These models are either based on biological processes (population growth, initial or maximal population) or statistical approaches; often with dynamic outputs. These models account for the specificities of the relation in the nematode-plant complex, e.g. through the root biomass fluctuation which represents the food resource for nematodes. After reviewing the existing models that simulates nematode dynamics in horticultural systems, we present the example of the SIMBA-NEM model dedicated to plant-parasitic nematodes in banana based systems. We highlight the way this modelling approach allows integration of existing knowledge and permits us to re-examine research about nematode-plant relationship. We emphasize how these models may help to optimize the effect of nematicide applications and participate to the design of sustainable and more environmental-friendly cropping systems. We also focus on the use of models to tackle issues surrounding new banana varieties. Finally, we discuss the relevance of the modelling scale from the root to the field, and its implication in the efficiency in forecasting population dynamics and plant damages. We examine the needs for spatially explicit models that take into consideration the spatial variability of soil moisture or the availability of host root biomass.

Plant-parasitic Nematodes of Coffee: Worldwide Status and Studies Conducted on *Meloidogyne exigua* at UENF

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In many coffee-producing regions worldwide, parasitic nematodes reduce the productivity and increase the production costs of this important commodity. In infested areas throughout the Americas, Africa and Asia, several species of *Meloidogyne* and *Pratylenchus* cause yield losses estimated in at least 15%, while many plantations have been decimated since late nineteenth century. In this complex pathosystem, nematode species, type of coffee grown (arabica or robusta and their cultivars) and edaphic-climatic conditions interplay to determine the damage level. Many other nematode genera have been reported associated with coffee, although in most case their parasitism has not been confirmed. *Rotylenchus reniformis*, *Radopholus* spp., *Hemicriconemoides* spp., *Xiphinema americanum* and *Helicotylenchus* spp. have been associated to coffee damage in some locations. Despite decades of research, in many cases the management options available to coffee growers are not satisfactory. In Rio de Janeiro State, Brazil, the widespread incidence of *M. exigua* has prompt the launching of several interdisciplinary projects, which covers evaluation of sampling strategies for assessment of field populations and epidemiology, assessment of productivity and resistance

of several arabica coffee genotypes, evaluation of cultural and chemical management strategies and investigations on the physiology of parasitism. This presentation will cover an overview of nematode problems on coffee production worldwide as well as results from the projects developed by our research group.

Does Nematode-resistance Breeding Deserve a Higher Priority in Tropical Agriculture? The Case of Banana

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Due to increasing awareness about environmental contamination by pesticides, the search for both plant resistance and/or tolerance to plant-parasitic nematodes is now a major challenge on most commercial crops. In subtropical and tropical agriculture, the concept of using resistant/tolerant cultivars is also gaining more attention since it is considered as a more sustainable management option not only for commercial producers but also for subsistence or smallholder farmers. However, efforts to screen agricultural crop germplasm for resistance to plant parasitic nematodes have mainly been focussed at identifying resistance to sedentary endoparasitic nematodes, such as root knot and cyst nematodes which have the most specialized host-parasite relationship. Concerning subtropical and tropical agriculture, many reasons not to look for nematode-resistance were mentioned by the past and reviewed here at the light of the research on bananas. This paper will review the past decades of research looking for resistance to nematodes in bananas up to the most recent results. Currently, screening for nematode resistance is still an ongoing process, particularly as newly developed banana hybrids become available. The results obtained in the Caribbean on dessert bananas with the new synthetic hybrids (AAA, *Musa acuminata*) from CIRAD, originally designed for their resistance to *Mycosphaerella* leaf spot diseases, have demonstrated their potentiality from laboratory to field experiments in their resistance/tolerance to burrowing and lesion nematodes.

Population Distribution of Plant-Parasitic Nematodes of *musa* spp in Peninsular Malaysia and Molecular Characterisation of Nematode Species

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Nematode infestation on bananas has been identified as the key contributing factor to increased crop losses worldwide. Different plant-parasitic nematode species exhibit different pathogenicity levels towards the hosts. Thus, knowledge of genetic diversity of the parasite is crucial for the development of effective resistance breeding programme for the hosts. As indistinguishable morphological variations were observed in nematode species, molecular-based identification method poses as an attractive alternative to the conventional identification procedures.

Nematode rDNA is comprised of multiple repeats of three ribosomal genes namely 18S, 5.8S, 28S and interspersed with internal transcribed spacer (ITS) regions. The highly conserved nature of nematode rDNA genes enables the design of universal primers to amplify orthologous ITS fragments from all nematode taxa. It was reported that copies of these spacer regions are similar in a single individual but distinct amongst different nematode species. Therefore, by using molecular approach, ITS regions from individual nematodes can be analysed

This study aims to investigate intraspecific and interspecific genetic variations demonstrated by the nematode isolates, thus, exploring the genetic diversity of the parasites. PCR was performed by using two sets of universal rDNA primers for comparison of the ITS regions of five nematode species isolated namely *Radopholus similis, Meloidogyne incognita, Helicotylenchus dihystera, Helicotylenchus multicinctus* and *Hoplolaimus* spp. Isolated amplified products were subjected to cloning and sequencing.

Initial results suggested that the size of amplified products could indicate the nematode species from which the DNA was obtained. Heterogeneity within individual was detected in *R. similis*, *M. incognita, H. dihystera* and *H. multicinctus.* ITS regions of all species showed high homogeneity level which ranges from 83.2% to 98% with the exception of ITS 2 regions amplified from *M. incognita.* In addition, interspecific heterogeneity was found in *Helicotylenchus* genus. These findings are significant for future development of species-specific primers or other DNA-based assays that could further ease nematode identification process.

SESSION EIGHTEEN – EDUCATION AND TRAINING FOR THE NEXT GENERATIONS OF NEMATOLOGISTS

CONVENORS: ROBIN HUETTEL & HARISH GAUR

Beyond SpongeBob SquarePants®

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The use of the cartoon SpongeBob SquarePants® shows that there are sources that introduce youth to the fact that there are nematodes. Unfortunately, aside from a few television programs, there are few sources to introduce the subject to university level students. *Caenorhabditis elegans* might be discussed in an introductory biology class but if so, it is generally referred to as 'worms' and only presented as a molecular model. Plant parasitic nematodes are rarely introduced until the student takes a general plant pathology course usually in the upper level years of college. Even then, plant pathology colleagues often devote only a couple of lectures to the students on nematodes. In North America, Nematology related to agricultural systems is fairly well represented at the university level. Most U.S. land-grant universities have one or more faculty members that are active in the discipline. Federal Agencies also employed many nematologists in both the U.S. and Canada. Even though there are more limited job opportunities for nematologists, these opportunities do exist and students need to be encouraged to pursue a career in nematology. Therefore, it is important to develop an introduction to these nematodes at an early stage in an academic career and encourage students to continue in the discipline. Undergraduate research opportunities that are meaningful can help develop an interest in continuing to working with these organisms. Various approaches that have been successful as well as ideas on creative new approaches to add nematodes to other curriculum will be discussed.

Nematological Research and Training in the Afro-Asian Region: Status and Opportunities

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Like nematodes, the distribution and development of the science of nematology in the world have been highly aggregated and fluctuating. Compared to sister disciplines of entomology and plant pathology, nematology is a relatively young. Nematologists have made significant contributions in various fundamental and applied aspects. Good beginning has been also made in application of DNA-based techniques for differentiating nematode taxa and understanding and even incorporating nematode resistance. The Indian Agricultural Research Institute, New Delhi started M.Sc. and Ph.D. degree programmes in Nematology in 1968. Ten State Agricultural Universities in India established nematology departments and started post-graduate teaching. A small course is also taught at the under-graduate level. These developments provided trained manpower in nematology and India today has one of the largest networks of nematologists. Many other nematology centres still teach and guide students on nematological aspects under entomology, plant pathology, botany, zoology, plant protection etc. Pakistan has a good Nematology Research Centre at Karachi. Good progress in nematological research has been made in South Africa, Egypt, Japan and China. Other countries in the region have very few or no trained nematologists. The centres of excellence in the areas of taxonomy, physiology, ecology etc., have got dwindled over time. Many nematologists are attempting to study entomopathogenic nematodes or molecular nematology. The expected and potential benefits of Plant Nematology have not been adequately realized. Recognition, documentation, disinfestation and management of nematode pests of crops have gained greater significance in post-GATT period involving much cross-border trade and movement of commodities, including phytosanitary, quarantine and biosecurity concerns. After the recent boom in IT and commerce, post-secondary talent is not getting attracted towards higher education in science; the situation has worsened more in nematology. Nematology graduates often face difficulty in securing employment since degrees are awarded under different subjects in various universities. The present scenario of nematological teaching and training facilities and techniques in Afro-Asian region will be reviewed in terms of emerging national and international needs and expectations.

Nematology Education in Europe: A Joint Effort

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In the past Nematology education and training in Europe started as several independent efforts in different countries, such as the UK, Germany, The Netherlands and Belgium. These trainings had different goals, frequencies, duration, timing and type of participants.

The current nematology education is characterized by on the one hand short training sessions, such as in the Netherlands and on the other hand by a university MSc programme at Ghent University in Belgium. The latter has evolved from a 1-year MSc programme into a 2-year MSc programme at Ghent University as well as into a 2-year European MSc course providing mobility of the students between partner Universities and Research Institutes located in different European countries. In this way new possibilities for cooperation and strengthening ties between nematologists are created.

Future perspectives are partnerships in education with Universities all over the world, including increased mobility for students between these partners.

Integrating Multifocial Microscopy Images of Nematodes into Nematology Training and Education

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Growth of nematology as a discipline is severely constrained by the relatively small numbers of students and young researchers interested in entering the field. Important causes for this lack of enthusiasm include the long hours behind the microscope traditionally needed to obtain a meaningful understanding of nematode anatomy, diversity, diagnostics and systematics. This in turn is largely due to the challenges of learning how to operate the microscope, as well as to the limited effectiveness of traditional monographs and identification keys as teaching tools. Based on our own experience as both trainees and trainers in nematode microscopy, we believe a more efficient approach requires a broad menu of teaching and self-teaching tools tailored to individual skill levels, prior experience and future goals of each nematology trainee. Because the learning curve is traditionally both steep and protracted, experts are often severely constrained in the kinds and amounts of personal knowledge they can transmit to trainees, while trainees are usually ill-equipped to receive and assimilate the information given by experts. Multimedia technology and online resources can mitigate and remedy many of these constraints, provided they are both robust and versatile, capable of helping trainees with differing needs and prior experience through a variety of computer platforms. We present some tools, prototypes and ideas developed in the course of our ongoing research and teaching, all of which are based at last partly on presenting trainees with multifocal image series that present nematode specimens and structures as they appear under the microscope. We outline a larger framework for experimentally testing and improving effectiveness of such tools, as well as potential strategies for improving and expanding them in wiki-like fashion by enabling addition and updating of information via internet tools such as email, ftp servers and dedicated websites.

SESSION NINETEEN – BIOSECURITY, QUARANTINE AND NEMATODE EMERGENCIES IN INTERNATIONAL TRADE

CONVENORS: SHASHI SHARMA & SUE HOCKLAND

SPONSORED BY CRC PLANT BIOSECURITY

Global Issues in Plant Biosecurity: Opportunities and Challenges for Nematologists

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‘Plant biosecurity’ refers to the safeguarding of plant resources from biological threats, in particular exotic threats, in managed and natural ecosystems. It has emerged as a major international issue directly influenced by an extraordinary increase in world trade and travel, and concerns about the effects of climate change. The plant health status of virtually all countries has become highly vulnerable to the invasion of potential biosecurity threats such as the species of plant parasitic nematodes. Although the majority of plant parasitic nematode species are soil-borne root feeders and are not normally spread via seed, fruit or flower pathways, some nematode species have spread widely and successfully established across continents, most probably via inadvertent transportation of nematode-infested soils. A study in 2004 showed that more than 80% of dust and dirt samples taken from 341 shipping container surfaces at New Zealand ports were infested with live nematodes. Predictive bioclimatic modelling studies indicate that some of the highly pathogenic nematode species such as the stem and bulb nematode, *Ditylenchus dipsaci* ‘giant race’ and the root-knot nematode, *Meloidogyne artiellia*, have the potential to establish in more than 25 additional countries. It is likely that unless concerted efforts are made to stop this seemingly unabated international spread of harmful nematodes, susceptible plant species in different regions will suffer significant damage particularly in those countries where farmers have sub-optimal level of awareness of diseases caused by plant parasitic nematodes. There is a need for a stocktake of plant health management strategies, policies, institutional arrangements and systems in terms of facilities, capacity and capability to minimise the introduction, establishment and spread of plant parasitic nematode. A global and shared approach to nematode risk management will be discussed together with opportunities for nematologists to contribute to biosecurity risk mitigation.

International Pathways for Introduction and Spread of Invasive Nematode Species and Options for Risk Management

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A diverse range of plant-parasitic nematodes is being intercepted by national plant protection organisations (NPPOs) around the world. Listed species are generally uncommon as a result of phytosanitary regulations, but increasingly action is taken against non-quarantine species which thus become regulated. Nematodes are being spread by a combination of both old and new pathways that reflect changing patterns of trade, both with regard to the type of consignment and the countries involved. A range of imported consignments are targeted for inspection but generally most regulated nematodes are found in the roots or growing media of ornamental plants. Nematodes are also being used as bioindicators as the finding of some species may indicate a contravention of international phytosanitary rules for the production of certified stock or derogations for certain types of trade. However, the personal baggage and footwear of international travellers is also being shown to be a pathway of increasing significance for quarantine and regulated non-quarantine species. But what determines a new threat? Countries around the world should adopt similar practices as set out in international standards. Intercepted nematodes often pose a challenge for identification but synchronisation of taxonomic expertise, as well as experience of eradication or other control procedures, is essential for simple or more complex pest risk analyses. However, the economic importance of new threats is often difficult to judge. Other species may pose a risk to national biodiversities but information is sparse. Methods of eradication usually entail destruction of consignments with consequent losses. Prevention strategies, the cornerstone of good plant health practice, will be discussed, together with other options for risk management, illustrating the mutual importance of exchange of information between nematologists in different climatic zones which should be encouraged.

Incursion Management of Potato Cyst Nematode and Restoration of Pest Area Freedom

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Since its original introduction(s) from South America into Europe in the mid-1800s, potato cyst nematodes (PCN) have spread to most regions of the world. PCN is now reported in over 65 countries of the world and considered endemic in many regions. For over 60 years extensive programmes in many countries have attempted to eradicate or limit the spread of PCN with varying degrees of success. In most instances PCN was identified after it had become established in a region and only a containment programme was achievable whist still allowing potato production. However, experience in Western Australia indicates that if PCN is detected soon after introduction then eradication can be achieved by implementation of extensive quarantine and control measures. With increasing global trade in potatoes PCN continues to be identified in new regions of the world, and eradication strategies will be dependant on species identification, infestation levels and resources available.

The pale potato cyst nematode *Globodera pallida* was confirmed in Idaho, USA in 2006 and presents an opportunity for an intensive eradication programme utilizing the greater knowledge and diagnostic techniques recently developed. This strategy involves application of chemicals and planting green manure crop for a number of years, together with the regular monitoring of PCN viability levels. This will prevent the introduction of PCN into a clean land and further spread within infested regions. Treatment will continue until no viable PCN is detected and is scheduled to continue for at least nine years. Long-term crop rotation followed by non-detection of PCN in extensive surveys could form the basis for restoring PCN freedom. Specifically, planting of oil radish in rotation with wheat after fumigation is considered as an effective method for PCN eradication under field conditions. Alternative management strategies will also be presented.

Quality Requirements for Accreditation and Standard Operating Procedures: What could be a Reference Method for Quarantine Nematodes Detection?

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The recent increase of trade in plants has stressed the need for standard operating procedures to get reliable and comparable results among diagnostic laboratories, preventing major dispute. For quarantine plant-parasitic nematodes, regional or international diagnostic protocols are already produced: e.g. IPPC international protocols or EPPO regional protocols. Even if helpful, these protocols often come closer to a compilation of most known or used tests, without elements about their performance (sensitivity, specificity, repeatability, reproducibility). These validation elements become more and more demanded as diagnostic laboratories develop quality management system and need to prove the validity of the method in use (accreditation - ISO 17025 standard). Thus consistent discussions began with different diagnostic protocols. Examples are given here from the IPPC protocol for *Bursaphelenchus xylophilus* with the assessment of the molecular identification. The specificity of published tests (PCR, PCR-RFLP) was evaluated against a set of *B. xylophilus, B. mucronatus* and other *Bursaphelenchus* species, chosen to represent part of the genetic diversity described. In practice most of the tests are reliable when compared to published results. Another example is given from the EPPO protocol for *Xiphinema americanum* and the limits of the different detection or identification methods. Performance of the different extraction methods would be discussed. Limits of the morphological or molecular identifications and the reliability of the final results would be presented and analysed. These results highlight that a diagnostic protocol should inform about the performance of the tests included to avoid mistakes and the best route to ensure reliable results (need for complementary tests). Moreover, at a regional or international level, definition of minimum performance criteria should be discussed and a validation process might be described. Then diagnostic protocols would offer more reliability and ease for all diagnostic laboratories undertaking official analysis, whether they are accredited or not.

Networking and Resources for Management of Biosecurity Risks Posed by Nematode Species

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Biosecurity risk management has emerged as an important aspect of international trade and exchange of genetic resources. The International Plant Protection Convention (IPPC), recognised by the World Trade Organisation as the source for international standards applying to phytosanitary measures which influence trade, covers international, regional and national arrangements for management of plant sector related biosecurity risk management issues. The international phytosanitary standards developed by the IPPC have direct relevance to the invasive alien species (IAS) including nematodes. Presently the limited information on invasive nematode species and pathways for their spread provides a challenge for pest risk assessment that might be improved with better networking and understanding.. One of the actions for nematologists is to develop protocols to identify invasive nematode species. The subject of identification of invasive nematode species as quarantine pests will be discussed using examples of potato cyst, pine wood, stem and bulb, and white tip nematodes. Issues regarding lack of reliable distribution databases, scientific justification for risk treatment, diagnostic protocols, inadequate capability and capacity to address the nematode risks and merits of developing regional and international nematology networks for information and expertise source sharing in the management of invasive nematode species will be presented.

Supply and Processing of Assimilates in Nematode-induced Syncytia

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The cyst nematode *Heterodera schachtii* induces specific syncytial feeding sites in the roots of its host, from where it withdraws its nutrients. Syncytia generate strong sinks in the host plant's transport system. In roots of Arabidopsis thaliana syncytia are symplasmically isolated during the first days of nematode development and solute supply relies on the activity of syncytial plasma membrane-bound transport proteins. Only later, plasmodesmata are formed connecting syncytia with the phloem. The expression of all sugar transporter genes annotated in the Arabidopsis Membrane Protein Library in young and fully developed syncytia was compared to non-infected A. thaliana roots by Affymetrix gene chip analysis. Three genes STP12 (At4g21480), ERD (At3g05400) and ANTR2 (At4g00370) were selected for in depth studies using qRT-PCR and in situ-RT-PCR. T-DNA insertion lines of STP12 and ERD both significantly affected nematode development. Further, the expression of STP12 and ERD in syncytia of male juveniles was compared to their expression in syncytia of female juveniles. In order to prove sugar transporter activity in syncytia fluorescence-labelled glucose was loaded into the phloem of leaves and readily translocated into syncytia.

Although the nematodes withdraw high amounts of nutrients, we found accumulation starch accumulation of starch in syncytia using high-performance liquid chromatography and microscopic analyses. Further, we monitored the expression of genes involved in the starch metabolic pathway by gene chip analysis and quantitative reverse transcription-PCR. Starch seems to serve as a carbohydrate buffer to compensate changing solute uptake by the nematode and as long-term storage during juvenile development.

Root-knot Nematodes Manipulate Plant Cytoskeleton during a Compatible Interaction

Favery, B., M.C. Caillaud, M. Quentin, P. Lecomte, J. De Almeda-Engler & P. Abad

UMR INRA 1301-UNSA-CNRS 6243 - Interactions Biotiques et Santé Végétale, Sophia Antipolis, France

Root-knot nematodes induce the redifferentiation of root cells into multinucleate and hypertrophied giant cells essential for nematode growth and reproduction. The distribution of microtubules and microfilaments in giant cells has recently attracted considerable attention. To understand how nematodes are able to manipulate host functions to their own advantage, we focused on the characterization of Arabidopsis genes early induced during giant cell development. We characterized the first plant candidate gene implicated in giant cell actin cytoskeleton reorganization (Favery et al., 2004). Three *formin* genes are induced in giant cells. Formins are actin-nucleating proteins that stimulate the *de novo* polymerization of actin filaments. We demonstrated that AtFH6 was anchored to the giant cell plasma membrane and was uniformly distributed. Suppression of the budding defect of a yeast formin mutant showed that AtFH6 regulates polarized growth by controlling the assembly of actin cables. Our results suggest that formins may regulate giant cell isotropic growth by controlling the assembly of actin cables. Actin cables would guide the vesicle trafficking needed for extensive plasma membrane and cell wall biogenesis. In addition, we will present a complete functional analysis *in planta* of a Microtubule-Associated Protein, MAP65-3. We showed that MAP65-3 is essential for giant cell development during root-knot nematode infection and that cytokinesis was initiated but not completed in giant cells (Caillaud et al., 2008). In developing giant cells, MAP65-3 was associated with a novel kind of cell plate — the giant cell mini cell plate — that separates daughter nuclei. In the absence of functional MAP65-3, giant cells started to develop but failed to fully differentiate and were eventually destroyed. These defects in giant cells impaired the maturation of nematode larvae. Subcellular localization of MAP65-3 and microtubule organization analysis in the *map65-3* mutant demonstrated that MAP65-3 played a critical role in organizing the mitotic microtubule array both during early and late mitosis.

Favery et al. (2004). Arabidopsis formin AtFH6 is a plasma membrane-associated protein upregulated in giant cells induced by parasitic nematodes. **Plant Cell** 16, 2529-2540.

Caillaud et al. (2008). MAP65-3 microtubule-associated protein is essential for nematode-induced giant cell ontogenesis in Arabidopsis. **Plant Cell,** www.plantcell.org/cgi/doi/10.1105/tpc.1107.057422.

Root-knot Nematode Management based on Solarisation

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In naturally infested soil with root-knot nematodes; *Meloidogyne* spp. two field experiments were carried out in the summer season (mid July–mid August) of years 2005 and 2006 at a two locations in Beheira governorate, Egypt, to investigate the role of solarization on root-knot nematode control .Five different polyethylene colour sheets (transparent, red, black, green and blue) were used to cover the infested soil. Control treatment was left without cover. Tomato seedlings were transplanted into holes which were done in plastic sheets, one month after covering. A great reduction of No. of J2, egg masses, No. of females and No. of galls per root system was occurred compared to the control at all type of plastic sheets. Transparent sheet showed the highest percentage reduction of total population and reproduction rates of RKN compared to the other treatment .Tomato plant growth parameters were enhanced markedly.

Pasteuria penetrans as a Biological Control Agent of Root-knot Nematodes in Egypt

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Experiments were carred out on nine *Pasteuria penetrans* isolates from Egypt, Japan, Malawi, Australia, Barbados, Ivory Coast, Great Britain, South Africa and Papua New Guminea for attachment rates to root-knot nematode *Meliodogyne* spp. Results indicated that the ability of attachment to second stage juveniles of RKN differed greatly. Only three out of nine: Pp Egypt, Pp Japan and Pp South Africa showed a great attachment, while no attachment was observed for the others .Under greenhouse conditions on tomato plants, only Pp Egypt and Pp Japan showed a high reduction of root-knot nematode gall numbers compared to the control. Infected females of RKN with Egyptian Pp were markedly significant compared to Pp Japan. Plant growth was greatly influenced when both Pp Egypt and Pp Japan were used: parameters of fresh shoot, root and shoot dry weights were increased markedly compared to the control.

SESSION TWENTY-ONE – NEMATODE MANAGEMENT IN SUBSISTENCE AND SMALLHOLDER AGRICULTURAL SYSTEMS

CONVENORS: JULIE NICOL & DANNY COYNE

Farmer Awareness of Plant Parasitic Nematodes on Maize in Uganda: A Basis for Assessing Nematode Resistance Breeding Needs in African Smallholder Maize Production

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Farmers in Africa have virtually no understanding of nematode pests, yet they are responsible for substantial losses, especially with intensified production. The current study first assessed nematode pest awareness by farmers in maize and also determined preferred traits, in order to establish the basis for breeding for nematode resistance in maize in Uganda. In two districts each, 60 farmers ranked pests as the major maize production constraint, both in Masaka (68.3%) and Iganga (48.3%). Most farmers (81.5%) were unfamiliar with nematodes as a maize pest, while 42.5% could identify nematode symptoms associated with tomatoes and 48.3% with bananas, compared to 10.8% on maize. Mostfarmers (84.2%) did not know the effect of nematodes on yield, but reported nematode associated symptoms of leaf chlorosis (85%), stunted growth (80.8%) and patchy growth (69.2%). However, 15.8% reported maize yield losses following tomatoes, eggplants, bringals or bananas affected by nematodes. *Pratylenchus zeae* and *Meloidogyne* spp. were recovered from maize roots in higher densities than other species. Root galling and blackened lesions, associated with *Meloidogyne* spp. and *Pratylenchus* spp. infection respectively, were recorded on some maize cultivars. Although mean nematode densities ranged between 166.7 to 845.9, and 833 to 21,042 per 100 g root fresh weight for *Meloidogyne* spp. and *P. zeae* respectively, all cultivars had similar levels of infection. Farmers ranked high yields (46.7%), pests and disease resistance (20.8%), and palatability (14.2%) as the most preferred traits in maize. Nematode resistant inbred lines 5057, 9450 (*P. zeae*), Mp712, Mp710, Mp711 and Mp709 (*Meloidogyne* spp.), and local susceptibles are therefore being evaluated for use in a combining ability study to develop high yielding multiple nematode resistant lines. Also, open pollinated cultivars of Longe 1, Longe 4 and ZM521 are being improved for resistance to nematodes using an S_1 recurrent selection procedure at Namulonge, Uganda.

Addressing Root-knot Nematodes in Horticulture: Diagnostics Resistance and Integrated Management Practices in Turkey

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Root-knot nematodes are the most important nematode species for the both protected vegetable, horticulture and potato cultivation in Turkey. When root-knot nematodes is not controlled, they cause significant yield losses (between 50% and 80%) every year. Identification of root-knot nematodes on various groups has been achieved by using morphological, host reaction and molecular techniques. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the common species found in Turkey. *Meloidogyne incognita* race 2 and *M. javanica* race 1 are widespread in vegetable growing areas estern Mediterranean Region of Turkey. Recently a new finding of *Meloidogyne chitwoodi* on potato has been identified in the middle Anatolian region.

With the phase out of Methyl Bromide in Turkey by 2008, a large integrated project supported by World Bank and UNIDO in Mediterrnean region of Turkey enabled alternative managment practices to be investigated in greenhouse horticultural production systems. A number of treatment and treatment combinations were investigated including Solarization + Trichoderma, Solarization + Dazomet, Solarization + Fresh Chicken Manure, Solarization Fresh Cow Manure, Grafting and Resistant varieties to control root-knot nematodes. The most cost effective alternatives to methyl bromide were Solarization + Trichoderma and Solarization + Organic Manures. Also, other treatments were found to be viable and cost effective alternatives to methyl bromide in greenhouses. Another management strategy was backcrossing breeding to incorporate Mi-1 resistant gene into a commercial fresh cultivar of tomatoes. The effective transfer of the gene was validated by using MAS (Marker Assisted Selection). However, one of the challenges with Mi-1 gene is the resistance has not been effective in several locations, and also other resistance cultivars have broken down in the West Mediterranean Region. In order to address this further research on tomatoes is being carried out on the Mi-gene breaking population of virulent root-knot nematode on tomatoes in West Mediterranean Region supported by TUBİTAK.

Impact of Nutrient Supply Systems in Rice-wheat Cropping System on the Dynamics of Nematode Community Structure in Sub-humid and Humid Agro-ecosystems

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The rice-wheat cropping system has become popular in parts of India, but depletes soil organic matter. Therefore, attempts are being made to devise nutrient supply systems including organic sources. Dynamics of nematode community structure was studied over a six year period (2001-07) in long term experiments in sub-humid (Indore, Madhya Pradesh) and humid (Kalyani, West Bengal) agro-ecosystems in India. The nutrients were either supplied at different doses entirely through mineral fertilizers (NPK) or substituted upto 25 or 50% equivalent of nitrogen through farm yard manure, crop residues or green manure. In clayey soil at Indore, in all paddy plots where NPK alone was applied showed increase in the population density of *Hirschmanniella oryzae* but when NPK was incorporated along with FYM, crop residue, green manures and compost the population density decreased. The equilibrium population density (EPD) of *H. oryzae* was significantly higher with 100% N through NPK, than in plots where 25-50% NPK was supplied through manures. In non-fertilized control EPD of *H. oryzae* was higher than in farmer's practice. Similar trend was observed in the EPD of *Helicotylenchus* spp. The EPD of saprozoic nematodes which belong to cp-1 and cp-2 grades, increased when organic matter was applied. At Kalyani, the EPD of *Hirschmanniella* spp. was 400-520 per 200 cm^3 soil in all the treatments. *Tylenchorhynchus mashhoodi* had moderate EPD of 200-300, while the root-knot nematode, *Meloidogyne graminicola* also prevailed at low–medium population densities (EPD 60-120) in all the treatments. No significant variations could be related to the nutrient treatments. The proportion of cp-1 and cp2 colonizers, hence, saprozoic nematode index (SNI), indicative of very active organic matter decomposition process and active soil microbial community in humid ecosystem. The contrasting observations on effects of similar nutrient supply systems at the two locations could be correlated to differences in soil organic matter and agro-ecosystem characteristics. The SNI emerged as a strong bioindicator of high organic matter and microbial activity, indirectly soil health.

Current Status of Plant Parasitic Nematodes on Banana and Plantain in Latin America: Biological Control and Agronomic Management for Sustainable Production Systems

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Recent surveys of plant-parasitic nematodes associated with banana and plantain in Latin America and the Caribbean revealed that *Radopholus similis* was the most important plant-parasitic nematode, followed by *Helicotylenchus multicinctus*, *Pratylenchus* spp. and *Meloidogyne* spp. Currently plant-parasitic nematodes are managed in commercial banana plantations by applying 2 or 3 nematicides applications per year, with a cost between US$ 400-450 / ha/ year. The use of nematicides has been criticised by the farmers due to the high cost and reduced efficacy to control nematodes due to enhanced biodegradation. In addition, banana farmers are looking for alternatives to nematicides, due to the expected market withdrawal in the following 5 years. Biological control and the use of organic amendments are potential alternatives to chemical control for the management of plant-parasitic nematodes. Over the past 10 years, we have found significant reductions in *R. similis* on bananas by inoculating *in vitro* plantlets with mutualistic endophytic fungi, mainly *Trichoderma atroviride* and non pathogenic strains of *Fusarium oxysporum,* as biocontrol agents in greenhouse conditions. The endophytes are currently being evaluated in field conditions on more than 35 ha in five countries in Latin America. Preliminary results in commercial banana plantations in Costa Rica indicate that a single inoculation of *in vitro* banana plantlets with endophytes, in the nursery phase prior to planting, gave better nematode control than 3 nematicide applications. Similar results were obtained in plantain in Panama and the Dominican Republic. The results from the use of organic amendments on bananas indicated that there was no direct effect on nematode control, but an increase in the root biomass as well as the percentage of functional roots.

Incidence and Economic Losses caused by PCN *(Globodera pallida and G. rostochiensis*) in the Andean Region of Bolivia and Peru

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Results of soil sampling during several years in main potato producing departments and information available on the distribution (incidence) and population densities (severity)of *Globodera spp*. was analyzed to estimate the economic losses that this nematode causes to the potato crop in Bolivia and Perú. All sampled departments in both countries showed the presence of PCN. Out of 3299 soil samples, 64.90 % were positives and infestation levels reached up to 2161 eggs/g soil in Peru. This percentage in Bolivia was higher (78.42 % out of 735 samples). The results obtained by the extrapolation of cultivated areas, PCN incidence and severity damage, and price of the tubers, allowed to estimate the economic losses of the gross value of potato production in Bolivia, reaching US $ 16 million. The dominating species in this genus was *G.pallida* in relation to *G. rostochiensis* and both species were most frequently found between 3,500 and 4,000 masl. Th effect of incidence and severity of PCN on the yield potato crop in Peru is also estimated although no gross value economic losses due to lack of more detailed information.

SESSION TWENTY-TWO – FUNDING APPLIED NEMATOLOGY, EXTENSION AND TEACHING: GOVERNMENT AND COMMERCIAL

CONVENOR: NICOLE SMOL

Governmental Influence (by Funding) on Research in The Netherlands and the EU

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Insight is given into how research funding is organised in The Netherlands; a distinction is made between public and private funding. Nematological aspects are covered in general plant health programs and in phytosanitary (statutory plant health) programs specifically related to quarantine matters. Some examples are given of policy supporting research related to the quarantine nematodes *Globodera rostochiensis/pallida* and *Meloidogyne chitwoodi/fallax*. Results of these projects are used for developing new policy and policy adjustments.

EU Framework Programmes are a source for financing plant health research. Within the EC 6th Framework Programme ERA-Net (European research area network) EUPHRESCO is funded, meaning European Phytosanitary RESearch Coordination. The aims of this project is to increase the co-operation and co-ordination of national phytosanitary research programmes and to fund at the EU level through networking of research activities and mutual opening of national programmes. The *Globodera* project will be used as an example to explain the process.

Funding for Applied Nematology in the UK

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A review of the public and commercial funding provided for applied and extension nematology in the UK is presented. In the UK, the public funding of applied nematology is largely limited to the responsibilities of statutory bodies in relation to the implementation of phytosanitary legislation. The most significant example of this is statutory pre-crop testing for potato cyst nematodes. Surveillance in relation to other quarantine species constitutes another important area of work, particularly in relation to the examination of imported plants and plant products. Research in applied nematology may be funded by either the public or commercial sectors and often by both. The extent to which public funding is likely to be involved will depend upon the degree to which the outcome is considered to be to the 'public good'. There is an increasing tendency for government funded research to be of benefit to government policy making.

Support for Nematology in Developing World Agriculture: How is the Future Looking?

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Globally, nematology tends to have a low profile with research organisations, universities and consequently donors, but especially in developing countries and Africa in particular. Within the consultive group for international agricultural research (CGIAR), designed to support and promote developing country agricultural research, nematology expertise has declined with nematologists a rarity in a system dedicated to supporting national programmes. This creates difficulties for nematologists to attract research funding and support for training, often through a limited critical mass and voice to champion the discipline. Fellowships, specifically designed to cater for needs of developing world scientists provide opportunities, but without the disciplinary advocates, nematology opportunities are often missed. Since the widely acclaimed International *Meloidodogyne* Project (IMP) (1975–1983), funded by USAID, which involved some 200 nematologists over 70 countries, there have been few substantial nematology interventions. Some notable examples include the Postgraduate International Nematology Course (PINC) (since 1992), now EUMAINE, funded by the Belgian Government through the Flemish Interuniversity Council (VLIR) and The Nematology Initiative in Eastern and Southern Africa (NIESA) (2005–2010), funded by the Gatsby Charitable Trust, both designed to build capacity in plant nematology. However, the weaknesses and needs of tropical and developing world nematology, such as basic knowledge and a lack of awareness among key actors, remain prominent, while becoming increasingly necessary to confront, as cropping intensification and cropping of more marginal lands exaggerate nematode problems. In particular, there is need to follow on the success of the IMP, especially in respect to meeting the challenge of the most important plant parasitic nematode group, *Meloidogyne* spp. With a bleak future perspective, there is urgent need to address nematology training and support to create greater disciplinary prominence and bring it more into line within agricultural research agendas.

Public, Government, Producer and Industry Funding for Nematological Research

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Most funding for nematological research, whether of an applied or basic type, comes from one of four main sources, public or state funding, governmental competitive granting agencies, producer associations, or members of the pesticide industry. The amount of funds from state sources varies considerable among the different states, but in general the amount is relatively low. The amount of funds obtained from competitive grants also varies considerably depending on the source. For example, funds from USDA National Research Initiative or National Science Foundation will generally be greater in amount than would be obtained from special grants sources. But on the other hand they will be far more competitive. One of the more stable sources of funding for nematologists is that procured via producer associations. States with large commodities, e.g., soybean, cotton, peanut, will have larger pools of funds available allocated for applied programs. For nematologists interested in working with the pesticide industry funding can be acquired but over the past 15 to 25 years there has been a large decrease in funds available for nematicide evaluations.

Who is Paying for Nematology Training and Education?

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For many years Nematology education was provided in different ways in nearly all continents of the world. Funding for nematology education consists of two parts: the costs of the organization of the training and the costs for the participants. A comparison of both aspects for different trainings reveals that depending upon the type of training the funding varied from personal, public, industry, international organizations to governmental. The organizational costs are mostly covered by the government or the university/institute where the trainings take place; the funds for the participants are provided either by governmental grants, industry, or even personal means. In the case of Europe, the European Commission has a specific programme: the Erasmus Mundus (EM) programme which promotes the European Union as a centre of excellence in higher education around the world and supports European top-quality Masters Courses. The *European Master of Science in Nematology* – EUMAINE is such a newly selected Erasmus Mundus course programme characterized by co-operation and mobility between different EU universities and institutes. The EM provides EU-funded scholarships for third country national students and scholars, as well as scholarships for EU-nationals studying at Partner universities throughout the world. Future EM funding possibilities will be announced.

PLENARY SESSION TWENTY-THREE – THE *C. ELEGANS* INHERITANCE: BIOINFORMATIC ANALYSIS AND NEMATODE GENOME DATA-MINING

CONVENORS: DAVID BIRD & STEPHEN TROWELL

The Genome of *Pristionchus pacificus* and Implications for the Evolution of Parasitism

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Pristionchus pacificus represents a nematode that shares with *Caenorhabditis elegans* many technical features and has been developed as a model system in evolutionary developmental biology. Many important differences on the genetic and molecular level of development, in particular vulva development, have been identified (for review see Hong & Sommer, BioEssays, 2006). Intriguingly, *Pristionchus* also occupies a distinct ecological niche. *Pristionchus* nematodes are associated with scarab beetles and have a necromenic life style: that is, worms invade the beetle as dauer larvae and wait for the insect's death to feed on the developing microbes on the carcass. It has been suggested that a close association with other organisms as seen in necromenic nematodes represent a pre-adaptation towards true parasitsm.

The *P. pacificus* California strain was selected as reference strain for high-coverage sequencing and the Washington strain was selected for low-coverage sequencing because it interbreeds with the California strain. The assembly of the *P. pacificus* California genome attains 9-10 fold coverage and is divided into 2,894 supercontigs. It contains 145 Mb of unique sequence and calculates a total genome size of around 169 Mb. The Washington polymorphic strain differs from the reference strain on average in 4.3% of all un-gapped positions in a whole genome alignment.

With 169-Mb and 29,000 predicted protein-coding genes the *P. pacificus* genome is substantially larger than the genomes of *Caenorhabditis elegans* and the human parasite *Brugia malayi*. Comparative analysis with *C. elegans* revealed an elevated number of genes encoding cytochrome P450 enzymes, glucosyl-transferases and ABC transporters that were experimentally validated and confirmed. *P. pacificus* contains cellulase and diapausin genes and cellulase activity is found in *P. pacificus* secretions, the first time cellulases have been identified in nematodes beyond plant parasites. The increase in detoxification and degradation enzymes is consistent with the *Pristionchus* life-style and is a pre-requisite for parasitism. Thus, comparative genomics of three ecologically distinct nematodes offers a unique opportunity to investigate the correlation between genome structure and life-style.

Current studies involve developmental and genetic analysis of various traits associated with the specific ecological setting of *P. pacificus*, such as dauer formation, insect recognition by olfaction, mouth form dimorphism and bacterial association. The talk will provide an overview on recent studies in *P. pacificus* genomics, proteomics and ecology.

Comparative Genomics of *Meloidogyne*: Genome Reorganization on the Road to Plant Parasitism

Bird, D.McK., C.H. Opperman & the M. hapla Genome Annotation Team

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Meloidogyne spp. are the most damaging and economically significant plant-parasitic nematodes worldwide. We selected *M. hapla* for sequencing based on its small genome (54 Mbp) and established genetic system (sexually reproducing diploid) and have obtained a whole genome assembly that spans >97% of the *M. hapla* genome. Extensive automatic and manual annotation has revealed that *M. hapla* encodes ~14,500 genes. In addition to gene discovery, a major motivation for sequencing plant-parasitic nematode genomes is to understand the processes that have lead to adaptation to the parasitic life style. Comparison of the genome sequence of *M. hapla* with those of other nematodes, including the non-parasitic species *C. elegans*, has revealed a pattern of gene loss and gene gain reflecting selective pressures both on individual genes and also on more substantial chromosomal reorganization; the role of these processes in the evolution of the genus will be discussed. The completion of the *M. incognita* genome by Abad *et. al*., coupled with partial genomes from cyst nematodes and those of other animal-parasitic and free-living nematodes, provides a unique platform for comparative genomics among the plant-parasitic Nematoda.

Worm and Fly: What Whole Genome Comparisons Can Tell Us about Taste and Smell

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Caenorhabditis elegans has 32 chemosensory neurons and possibly as few as eight interneurons dedicated to processing chemosensory information[1]. In contrast, *Drosophila melanogaster*, which has the simplest olfactory system of any insect studied in depth, has more than 2,500 chemosensory neurons[2] and at least twice that number of interneurons[3] dedicated to processing chemosensory information. Neural networks that match the scale of the Drosophila mushroom bodies are capable of robust classification of many tens or even hundreds of input classes[4] but it is not conceivable that the nervous system of *C. elegans* has this level of integrative power. Despite theory suggesting that the lack of neural processing power will impose severe restrictions on the nematode's ability to identify and discriminate amongst chemosensory cues, the nematode displays a range of sophisticated chemosensory behaviours, raising the question of how this is achieved. Approximately 1,500 chemoreceptor genes have been identified in the genome sequence of *C. elegans*, a seemingly extravagant number compared with the 62 olfactory receptor genes and 60 gustatory receptor genes known from *D. melanogaster*. It may be that the nematode's larger repertoire of chemoreceptor genes reflects a radically different chemosensory architecture from insects and more complex animals. If so, it would lead to some precise predictions regarding the molecular pharmacology of nematode ORs. Furthermore, the real-time performance of even such a modest nervous system as that of *D. melanogaster* cannot yet be replicated *in silico* cost effectively. Therefore chemosensing strategies adopted by the nematode may provide some useful learnings for human engineers.

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SESSION TWENTY-FOUR – CLIMATE CHANGES, SOIL HEALTH MONITORING AND NEMATODE BIOINDICATORS

CONVENORS: ROY NEILSON & THOMAÉ KAKOULI-DUARTE

Nematofauna Analysis for Assessment of Soil Ecosystem Functioning in No-till *versus* Conventional Agricultural Systems

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Soil fauna through its activities driven by key species or by complex assemblages of organisms realizes several life-supporting functions such as the decomposition of organic matter, the mineralization and recycling of nutrients, the regulation of diseases and pests, the formation of soil structure. In conventional agriculture most of these functions are achieved through the inputs (structure and weed control: tillage -and herbicides-, availability of nutrients: mineral fertilizers, control of pests and disease: pesticides). In integrated or organic agriculture, parts of these functions are achieved by the biological actors.

The aim of our study was to determine how soil nematofauna reflect the soil biological functioning in cropping systems with or without tillage and with or without organic and/or mineral fertilizers.

During three years, we analyzed the effect of no-tillage and permanent cover crops on nematode functional diversity in two long-term (15 years and 9 years) trials in Antsirabé (Madagascar) under a subtropical climate (16°C, 1 200 mm y^{-1}) and in a clayey soil. Correlations between nematofauna and other soil organisms (micro-organisms and macrofauna) were assessed, as well as relations with soil physico-chemical parameters (mineral Nitrogen, C and N content, Nirs spectrum) in the rhizosphere, and crop parameters (yields, quality of the litters). The potential of indicators of soil functioning of different nematode functional groups and indices was demonstrated.

Nematode Indicators of Soil Quality in Forest to Pasture Conversion

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In New Zealand present and projected decline in the price of primary forest products has lead to conversion from forest to pastoral farming in many areas. Several decades of forest cover coupled with intrusive harvest operations, and mulching of residue wood material have produced a remnant soil that is acidic, contains toxic levels of exchangeable aluminum and low available nitrogen, very high C:N ratio and, is devoid of earthworms and structural integrity.

An experiment was established in April 2005 to determine the best regime of lime and nitrogen to be used for successful conversion. However, concerns grew of the short and long-term biological impact of such interventions to the conversion process. Use of worm counts as an evaluation of biological soil quality was precluded by the absence of earthworms. Nematode assessments became an attractive surrogate since several researchers provided conclusive evidence of the association of specific nematode groups and community structures with particular soil conditions and habitats. We hypothesized that an assessment of the nematode community would discriminate soil management practices (application of lime and nitrogen fertilizer) in a recently converted experimental pasture block.

The results indicate that lime (10 tons/ha) and nitrogen fertilizer (200 kg N/ha) did not influence nematode abundance or community structure at the conversion site after 2 years. A total of 22 nematode families were identified and placed into six feeding groups. Faunal assessment of the food-web condition also showed similarities between all treatments in the converted site. However, the converted land (whether treated or untreated) was very different from two reference sites (long-term pasture and a 60-year forest stand) located within a 500 m radius of converted site. This suggests that land use rather than lime or nitrogen fertilizer influenced the nematode community and probably other soil biological properties.

A Subtracted cDNA Library of Putative Chromium Influenced Genes: A Nematode Generated Resource in Ecotoxicology

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Nematodes constitute the most numerous phylum on the planet and are a valuable resource in determining the health of an environment. Covering wide ranging and diverse habitats, they have adapted to feed on almost any available food source. Therefore, they can be affected by and reflect food chain contamination at many levels.

The entomopathogenic nematode (EPN) *Steinernema feltiae* is ubiquitous in Ireland, and it can be easily isolated from the soils and cultured in the laboratory. At the Institute of Technology Carlow we have been investigating this nematode as a bioindicator for the contamination of soils with hexavalent chromium (chromiumVI). Subsequently, we have discovered that the nematode demonstrates considerable resistance to this chemical at high concentrations and that there appear to be tangible molecular effects associated with exposure to chromium VI. Chromium is a widely documented carcinogen affecting a wide variety of organisms including humans. It is widely used in industrial activities and is often disposed off inappropriately leading to environmental contamination.

Consequently, we have begun to develop a subtracted cDNA library that will contain genes expressed in *S. feltiae* in the presence of chromium VI. We envisage that this library will lead to the development of useful and reliable molecular markers that can be employed in the field as detectors of chromium induced molecular damage. This in turn will yield information about the levels of contamination and its effects in the ecosystem. In addition, this resource can be developed further with the development of 'universal' PCR-based molecular probes that can retrieve homologous genes from other organisms for similar purposes.

Nematode Biosensors to Identify Toxicants and to Discover their Antidotes

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The toxicity testing of food or environmental contaminants or the risk assessment of new drugs is generally both time-consuming and costly. Because today hundreds of thousands of new chemicals and drugs with unknown toxicities have been discovered or synthesized, fast and inexpensive methods are urgently needed to evaluate these compounds. Through DNA microarray and protein 2-DE screens for *Caenorhabditis elegans* genes up-regulated by the harmful food substance acrylamide[1], we selected four detoxification enzymes, GST-4 (Glutathione S-transferase), UGT-13 (UDP-glucuronosyl transferase), F55E10.6 (Short-chain type dehydrogenase, SDR), and C55A6.7 (SDR), for the construction of *gfp* fusion genes, which were used to transform *C. elegans* into biosensors[2]. These enzymes, conserved from bacteria to humans, function in the phase I and II xenobiotic metabolic pathways. A nematode biosensor with the *gst-4::gfp* fusion gene detects many food and environmental toxicants such as acrylamide, 3-MCPD, 1,3-DCP, and methyl mercury by emitting a GFP-expression signal in a dose- and time-dependent manner. Because the nematode biosensor uses the natural biological responses of a whole living animal to toxicants, we could rapidly test the toxicity of not only known but also unknown chemicals.

Furthermore, using the same biosensor we have developed a method to screen for substances that reduce the acrylamide-induced *gst-4* expression. When the biosensor was exposed to acrylamide together with commercially available green tea, GFP signal levels were reduced to the control level[2]. The method thus identified green tea as a candidate that might have reduced or inactivated the action of acrylamide, an oxidative-stress-producing xenobiotic.

[1] Hasegawa et al., (2008) Toxicol. Sci. 101: 215-225; [2] Hasegawa et al., (2007) Toxicol. Lett. 175: 82-88.

Molecular Nematology as a Tool for Soil Monitoring

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Climate change is now recognized as one of the most important challenges for the planet. Whilst the gross geophysical changes such as receding glaciers capture the headlines and are in relative terms simple to measure, the impact upon soil ecosystems is potentially more difficult to detect due to the resilient nature of soil. The combination of climate change and constant perturbation by agronomic practices may accelerate degradation of soils in agricultural zones with soil erosion for example an increasing issue.

Intensive land use has been implicated in declining soil health with concomitant concerns over sustainability of agronomic production. Monitoring soil health is problematic although soon to become a reality in the European Union due to forthcoming legislation.

Nematodes have been proposed as indicators of soil health due to their ubiquity, short generation times and trophic composition, however identification based on morphology is time consuming and challenging. Previously univariate and multivariate analysis of classical morphological data have been used to interpret nematode assemblage data with limited success.

Alternative molecular approaches to profiling soil nematode assemblages have been applied here, based on Terminal Restriction Fragment Length Polymorphism (T-RFLP) of small subunit ribosomal DNA. Two approaches are described, the first entailing digestion of fluorescently labelled PCR product with a single enzyme, combined with multivariate analysis of the resulting fragment profile. Application of this method on agricultural sites under differing management regimes revealed significant differences in assemblage composition by agronomic treatment. The second approach utilises a directed method where, from collected sequence information, a restriction digest has been designed to separate nematode taxa present at the study sites into terminal restriction fragments of known size. We envisage the resulting semi-quantitative profiles may be combined with existing nematode diversity indices and other soil (a)biotic data as a potential tool to monitor soil health.

Session Twenty-Five – Molecular Interactions in Nematode-bacteria Asssociations and Symbiosis

Convenors: Keith Davies & Aurelio Ciancio

Somaclonal Variation in Plant Parasitic Nematodes as Revealed by *Pasteuria*: The Potential Importance of Innate Immunity

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Pasteuria penetrans is an endospore forming bacterial parasite of root-knot nematodes (*Meloidogyne* spp.) that is host specific. Electron micrograph studies suggest that small fibril-like structures on the surface of the nematode cuticle are involved in attachment. Endospores can consistently attach to one population of nematode but not another and this represents a first order interaction. To look at this in more detail, endospore attachment studies were undertaken between clonal lines of juveniles of *M. incognita* and *M. hapla*, that reproduce by obligate parthenogenesis and facultative meiotic parthenogenesis respectively. Results revealed cuticle variability between second-stage juvenile broods of individual females derived from a single individual in both sexual and asexual nematode species. This suggests that second order mechanisms exist that produce functional differences in the cuticle surface that affect *Pasteuria* adhesion.

The IGF1 signalling pathway in mammals is related to the DAF2 pathway in *Caenorhabditis elegans* and influences, amongst other things, fecundity, longevity and the stress response. The DAF2 pathway, involved in dauer formation, is also important in the worm's innate immune system. Changes to the cuticle surface of *C. elegans srf* mutants have been shown to affect microbial adhesion and the worm's innate immune system has been implicated. Peptides inhibitory to IGF1 have been shown to alter *C. elegans* fecundity and longevity and were tested to see if they had any effect on the attachment of *Pasteuria* endospores to second-stage juvenile cuticle. Preliminary experiments suggest that the attachment of endospores to second-stage juveniles of root-knot nematodes exposed to peptides significantly affected attachment at 18 – 21 hours post-exposure. This result shows the potential importance of innate immunity in generating functional variation to the cuticle surface that affects *Pasteuria* adhesion.

Uncovering the Parasitic Interaction between *Meloiodgyne hapla* and *Pasteuria penetrans*

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Pasteuria penetrans is an endospore producing, Gram-positive bacterium that is an obligate parasite of root-knot nematodes (*Meloidogyne* spp.), which are themselves obligate parasites of plants. This bacterium has been associated with nematode suppressive soils and also shown to have tremendous potential for the biological control of root-knot nematodes. However, the fastidious nature of the organism and the lack of ability to perform forward genetic experiments have hindered our ability to make progress on culturing, host range expansion, and mass production techniques. With the completion of the genome sequences of both organisms, *Pasteuria penetrans* (Davies *et al*, 2008) and *Meloidogyne hapla* (Opperman *et al*, 2008) experiments investigating global gene expression, proteomics and indeed metabolomics studies of this parasitic interaction have become possible. Here we describe our experimental approach to studying these difficult organisms and present preliminary results from our studies.

Interactions between Nematodes and Rhizobia: from Proteomics to Plant Distribution

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The establishment of legumes depends on mutualistic interactions with rhizobial bacteria, but plant performance can be affected by plant-parasitic nematodes (PPN). Previous studies indicate that PPN may reduce nodule formation and that rhizobial strains can induce plant resistance against PPN. Nematodes and rhizobia share biochemical pathways of infection and interact in the rhizosphere, but the outcomes of their interactions for plant performance and distribution are still not clear. We aim to assess the role of these interactions in the distribution of legumes in their native and exotic habitats. The model legume *Medicago truncatula* and laboratory cultures of the root-knot nematode *Meloidogyne javanica* and the rhizobial bacteria *Sinorhizobium melioti* were used in controlled experiments. The axenically grown legumes were inoculated with PPN and rhizobia, alone or in combination, at distinct time lags (0, 1 and 3 days). Root and shoot fresh weight, nodulation and gall formation were determined after 3 weeks. The nematodes significantly stimulated nodulation and rhizobia significantly reduced nematode infection ($p<0.05$). In a parallel similar experiment, plants were harvested 3 days after the last inoculation, root samples were homogenised and proteins extracted for 2-DGE. Protein expression was analysed by Differential In-Gel Electrophoresis, gels were scanned on a Typhoon fluorescence UV scanner and data were analysis using the DeCyder software. A total of 141 protein spots showed differential expression patterns in different treatments (grouped per treatment and harvest age), with either reduced or increased expression as compared to control ($p<0.05$). Proteins of interest, putatively affected specifically by either PPN or rhizobia, were excised from gels and are being identified through mass spectrometry. We discuss the potential ecological consequences of the molecular interactions between PPN and rhizobia on plant performance in natural ecosystems and ultimately on the invasive potential of exotic legumes.

Production and Efficacy of *in vitro Pasteuria* spp Parasitizing *Belonolaimus longicaudatus*

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Production of *in vitro* endospores of *Pasteuria* spp. that parasitize *Belonolaimus longicaudatus* began with collecting primary isolates from field soil. Sting nematodes encumbered with spores were handpicked, surface sterilized and placed into culture media in 6-well plates. The nematode bodies were immediately crushed to release cells. Cultures were incubated for 2 days to check for contamination and transferred to shaker flasks with more media added and allowed to shake for an additional 2-3 days. The cells from the shaker flasks were transferred to 500 ml fermentation reactors and grown for 3 or more days. When cell count was between 10^6-10^7 cells/ml cultures were transferred to a 20 liter fermenter and allowed to grow to 10^9 cells/ml , at which time sporulation was induced. Efficacy of *in vivo* produced spores was tested for attachment and infection rate. The outer exosporium spore coat was removed by passing the spore suspension through a French Press. The spore attachment rate was compared to *P. penetrans* (*in vivo* spores) on root knot nematodes using a centrifuge technique. The sting nematode *in vitro* spore attachment rate (100,000 spores/tube) averaged 89% compared to 100% for *in vivo* root-knot. *In vivo* spore encumbered sting were used to determine the infection rate. Spore encumbered nematodes were placed in clean moist sand at room temperature 23 C for 48 hours. Nematodes were retrieved, surface sterilized, and placed in media as described above. Approximately 48 hours later, wells were observed for presences of *Pasteuria* spp. cells and sporulating structure. Spore encumbered sting nematodes had an average of 100% infection. Presently, efficacy of spore penetration, attachment, and infection in soil is being tested.

Molecular Mechanisms of Symbiosis Establishment in Marine Nematodes

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Laxus oneistus is a marine nematode whose cuticle is obligatorily coated by sulfur-oxidizing bacteria. It lives few centimetres below the sea bottom, where it migrates between upper sand layers and deeper ones. These migrations allow the bacteria to alternatively obtain oxygen and sulfide. In turn, the symbionts are the main component of their host diet. We are interested in the molecular mechanisms of symbiont recruitment from the environment by newly hatched or moulted worms. By homology cloning, we identified Mermaid, a Ca^{2+}-dependent mannose-binding protein. This lectin is secreted onto the worm's cuticle and localizes to the symbiont coat. Mermaid mediates both bacteria-bacteria and worm-bacteria attachment and even shows striking structural and functional similarities to a human dendritic cell-specific immunoreceptor. To identify other genes involved in symbiosis, we created an EST collection and screened for abundantly expressed transcripts. 21% of the ESTs corresponds to a gene encoding for a novel, secreted, extremely hydrophilic protein which we provisionally called Pesto. Pesto localizes to the exit channels of the sub-cuticular glands which also secrete Mermaid, but does not localize to the bacterial coat. Preliminary results show that Pesto blocks Mermaid-mediated bacterial agglutination, possibly by chelating Ca^{2+}. The biological significance of this, as well as the function of other abundantly expressed transcripts, is currently under investigation.

SESSION TWENTY-SIX – MOLECULAR APPLICATIONS IN DIAGNOSTICS AND NEW NEMATODE THREATS

CONVENORS: ANDREA SKANTAR & SERGEI SUBBOTIN

Progress and Challenges in the Molecular Identification of Plant-parasitic Nematodes at the USDA ARS Nematology Laboratory

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One mission of the USDA Agricultural Research Service Nematology Laboratory in Beltsville, MD is to provide nematode identifications that are urgently required by ARS scientists, federal and state researchers, and regulatory agencies for research, regulatory actions, and control purposes. In addition to traditional morphology-based taxonomic approaches, molecular methods are often required to confirm diagnoses or to provide conclusive identification in those instances when species morphology is ambiguous or when microscopic examination is impractical to perform. Here we will highlight current approaches, challenges, and new developments in molecular identification of plant-parasitic nematodes within the context of some recent diagnostic scenarios involving *Globodera, Meloidogyne*, and *Pratylenchus* spp.

Specific Diagnostic and Genetic Variability of *Meloidogyne mayaguensis* Isolates Revealed by Molecular Markers

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Meloidogyne mayaguensis is an extremely polyphagous root-knot nematode. Disease caused by this species is a matter of grave concern in Brazil where it is responsible of serious economic damages on guava crops. Based on sequence analysis of a highly repetitive satellite DNA family characterized in the genome of *M. mayaguensis*, primers were developed that provide a specific and very sensitive PCR diagnostic methodology. In order to further analyze the intraspecific genetic variability of the nematode, sixteen *M. mayaguensis* isolates from different geographical regions and hosts were compared using a variety of biochemical and molecular markers. All the isolates showed the same phenotype based on either isoesterase electrophoresis or amplification of. ribosomal and mitochondrial DNA particular regions. Further analysis with different neutral molecular markers (RAPD, ISSR and AFLP) showed a high level of homogeneity among the isolates, and resulted in very similar clustering of the isolates in the individual trees obtained with each marker. Although this root-knot nematode displays a very wide host range and worldwide distribution, these molecular data confirm that *M. mayaguensis* is genetically a very homogenous species, in agreement with its mitotic parthenogenetic mode of reproduction.

Molecular Characterization and Diagnosis of the Important Plant Parasitic Nematodes in China

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The soybean cyst nematode *Heterodera glycines,* potato rot nematode *Ditylenchus destructor,* root-knot nematodes *Meloidogyne* spp. and dagger nematodes *Xiphinema* spp. are major indigenous nematode pests in China. *Bursaphelenchus xylophilus* and *Radopholus similis* are considered to be introduced to the country. Molecular methods using PCR are successfully applied for rapid, fast and reliable diagnostics of several of these pests. During the last years we developed PCR-ITS-RFLPs for the identification and detection of *B. xylophilus, R. similis, H. glycines, D. destructor, Meloidogyne* spp. and *Xiphinema* spp. in China. Duplex PCR procedures with species-specific primers amplifying ITS-rRNA fragment were designed to detect *B. xylophilus* and *R. similis*. SCAR PCR (sequence characterized amplified regions) was successfully used for detection of *H. glycines*. Comprehensive sequencing and RFLP analysis of the ITS-rRNA from 21 populations of *D. destructor* from sweet potatoes in China and one Korean population from garlic revealed at least two types of the ITS-rRNA. Results of this study allowed developing the duplex PCR with specific primers distinguishing between type A and type B of ITS-RNA for *D. destructor*. Present and future projects target at the development of new molecular diagnostics tools for other nematodes important for Chinese agriculture.

The Application of Molecular Diagnostics in the U.S. Cooperative Agricultural Pest Survey Program

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The Cooperative Agricultural Pest Survey Program in the U.S. is a federally funded program that targets specific exotic pests for regulatory action. Phytosanitary certification is issued by state agencies once it is determined that production fields and the agricultural commodity is free of regulated pests. The species-level identification required by the program provides challenges to the nematode diagnostician. Molecular techniques add resolution to identifications, but also focus attention on specific problems. These include the determination of species boundaries, the need to characterize closely related non-target species, mixed-species field populations, and lack of relevant molecular information from many exotic species. Examples derived from 2002-2007 laboratory analysis will be presented with special emphasis on identification of *Meloidogyne* and *Heterodera* species.

Session Twenty-Seven – Nematode Management in Protected Cultivations Systems in Temperate Agriculture

Convenors: Brent Sipes & Antonio Bello

Plant Growth-Promoting Rhizobacteria (PGPR) in Transplant Mixes: Benefits for Nematode Control

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Many high-value crops including vegetables, melons, and strawberries are propagated from transplants. In the U.S., plug transplants are typically used for vegetables and melons, while strawberries are predominantly planted from bare-root material. However, use of strawberry plug transplants has the potential to eliminate the need for fumigation of soil in strawberry nurseries, where it has been difficult to identify alternatives to methyl bromide that do not negatively impact runner plant production. Plug transplants also allow for the introduction of biological agents such as plant growth-promoting rhizobacteria (PGPR) into the planting medium. PGPR induce systemic resistance and/or increase tolerance to pathogens in the host plant, resulting in increased plant growth and yield. In experimental field trials conducted in Florida, two Gram-positive PGPR isolates (*Bacillus subtilis* strain GBO3 and *B. amyloliquifaciens* strain IN937a) in a formulation containing chitin, reduced galling caused by *Meloidogyne incognita* and improved root condition of pepper (*Capsicum annuum*) and muskmelon (*Cucumis melo*) when added to transplant medium at seeding. In subsequent field trials on pepper, the population dynamics of the same PGPR strains were monitored throughout the growing season and it was determined that both PGPR strains established stable populations in the rhizosphere that persisted through harvest. Additional aqueous applications of PGPR during crop production did not increase the populations of applied strains compared to treatments only receiving bacteria in the potting medium. However, in-field applications did increase plant growth compared to the untreated control. Additional advantages of PGPR-amended plug transplants include improved stand establishment and vigor, and earlier flowering and fruit set. It has also been demonstrated that under stress, PGPR amended plug transplants produce higher yields.

Characterization and Management of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Nematoda: Meloidogynidae) Biotypes in Spain

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The distribution of *Meloidogyne arenaria* in Spain was revised and new samples were taken in Central Spain in areas where grapevine is the main crop and this species happened to be the main root knot nematode. In order to characterize the race and biotypes of the populations, morphometric, differential host plant test and SCAR-PCR have been carried out. A total of 120 citations were found, corresponding to 45 different host plants, of which 42 citations (35.0%) are new from this study. The populations studied belonged to race 2, which reproduced on tomato plants carrying the *Mi* gene, or race 3 that reproduces on both resistant pepper and tomato. The nematodes were mainly found in vegetables, fruit trees, tobacco, grapevine, and weeds. The integrated crop management for nematode control in protected cultivation systems and open field and is proposed. The species shows a preference for warm areas, but it can appear on the Northern area of Spain under greenhouses.

Evolution of Control Strategies for the Citrus Nematode, *Tylenchulus semipenetrans,* in South African Citrus Orchards

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The citrus nematode, *Tylenchulus semipenetrans,* is the only major nematode pest that causes significant economic losses in all citrus producing regions in Southern Africa. Chemical control of the citrus nematode forms part of an integrated pest management approach that is currently been utilised by the South Africa citrus producers. Nematicides have been used effectively for several years to reduce citrus nematode populations in the soil and roots of citrus trees. Three applications of cadusafos applied in one season reduced nematode female population counts to undetectable levels for a period of three years. Other nematicides such as aldicarb, fenamiphos, ethoprophos and fosthiazate were also effective but annual follow-up applications were needed for effective control. Variable results were, however, reported by citrus growers. Insufficient irrigation, poor application of the products and accelerated microbial degradation were identified as the three main factors affecting the efficacy of the registered nematicides. Worldwide concerns of mammalian toxicity due to the use of extremely toxic nematicides motivated researchers to look for safer and more cost effective measures for the control of nematodes. Sole or integrated use of less toxic, biological and GRAS chemicals were therefore evaluated. In general, products such as PL+ and furfural were less effective than the registered nematicides which necessitated more frequent applications and/or integration with nematicides in programmes. Further research will incorporate these and other products in an integrated root health approach.

Host-plant Resistance in Crops Cultivated in Protected systems

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In Spain the most important crops grown in protected systems are tomato and pepper. Among the possible non-chemical control strategies, the use of resistant cultivars is preferred, but in order to be successful it is necessary to characterize the virulence range of the nematode population. Pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) cultivars were chosen for this study due to their economic importance, and also because there are few crops in which nematode resistance is available. The dominant resistance gene *Mi* in tomato confers resistance to *Meloidogyne incognita*, *M. arenaria* and *M. javanica* but not to *M. hapla*. Different populations of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* from representative horticultural regions of Spain were evaluated using a bioassay designed to characterize the virulence. Seventy four percent of the *M. incognita* populations that were virulent on resistant tomato did not parasitize the resistant pepper cvs. In the case of resistant peppers none of the of *M. arenaria* race 2 or *M. javanica* populations parasitized any of the resistant pepper cultivars used, but all of the *M. hapla* populations reproduced on resistant peppers. Fortythree populations were found to parasitize both susceptible and resistant pepper cultivars, of those, 37 populations belonged to *M. incognita* (all races), one to *M. arenaria* (new race 3), and five to *M. hapla* races A and B. Seventeen of the *M. incognita* populations that were virulent on resistant pepper did not parasitize the resistant tomato cv Nikita containing the *Mi* gene. The results also demonstrate that there exists resistance gene breaking populations of *Meloidogyne incognita*, *M. arenaria* and *M. javanica* throughout Spain and those local nematode populations should be screened before planting such resistant varieties. The results obtained have important implications for the design of alternative nematode management strategies using resistant cultivars.

Disinfecting Planting Material of Nematodes

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Many crops cultivated in protected systems are vegetatively propagated. This propagation method can lead to unintentional transfer of plant-parasitic nematodes and exacerbate nematode damage in the new planting. Several options can be utilized to stop the continual reintroduction of nematodes into new plantings. Tissue culture eliminates nematodes like *Meloidogyne javanica* and *Radopholus similis* from anthuriums. Tissue culture is available for many but not all plants. Heat treatments are effective across a range of plants and nematodes. In anthurium, a 12 minute bath in 49̊C eliminated *R. similis* from plants. However, heat treatments must be adjusted for each crop because damage can occur at high temperatures or with lengthy exposure times. An other option to disinfect planting material of nematodes is to used nematicides. Crops cultivated in protected environments may be grown in individual pots and therefore lend themselves to dip treatments. Foliar nematodes were eliminated from orchids after a single dip in recommended rates of Avid or Pylon. As high value crops, many options are viable to eliminate plant-parasitic nematodes from planting material used in protected cultivation systems.

Control of *Meloidogyne incognita* on Tomato Grown in Artificial Substrate, using Bio-rational Pesticides

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To avoid soil-borne pests and diseases in greenhouse production systems, crops can be grown in different types of soilless artificial substrates. Although providing a clean substrate at planting, there is a serious risk of spreading diseases or pests that are introduced into the system throughout the culture, because irrigation water is commonly re-circulated. Little is known about the suitability of artificial substrates as an environment for plant-parasitic nematodes. However, we recently encountered a severe infestation of root-knot nematode (*M. hapla*) on roses grown in glass wool (Rockwool™) mats. A study was initiated to evaluate the efficacy of several biologically based (bio-rational) products to control root-knot nematodes post-plant in glass wool. Tomato and *M. incognita* were used as a model system. Of the products tested, abamectin was most effective in controlling *M. incognita* infestation of tomato, but only when applied shortly after nematode inoculation. Soaking transplants in a product containing Neem tree extracts, 1 week before transplanting into nematode inoculated glass wool blocks, also reduced infestation. Only the oxamyl control treatment reduced nematode numbers and tomato root-galling in an established infestation.

Chemical Controls for Nematodes in Protected Cultivation in Israel

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Several effective soil fumigants and nematicides were developed between 1940 and 1970. Without a doubt, these chemicals were instrumental in ensuring food supply for the growing human population during the last half century. However, several effective nematicides have been withdrawn due to environmental and food-safety considerations. Since the phase out of methyl bromide, the most effective soil fumigants for a wide range of soil-borne pathogens and weeds, alternative chemicals and control methods have been evaluated for their effectiveness in control of nematodes and other soil-borne pathogens. Only a small number of new nematicides and soil fumigants have been released or are now in the registration process. In Israel, combinations of these few chemicals are currently used for control of nematodes in protected cultivation of crops including: tomato, bell pepper, cucumber, herbs and flowers. These crops are often attacked by root-knot nematodes, mainly *Meloidogyne javanica*. The most common strategy is pre-planting application of methyl isothiocyanate (MIT) or 1, 3-D based soil fumigants combined with non-fumigant nematicide application during the growing period. Formalin, which may suppress biodegradation of fumigants, is sometimes used to enhance the efficacy of MIT based fumigants. However, soil fumigation alone does not always keep nematode populations below threshold levels until the end of growing periods. Non-fumigant nematicides based on organophosphates or carbamates, such as fenamiphos, cadusafos and oxamyl, are applied during the growing season, if allowable according to chemical residue levels. A new nematicide belonging to the fluoroalkenyle group has been tested for its efficacy against *M. javanica*. The nematicide kills the nematode rather than nematostatic action, and is in the registration process. Registration of methyl iodide, a promising soil fumigant with broad-spectrum control of soil-borne diseases, weed seeds, insects and nematodes, is to be initiated this year.

Session Twenty-Eight – Panel Discussion: Understanding Challenges to Creating Bridges with Developing Regions

Convenors: Julie Nicol & Haddish Melakeberhan

Understanding Challenges to Creating Bridges with Developing Regions

Melakeberhan, H. (1) & *J. Nicol* (2)

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The goal of this colloquium is to build more two-way bridges between developed and developing nations through understanding global issues and collaborative processes for the benefit of all. Globally, research funding has become increasingly competitive and often requiring cross- and multi-disciplinary collaborations. This is true with governmental and non-governmental organisations (NGO). Due to obvious economic, prioritization and other restrictions, the participation of nematologists from developing regions in collaborative projects or sharing their work at international meetings is limited. Thereby, affecting developed nations' nematologists ability and desire to interact with the developing world as well. With many intertwined global issues affecting developed and developing regions, the will to help and to collaborate for developing global actions that benefit all have never been greater. For example, nematodes' abundance (80% of multi-cellular animals on the planet) provides nematologists unique opportunities to obtain funds from many agencies including those focused on global warming issues. However, meshing the North-South's multi-dimensional needs with the different organisations' mandates and timing to fund short- and long-term collaborative projects has many obstacles. For example, many of the developed countries have some forms of international research components administered separately or as part of within their national research programs. However, tapping into opportunities within many of the funding agencies requires in depth understanding of their mandates and timelines. In order to facilitate such collaborative projects the following topics will be discussed: Knowing NGO's priorities (Julie Nicol, CIMMYT-Turkey), government agencies' mandates and research priorities (David Chitwood, USA), developing regions voices and needs (Herbert Talwana, Uganda and others), lessons learned in the process of fund raising (Haddish Melakeberhan, USA), private industry perspectives (Prem Warrior, USA), and ways forward from here on (Vivian Blok, UK). We anticipate developing a working document that will lead to better collaboration bridges between developed and developing regions' nematologists.

SESSION TWENTY-NINE – SOIL FOOD WEBS AND RHIZOSPHERE COMPLEXITY

CONVENORS: LILIANE RUESS & BRYAN GRIFFITHS

Multitrophic Interactions in the Rhizosphere Shaped by Root-feeding Nematodes

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Plant parasitic nematodes, often referred to as 'hidden enemies', are among the most widespread and important herbivores causing crop loss. They influence host plants mainly directly by their carbon demand. These primary effects expressed at the cellular, tissue and whole plant level have been extensively investigated. However, indirect nematode-plant interactions may be at least as important as direct ones, as:

1. Root feeders can facilitate the allocation of photoassimilate carbon to roots, resulting in higher exudation rates.
2. Nematode induced leakage of plant metabolites from damaged root cells may increase carbon translocation to the soil microbial biomass.
3. The enhanced carbon supply to the rhizosphere likely promotes microbial populations, and fosters mineralisation processes and plant growth.

This indicates a tight link between root-feeding nematodes and soil microorganisms, and parallels can be drawn between nematode-plant and microbial-plant interactions. Moreover it implies that the activity of root feeders imposes similar mechanism on microbial turnover than does microfaunal grazing. Recent studies support these hypotheses. They revealed additional indirect effects such as changes in root morphology, which modified microbial communities in the rhizoplane. Overall, these multitrophic interactions in the rhizosphere were dependant on the specific nematode-plant association and the developmental stage of the nematode in the host.

What Nematodes Do for the Microbial Loop in the Rhizosphere

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Conceptually the hypotheses for signaling and positive feedback loops in the rhizosphere are well advanced. The stimulation of bacterial growth by root exudation attracts bacterial-feeding fauna, of which nematodes are a significant component. Grazing causes a change in the rhizosphere microbial community structure and is specifically thought to encourage the activity of hormone producing bacteria around the roots. The subsequent stimulation of root growth and lateral root initiation leads to a positive feedback – more exudation stimulates more bacteria and leads to more fauna, etc. The practical demonstration of these phenomena is harder to come by. Most convincingly shown for protozoa in gnotobiotic systems, recent studies with bacterial-feeding nematodes in field soil with mixed microbial communities will presented and discussed.

Soil Nematode Communities under Climate Change

Kardol, P.

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Through their role in decomposer food webs and plant root / rhizosphere interactions, nematodes play an important role in climate change effects on ecosystem functioning. I used the long-term multi-factor Old-Field Community Climate and Atmosphere Manipulation (OCCAM) experiment to examine single and interactive effects of elevated CO_2, temperature, and water availability on soil nematode community composition and diversity. The OCCAM experiment consists of constructed plant communities (including C3 and C4 grasses, forbs, and legumes) in open-top chambers. After four years, it was found that the relative importance of single factor vs. interactive effects on abiotic and microbial soil properties varied among years and depended on the response measured. Importantly, plant species differed in their responses to the climate change factors, resulting in changes in plant community composition. Such shifts in plant dominance patterns, which can affect quantity and quality of rhizodeposition and litter input to the soil, probably have much larger effects on soil nematodes communities than direct effects of climate change factors. In each chamber, I collected bulk soil samples as well as soil samples under the two dominant plant species, *Lespedeza cuneata* (an exotic legume) and *Festuca pratense* (a native grass), whose cover were significantly affected by the climate change treatments and thereby reflect the changes in plant community composition. Preliminary results show that, in general, number of nematodes were lower in dry than in wet treatments and were lower under *Lespedeza* than under *Festuca*. [CO_2] and temperature had less effect. Multivariate analyses indicate plant species × treatment interactions, which suggest that direct effects of climate change on soil nematode communities can depend on, or be overruled, by changes in the plant community composition. I will discuss the latest results using a conceptual framework comprising plant community dynamics, rhizosphere interactions, and decomposer food webs.

Tardigrade-nematode Interaction: Predator and Prey Behaviour and their Impact on Feeding Rates and Prey Numbers

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Many predators prey on nematodes, but little is known about the strength of these predator-prey interactions and the regulatory top-down influence on nematode species composition and population growth. In order to provide insights into the structuring forces of predator – nematode interaction, we studied a tardigrade (*Macrobiotus richtersi*) - nematode (*Pelodera teres, Acrobeloides nanus*) system in detail, quantifying feeding rates, environmental influences and the importance of predator and prey behaviour. Intensive laboratory and microcosm investigations revealed that the tardigrade is an efficient predator of nematodes, a single adult consuming up to 104 juvenile nematodes in four hours. Tardigrade feeding rates were influenced by body sizes of predator and prey, nematode densities, temperature, substrate type and environmental richness. Additionally, specific behaviour of both, predator and prey, affected consumption rates and prey numbers: 1) Food-rich conditions as well as satiation led to wasteful feeding, i.e. low exploitation of single prey item. As a consequence, for the same biomass uptake tardigrades reduced prey numbers up to three times as much as under food-poor conditions. 2) Vigorous defence behaviour of the prey *Pelodera teres* reduced feeding-rates, but on the other hand it increased the number of nematodes that were killed by the tardigrade. In order to correctly interpret predator-prey interaction in the field and to assess the actual reduction in prey numbers caused by a predator, it is therefore decisive to consider animal behaviour as well as environmental factors.

Modeling Soil Properties and Organisms' Interactions by Food Webs

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In the Netherlands, we developed a biological indicator for the sustainability of soil use and ecosystem resilience, based on belowground food-web interactions and ecological processes of microorganisms, microfauna and mesofauna. We wish to introduce a new approach to the diversity-stability debate using a method for complete comparison of the structure and functioning of allometrically-scaled food webs, properties of soil systems and environmental impact at different locations.

We evaluated the biodiversity, multi-trophic dynamics, trait distribution, and numerical abundance of the belowground community food webs using non-metric multidimensional scaling and cluster analysis, and investigated the influence of management regime on the nematofauna with multiple regression analysis and Mantel tests. Soil characteristics did not differ significantly among the grasslands on sand, or between mature, highly-productive, and undisturbed sites. In contrast, at all sites the taxocenes react in a significantly different way to nutrient supplies and soil chemistry.

Our soil food webs, plotted according to a new methodology, show that prey and predators fall near a diagonal where the mean slope of their body-mass ratio as function of their specific abundance-ratio equals – ¾. As this negative scaling with body size is nearly universal, we can easily predict by metabolic scaling theory the cumulative temperature-dependent respiratory rate for multiple invertebrates within the two independent energy pathways: the fungal channel and the bacterial channel. With this method, the decrease in the biodiversity and populations of microbial-grazing nematodes under specific environmental conditions can be easily illustrated by a simple comparison of regression slopes. Even the existence of different patterns between community food webs appears to be in clear agreement with functional differences at higher trophic level between species-poor and species-rich soil systems.

Session Thirty – Role and Expression of Nematode Parasitism Genes

Convenors: Eric Davis & Ann Burnell

Hookworm Genes and the Infectious Process

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Third-stage larvae (L3) of the canine hookworm, *Ancylostoma caninum*, undergo arrested development preceding transmission to a host. Many of the mRNAs up-regulated at this stage are likely to encode proteins that facilitate the transition from a free-living to a parasitic larva. The initial phase of mammalian host invasion by *A. caninum* L3 (herein termed 'activation') can be mimicked *in vitro* by culturing L3 in serum-containing medium. The mRNAs differentially transcribed between activated and non-activated L3 were identified by suppression subtractive hybridisation (SSH). The analysis of these mRNAs on a custom oligonucleotide microarray printed with the SSH expressed sequence tags (ESTs) and publicly available *A. caninum* ESTs (non-subtracted) yielded 602 differentially expressed mRNAs, of which the most highly represented sequences encoded members of the pathogenesis-related protein (PRP) superfamily and proteases. Comparison of these *A. caninum* mRNAs with those of *Caenorhabditis elegans* larvae exiting from developmental (dauer) arrest demonstrated unexpectedly large differences in gene ontology profiles. *C. elegans* dauer exiting L3 up-regulated expression of mostly intracellular molecules involved in growth and development. Such mRNAs are virtually absent from activated hookworm larvae, and instead are over-represented by mRNAs encoding extracellular proteins with putative roles in host-parasite interactions. One family of proteins inparticular, the Ancylostoma Secreted Proteins (ASPs), has undergone enormous expansion in the hookworm genome and seventeen of the thirty most highly upregulated genes encoded for ASPs. The functions of ASPs are mostly unknown, but structural and modelling studies show a large peptide-binding groove which likely accommodates host-derived ligands. Although the data presented here should not invalidate *C. elegans* dauer exit as a model for hookworm activation, it highlights the limitations of this free-living nematode as a model organism for the transition of nematode larvae from a free-living to a parasitic state.

Plant Parasitic Nematode: Restricted Insertions and Deletions Inferred from Nematode Transcriptomic Data

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The wealth of sequence information from nematodes enabled us to perform a systematic comparative analysis of coding sequences with a focus on insertion and deletion restricted to nematode proteins. Insertions and deletions are rare events but they may affect proteins' structure and function. In this study we analyzed insertions and deletions in over 2,000 protein families, of which ~1,000 contained insertions and deletions restricted to nematode-originated proteins (studied deletions and insertions ranged in size from 5 to over 100 bp). Insertions and deletion were distinguished by utilizing homologous sequences from other metazoan and fungi. Overall, there were more deletions than insertions, and there are indications that clade V members have undergone an insertion/deletions burst. Furthermore, the majority of insertions and deletions were generated after the divergences of the major 4 studied clades, but most of the insertions shared by more than one clade were observed on the most common ancestor of the Phylum Nematoda. There were three sizable deletions unique to and shared by all parasitic nematode proteins sequenced. All protein families were functionally classified in pathway context. Proteins involved in multiple cellular pathways tend to have more deletions and insertions. The insertions and deletions shared by plant parasitic nematodes are biased to proteins involved in metabolism, while those proteins involved in cellular pathways shared deletions but no insertions. The 26 protein families that contained a total of 31 insertions and deletions (using our cut-off) restricted to plant parasitic originated proteins will be discussed in more details.

Genes Expressed during the Early Stages of Infection by the Entomopathogenic Nematodes *Heterorhabditis bacterioprhora* and *Steinernema carpocapsae*

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The dauer juvenile stage (DJ) stages of the entomopathogenic nematodes (EPN) *Heterorhabditis* and *Steinernema* are adapted for dispersal, host finding and infection. Once inside the infected insect the DJs release cells of a bacterial symbiont which they carry in their intestines. The genome of *Photorhabdus luminescens*, the symbiont bacterium of *Heterorhabditis bacteriophora* has been sequenced (Duchaud *et al.,* 2003). Analysis of this genome indicates the importance of this symbiont's role in insect killing and bioconversion of the insect cadaver into a suitable food medium, as it encodes a large number and variety of toxins and lytic enzymes. The genome of *H. bacteriophora* is currently being sequenced (http://genome.wustl.edu/genome.cgi/). *H bacteriophora* needs its symbiont (*Photorhabdus luminescens*) to kill its insect host, but axenised *Steinernema carpocapsae* are able to infect and kill insects unaided. Thus it seems that *S. carpocapsae* produces the necessary insecticidal toxins independently of its bacterial symbiont. The genome of *Xenorhabdus nematophila* the bacterial symbiont of *S. carpocapsae* is currently being sequenced (http://xenorhabdus.danforthcenter.org/), but no *Steinernema* genome sequencing project has been initiated to date. We tested the ability of *S. carpocapsae* strains from around the world, when cultured axenically, to kill insect larvae. The most virulent was found to be the Breton (France) isolate. We constructed a cDNA library from *S. carpocapsae* DJs 4 hours post infection. We sequenced 4608 ESTs from this library. Abundant and novel nematode sequences also some proteases and elastase that have predicted signal peptides were selected from this dataset for further investigation. The entire library of 15,360 clones was spotted onto high density filters. These have been probed with radiolabelled cDNA from Breton strain 4 hours after recovery and also with cDNAs from non-recovered Breton DJs and from an avirulent strain of *S. carpocapsae*. In our presentation we will provide a summary of these analyses.

Parasitism Genes of Root-Knot and Cyst Nematodes

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Root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.) secrete the protein products of parasitism genes (PG) expressed within their two subventral and single dorsal esophageal gland cells from their stylet into plant tissues during host infection. Over fifty candidate PG have been identified from both root-knot and cyst nematodes that are differentially regulated in expression during host root penetration and subsequent transformation of plant root cells into elaborate multinucleate feeding sites. The majority of PG encode proteins novel to either cyst or root-knot nematodes, consistent with the differential ontogeny of feeding cell formation between the two nematode groups. A battery of PG that encode cell wall-modifying proteins expressed during migratory parasitic stages is common among root-knot and cyst nematodes, including evidence that the origins of these genes represent ancient horizontal gene transfer. The model plant species, *Arabidopsis thaliana*, has been useful for functional analyses of root-knot and cyst nematode PG, including confirmation that a *Heterodera glycines* PG product can mimic the function of the endogenous plant signaling peptide CLAVATA3 that is involved in plant cell differentiation. Both overexpression of PG in Arabidopsis and plant host-derived RNA interference (RNAi) targeted to nematode PG are providing an assessment of the biological significance of PG products in nematode parasitism of plants. For example, the root-knot nematode 16D10 PG encodes a novel 13-amino acid peptide that induces accelerated root growth when overexpressed in Arabidopsis and interacts with a specific domain of SCARECROW-like plant transcription factors. Plant host-derived RNAi targeted to the 16D10 PG resulted in dramatic reduction in number of females of the four major species of root-knot that developed on plant roots, suggesting an essential role of this nematode PG product in the parasitic interaction and a potential means of developing broad-spectrum resistance to root-knot nematodes in multiple crop species.

Expression Profiling and the Search for Parasitism Genes in Animal Parasite Nematodes

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The nature of the genetic basis of parasitism in nematodes, and the identity of "parasitism genes" that may have been subjected to selection during the evolution of parasitism, remain unknown. Identifying those genes is of both academic and practical significance. Furthermore, the hypothesis that parasitism has most likely evolved independently several times in nematodes raises the question whether these separate events have followed similar or different paths i.e. has the same set of parasitism genes been selected in different parasites? In animal parasites, a longstanding hypothesis is that the dauer pathway from free-living nematodes played an important role in the evolution of parasitism. We have examined this hypothesis by cloning and characterising the expression of some of the key dauer genes from parasites of the genera *Strongyloides* and *Parastrongyloides*, and have sought more broadly for candidate parasitism genes by microarray comparisons of gene expression between the parasitic and non-parasitic life cycles. In general, these data support a role for dauer genes but have so far failed to discover novel genes that may required for parasitism in these two genera.

Session Thirty-one – Molecular and Applied Systematics and Taxonomy

Convenors: Alex Holovachov & Manuel Mundo-Ocampo

Molecular Systematics of the Order Tylenchida: From Ribosomal RNA Genes to Genome Analysis

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The order Tylenchida (*sensu* Siddiqi, 2000) includes the largest and most economically important nematode parasites of plants, insects and mites, as well as mycophagous species. The wide range of taxonomic problems, from the level of species to suborder, using classical morphological analysis, is well known. Many of these problems have been addressed and solved by molecular analysis and new insight in to phenotypic evolution. These molecular approaches, taking into account ribosomal genes (D2-D3 of 28S rRNA, 18S rRNA and ITS-rRNA), are reviewed. The importance of the congruence of extant morphologically and biologically-based tylenchid classifications with molecular phylogenies, and tests of monophyly of some superfamilies, families and genera is emphasized. Hypotheses concerning the ancestral form of tylenchids and origins of specialized sedentary parasitism also have been tested with these molecular approaches. Several examples of estimating species boundaries for plant parasitic nematodes using molecular methods are given. It appears that multiple gene sequence and phylogenetic analysis could provide a rigorous system to critically examine species boundaries originally proposed on traditional morphological/taxonomic approaches. The forthcoming results of the genomic projects for tylenchids will have a profound impact on inquiries at all taxonomic levels, providing better tools for estimating phylogeny and population structure and ultimately for allowing the identification of genes and mechanisms which regulate the evolution of plant parasitism.

Nomenclator Nematologicus: An Online Source of Nomenclatorial Information

Holovachov, O. (1), G. Karssen (2, 3), P.A.A. Loof (2), V. Demchuk (4) & T. Bongers (2)

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For taxonomical purposes it is important to know which species are described in a given taxon, the status of those species, references to the most recent identification keys and references to descriptions which appeared after the most recent key was published. For that reason, Dr. P.A.A. Loof of the Wageningen University started to register all new species descriptions and nomenclatorial changes of terrestrial, freshwater and marine nematodes, excluding animal parasites; and his efforts are now taken over by Dr. G. Karssen. This unique nomenclatorial data system refers to the name of a genus, the type-species of that genus, species included, and identification keys for the species. At species level information is available regarding all nominal species, references, authors, original name, additional descriptions, synonyms, homonyms, status and opinions. Up to this year the data were available as a card file only for those working or visiting Wageningen University. The next step was to upgrade the data system to be electronically available on the World Wide Web so that new homonyms caused by overlooking existing names will be things of the past, and to offer every author, describing a new *Aphelenchoides*, *Eudorylaimus*, *Rhabditis* or member of another taxon the possibility to compare the specimens at hand with all hitherto described nominal species, including the doubtful ones, and to provide them of a list with all occupied names. This year the online version of the database will be released for free access under the name 'Nomenclator Nematologicus'. The only way to keep this system up to date is to check the literature regarding new species descriptions and nomenclatorial changes weekly. Therefore we would be very pleased to receive reprints of new species descriptions, additional morphological descriptions and nomenclatorial changes especially if they are published in hardly accessible local journals and books.

Missing the Unseen: Morphological and Ultrastructural Characters in Genomic Era

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Molecular phylogenetics are undoubtedly providing critical information to be incorporated in diagnostics and phylogenetics of nematodes. In spite of this, comparative morphological diversity remains crucial to understanding functional biology and phenotypic evolution. Unfortunately, for most nematode taxonomists, morphological diversity and classical morphological characters selected for diagnostics among nematodes (i.e. Tylenchomorpha) often does not provide very reliable characters to support solid phylogenetic analyses. This in part may be because many characters are at the limits of light microscope resolution and are subject to misinterpretation. The 'known' diversity of nematodes (in particular free living and marine taxa) when analyzed with the aid of modern light, electron microscopy (SEM & TEM), 3D reconstruction and multi focal video imaging, usually provides new character information never included in classical taxonomic systems. Moreover, new taxa are discovered at a steady pace and often further expand the collective range of potentially diagnostic features. Resulting observations increase the relevance and accuracy of morphologically supported phylogenies, classification and identification. They further provide a basis to establish hypotheses of character homology across taxa and to understand specific functional adaptations. Case examples are presented and discussed.

Taxonomy and Systematics of *Hirschmanniella*

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Hirschmanniella species are versatile and taxonomically confounding nematodes. The genus is composed of 36 nominal morphospecies distinguished mainly by a few phenotypic characters that are oftentimes highly variable and difficult to resolve with light microscopy. A case in point is the recently described cryptic species *Hirschmanniella santarosae* which is morphologically very close to *H. pomponiensis* and only differs in excretory pore position and shape of stylet knobs, even though both species showed 1% and 4% sequence differences in small subunit (SSU) and partial (D2D3) large subunit (LSU) nuclear ribosomal DNA (rDNA), respectively.

We have obtained six additional *Hirschmanniella* isolates from both sides of the Atlantic and present their phenotypic and genetic character suites as well as phylogenetic analyses in order to provide better resolution of the evolutionary relationships and species delimitation among congeners. Combined analyses of these and other isolates suggest the likely discovery of additional cryptic species and species complexes, including further evidence of extensive radiation among Californian lineages. Morphological and molecular data must clearly be analysed jointly in order to eventually produce a more reliable estimate of species diversity in this problematic genus.

Free-living Marine Nematode Communities from Patagonian Littoral Coasts

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This talk will describe a baseline study on nematode biodiversity from three different latitudes: San Antonio Oeste, 40ºS (Rio Negro); Puerto Madryn 43ºS (Chubut) and Comodoro Rivadavia (Chubut) 45ºS, in four urban coastal cities, in contrast with pristine areas.

This project titled: 'Biodiversity of meiofauna communities in sand beaches of Río Negro and Chubut' had three main objectives: a) to contribute to the biodiversity knowledge of meiofauna and nematodes on Rio Negro and Chubut province coasts; b) to characterize, using communitarian structures the degree of disturbance in four urban coastal cities; c) to form doctorate students.

Seven littoral areas have been sampled at the three latitudes. The samples have been fixed in 5% formalin with rose Bengal in the field. The geographical position (GPS), salinity, temperature, granulometry, organic matter, DPR layer and dissolved Oxygen have been taken. Nematodes were extracted using the elutriation/decantation/LUDOX TM method and specimens mounted on slides.

The Nematodes found were identified up to the lowest taxonomy level possible, and photographs were taken using a Photomicroscope. For nematode identifications the keys of Platt & Warwick and Warwick et al. were used.

The differences in meiofauna and nematode species composition between latitudes and environments were analyzed by multivariate methods.

As main results we found that Nematodes were the most abundant taxa at the three latitudes. The environmental parameter showed in urban areas, a decrease in salinity at high littoral levels and a DPR layer near to the sediment surface, contrary to the expected for Patagonia coasts. Detailed information about species found plus dominant genera/species at each latitude will be presented as well.

The paradigm of two different biogeography units for Patagonia coasts is confirmed for meiofauna and in particular for free-living marine nematodes.

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SESSION THIRTY-TWO – NEMATODE MANAGEMENT IN INDUSTRIAL AND ENERGY CROPS

CONVENORS: HADDISH MELAKEBERHAN & GERARD KORTHALS

Roles for Nematology in Understanding Renewable Energy Needs and Balancing Food and Socio-economic Interests

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The finite nature of fossil fuel combined with greenhouse gas effects on global warming are factors that affect every living organism and make a strong case for alternative and renewable energy resources. The technology to achieve short- and/or long-term renewable energy sources either exists or it is at varying stages of development. However, most of the current and potential plant-based renewable energy sources like sugar cane, corn, soybeans, rapeseed, palm, cassava and yam (to mention a few) are crops where hundreds of millions of people depend on for many aspects of their daily life. Specifically, food security, land use, biodiversity and socio-economic factors associated with the production of the crops in question are major issues in developing countries. Thus, there is a need for sustained understanding of the issues for the benefit of all. Against this background and regardless of what controversies may exist, this presentation will discuss what roles nematology can play in creating bridges towards biological solutions for some of the challenges associated with production practices. These include: **i)** Getting nematology to proactively participate (along other disciplines) in the development of the genetic improvement of the target crops. In addition to limited knowledge of the status of most of the potential renewable energy crops against nematodes (e.g. switch grass), there is limited polygenic resistance to nematodes in current crops, and the renewable energy crops are being selected for other than nematode resistance. At the very least, proactive participation will help to identify nematode management options that will increase the returns from the technological investments on the potential crops. **ii)** Using nematodes as indicators of biological land degradation due to monocropping and other practices, and incorporating soil fertility use efficiency models that assess for agronomic, economic, environmental and nematode management factors when changing soil conditions to improve crop production.

European Approach to Nematode Management in Biobased Economy

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The increasing interest for energy crops and new industrial crops will change the agricultural landscape throughout Europe. Many crop rotations, such as sugar beet-wheat-potato, which were common for decades may change in favour of energy crops, such as maize. These changes will be seen above ground, but will certainly have their impact belowground as well. It can be hypothesized that these changes will have an impact on the control of soil born pathogens, like nematodes. PPO revitalised the idea of a Nematode Control Strategy (NCS) on farm level.

The Nematode Control Strategy is based on:

- an inventory of potential problems considering soil type, cropping history and planned crops within the rotation;
- an inventory of actual problems through soil sampling and crop inspection to determine nematode species and population densities for each of the growers fields;
- the design of a sound crop rotation scheme based on potential and actual problems and economic feasibility;
- prevalence of other soil-borne diseases e.g. *Rhizoctonia solani* and *Verticillium dahliae*;
- additional measures like black fallow, biofumigation and other biological control measures

To get a NCS implemented on European level, interaction is needed between farmers, farmer organisations, agro industry, extension services, plant protection services and nematologists. Farmers themselves have to be aware of the effects of nematodes on both yield and quality. This is only possible when farmers and their intermediates can recognize nematode symptoms and have an infrastructure available to process soil and root samples to confirm findings and to make an inventory of farmer fields. Furthermore research and extension infrastructure is needed to develop technical and economical feasible measures to solve encountered problems. To aid the development and implementation of European Nematode Control Strategies, several tools are presented and discussed. A first tool in which qualitative knowledge on host status and tolerance of crop nematode combinations is summarized can be found as www.aaltjesschema.nl (In Dutch). An second tool, which is a quantitative Decision Support System on the control of potato cyst nematodes, called NemaDecide, will be demonstrated as well. There is a challenge for nematologists of the ESN and SON to cooperate in developing an international Nematode Control Strategy and to develop awareness and knowledge to bring solutions on farm level.

Sugar Cane Production and Nematode Management Challenges

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Sugar cane production in Brazil has been expanding rapidly and the projections point to a 100% increase in production over the next 10 years. Each year new areas have been used for the crop establishment; some of them are infested with nematodes and have poor sandy soil. As the crop expands to nematode infested areas associated with low fertility soils, the nematode virulence will be higher and the crop yield will be lower, especially in soils with low levels of phosphorus, calcium and magnesium. The nematode populations in sugar cane (*Pratylenchus* sp. and *Meloidogyne* sp.) have usually been controlled by the use of nematicides. Most studies, especially in the Southeast and Northeast of Brazil, where the sugar cane crop has been traditionally planted for centuries, has shown a high efficiency of the chemical control on reducing nematode population densities and enhancing the crop yields. Yield gains, ranging from 8 to 40 tons per hectare, have been reported depending on the variety planted. The studies in those regions have also found some benefits from the use of sugar cane filter cake, although there is a lack of information on the actual effect of this residue on nematodes. In the central part of Brazil, the new frontier for sugar cane production, the nematode population densities aren't as high as in the other regions. This could be the reason why the studies on chemical control have not shown high crop yield enhancement and the alternative methods of nematode management should be more efficient. Studies have been carried out in order to test the effect of chemical and some natural products, such as neem oil (*Azadirachta indica*) and filter cake, on the nematode populations. We have also studied the effect of crop rotation on nematode populations and the evaluation of sugar cane genotypes reaction to *M. incognita*.

Nematode Management in Tropical Oil and Energy Crops

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There is a wide array of oil and prospective energy crops grown in the tropics. Due to time constraints, we will focus on two oil crops, oil palm (*Elaeis guineensis*) and peanut (*Arachis hypogaea*), and two tuberous starch crops, cassava (*Manihot esculenta*) and yam (*Dioscorea* spp.). Although primarily grown for cooking oil and as a staple food, these crops have also been proposed as a feedstock for biofuels. We will discuss the major nematode parasites and current management practices for each crop, and the implications of their candidature as biofuel feedstock to their production, nematode infection and damage, and management. Management of the red ring nematode, *Bursaphelenchus cocophilus*, in oil palm is primarily through removal of infested plants and control of the weevil vector. For the remaining crops, rotation with non-hosts and nematicides can be used to reduce nematode damage. Peanut and yam are the only crops for which nematode-resistant cultivars are available. Regardless of whether the crops are used for food or to produce biofuels, we should strive for management options, such as host-plant resistance, that are effective in reducing nematode populations and are inexpensive. However, challenges to developing nematode-resistant cultivars are the lack of genetic variability for resistance in existing germplasm and crop species are that are plagued by multiple nematode parasites.

Nematode Management in Temperate Oil and Energy Crops

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Nematode management in temperate oil and energy crops is based on resistance breeding of the main crop, adapted crop rotation and using trap crops. The restriction of using chemical nematicides in Europe claimed close collaboration of scientists and breeders to find acceptable solutions for the farmer. Starting in the nineteen eighties with controlling beet cyst nematodes *(Heterodera schachtii)* in sugar beet crop rotation with trap crops like resistant fodder radish *(Raphanus sativus)* and mustard *(Sinapis alba*) the system of Integrated Pest Management has increased stepwise by involving more crops and different nematodes.

Nowadays varieties exist in nearly all important main crops, which resist or tolerate nematodes with serious economic damage. Many of these varieties have a no longer disadvantages in cultivation characteristics and yield potential compared to susceptible varieties. But changing climatic conditions and more intensive growing systems with limited rotation and fewer times of black fallow result in new nematode problems (*Pratylenchus sp., Trichodorus sp.*). Also the careless exchange of machines, seeds and plants contributes to the spreading of nematodes and leads to new difficulties.

A practical and effective solution of this challenge will be reached in the future only by a close co-operation of scientists, breeders and farmers and has to maintain the current high standard of intensive agriculture not only ecological but also highly economic. Competent knowledge, large flexibility and enhanced specification are required from all took part to find new, innovative solutions which meet with the complexity in nematodes and sustainable plant production.

SESSION THIRTY-THREE – NEMATOLOGY IN DEVELOPING NATIONS

CONVENOR: JULIE NICOL

SESSION THIRTY-FOUR – NEMATODE DETECTION AND QUARANTINE

CONVENORS: SARAH COLLINS & MICHAEL JONES

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The Quest for Area Freedom from Potato Cyst Nematode in Western Australia

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Potato Cyst Nematode (PCN), a devastating plant pathogen worldwide, was detected between 1986 and 1989 on six properties (a total of 15ha) in the metropolitan area of Perth, Western Australia (WA). A strict quarantine and eradication programme was immediately implemented, and no PCN has been detected anywhere in the state since. Despite strong indications, through continued monitoring and testing, that WA is now free of the nematode, the state is still subjected to strict quarantine protocols and restricted access nationally and internationally to export markets.

With almost 20 years since the last detection, WA is now in an excellent position to re-claim Area Freedom from PCN. This will provide the WA potato industry with greater access to international and domestic markets for fresh and seed potatoes. This research project aims to substantiate Area Freedom through: a) an intensive survey of the six original infested sites; b) bioassay of soils from the original sites; c) intensive survey of all potato growing areas in the state; and d) collation of all data for soil sampling, fork testing, machinery and bin inspections, and export phytosanitary testing conducted since 1986.

Proving a 'negative' is always a challenge. With this in mind, survey methods were tailored to generate data to show with the highest possible confidence that PCN no longer occurs in WA. At all survey sites, 50g soil samples to a depth of 15 cm were collected on a 5 x 5 m grid pattern across entire fields. This resulted in collection of approx. 20 kg/ha, all of which was processed (without sub-sampling) by the Fenwick method for total organic matter extraction. This sampling regime is far more intensive than any standard worldwide. A new and innovative molecular method enabling detection of PCN by PCR from large volume soil samples has been developed.

Records of *Bursaphelenchus* species in Pines in Ningbo, Zhejiang, China

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The continuous spreading of the pine wood nematode by wooden packaging and the serious damage caused by it to pine forests in several countries, forced the worldwide establishment of quarantine measures for wooden packaging. In China, almost all imported wooden packages have been inspected and sampled in recent years, and around 20 *Bursaphelenchu*s species (including seven new species) were detected from wooden packaging imported through Ningbo port. However, packaging wood is a circulating product, and the presence of a *Bursaphelenchus* species in packaging wood is not always dependent on the origin of the consignment accompanied by the wood. Therefore, the investigation of the real distribution of *Bursaphelenchus* species, in this case in Ningbo, China, will contribute to a better understanding of the findings in imported packaging wood and *Bursaphelenchus* study.

From 2005-2007, 214 pine wood samples from different counties of Ningbo region in Zhejiang, China were sampled. They were nematologically studied in lab with morphological and molecular methods. Eleven *Bursaphelenchus* species were found: *B. xylophilus*, *B. lini*, *B. ranulfi*, *B. mucronatus* (East Asia Type), *B. hunanensis*, *B. sinensis*, *B. thailandae*, *B. hylobianum*, *B. leoni*, *B. yongensis*, *B.chengi*. Among them, *B. xylophlus*, *B. lini*, *B. rainulfi*, *B. mucronatus*, *B. hunanensis*, *B. sinensis* and *B. thailandae* were widely spread. A new discovered species , *B. yongensis* was found in samples from Beilun and Yuyao county.

In this investigation, we observed the phenomenon of *B. xylophilus* and *B. mucronatus* living in the same tree (found in four samples). We also observed *B. hunanensis* (female) eating *B. mucronatus* (juvenile)and *B. rainulfi* (male).

Numerous *Bursaphelenchus* species detected in packaging wood are obviously not occurring in Ningbo, whereas the presence of others both in packaging wood and Ningbo region indicates their possible origin in China. Most *Bursaphelenchus* species are known to live only fungivorously. The mode of life of newly dicovered species should be studied. However, the presence of any *Bursaphelenchus* species in packaging wood indicates that proper quarantine treatment according to international rules was not carried out. Since up to now the percentage of living nematodes detected from treated packing wood is still high, it is important to carry out the packing wood quarantine measurements.

Revision of Quarantine Nematodes Reported in Spain

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In revising the quarantine status of nematodes in Spain, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 is widespread on many different crops, including garlic, onion, strawberry, sugarbeet, cereals and legumes. Reports from other crops and uncultivated areas should be reviewed. *Globodera pallida* (Stone, 1973) Berenhs, 1975 is present, the main pathotypes being Pa2/Pa3 and also Pa1 in Tenerife (Canary Islands). The pathotypes Ro1/Ro4 of *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 occur throughout Galicia, La Rioja, Mallorca and Tenerife (Canary Islands). *Xiphinema rivesi* Dalmasso, 1969, a nematode vector of the Tomato Ring Spot Virus (TomRSV) in grapes in the USA, is widely distributed in the Spanish peninsula. Although cited, *Ditylenchus destructor* Thorne 1945; *Nacobbus* Thorne & Allen, 1944 and *Xiphinema americanum* Cobb, 1913 *sensu stricto* have not been found in Spain. However there are reports of *X brevicollum* Lordello & Dacosta, 1961, and *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 which are species belonging to the *X. americanum*-group. The other quarantine nematodes *Aphelenchoides besseyi* Christie, 1942; *Bursaphelenchus xylophilus* (Steiner & Buhere, 1934) Nickel *et al*., 1970, *Heterodera glycines* Ichinohe, 1952, *Hirschmanniella* Luc & Goodey, 1964, *Longidorus diadecturus* Eveliegh & Allen, 1982, *Meloidogyne chitwoodi* Golden *et al.,* 1980, *M. fallax* Karssen, 1996, *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944, *Radopholus similis* (Cobb,1893) Thorne 1949 (syn.: *R. citrophillus* Huettel *et al.,* 1984), *Xiphinema bricolense* Ebarsy et al., 1989, and *X. californicum* Lamberti & Bleve-Zacheo, 1979 have not been reported from Spain. Due to the importance of quarantine measures, an in-depth study of the distribution and epidemiology must be carried out to increase our knowledge on the existence of biotypes, their host range, and other plant pathogen interactions as in the case of *D. dipsaci* and *X. rivesi*.

Detection and Quantification of Root-lesion Nematodes from Field Soil by Conventional and Real Time PCR

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It is challenging to detect and quantify nematodes from soil extracts by PCR due to PCR inhibitors in soil. We have developed a protocol for detection of root lesion nematode *Pratylenchus spp.* in soil extracts using conventional PCR with specific primers. Nematodes are extracted from soil using Baermann funnels and centrifugal flotation in a sucrose gradient that alleviates the inhibition and increases the sensitivity of PCR detection. The nematode extract is then digested with proteinase K and the digestion used as a DNA template in the PCR assay. With this protocol and species-specific primers designed from ITS sequences obtained through cloning and sequencing technologies, we diagnosed *P. penetrans, P. vulnus, P. scribneri* and *P. thornei,* four major species in California fields by multiplex PCR. Analysis of unknown samples indicated that the detection was sensitive and specific. It detected samples at levels as low as a single target nematode among hundreds of thousands of other plant parasitic and free-living ones. We quantified *P. vulnus* from orchard soil by real time PCR. Grinding prior to proteinase K digestion was used to prepare nematode DNA. DNA quantity per female is about 1.5 times higher than for other stages, but quantities are similar for males and juveniles. All stages are present in California field and females comprise about 15-20% of the population. Quantification using real time PCR corresponded to that using microscopic examination ($r^2 \geq 0.95$) without concerns for variation in DNA quantity of nematodes at different stages. The effect of DNA quantities in lesion nematode at different stages and the stage structures in field samples on quantification will be discussed. The method we developed should be efficient and cost effective particularly for large numbers of samples for diagnostic services and research plots.

Reliability and Limits of Published Molecular Tests for the Specific Identification of Potato Cysts Nematodes (PCN) of Quarantine Concern

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In the context of international trade and international or regional quarantine requirements, diagnostic laboratories need reliable identification tools. In the European Union *Globodera pallida* and *G. rostochiensis* are the only *Globodera* species considered as quarantine pests (EU 2000-29 directive). For national survey or import controls, identification is often based on morphological and molecular tests. But which the reliability for the result whether we choose one tool or another and when faced to the wide diversity observed in South-America where *Globodera* originated from? The specificity of nine published molecular tests (PCR, PCR-RFLP) was evaluated against a set of *G. pallida*, *G. rostochiensis, G. tabacum*, '*G. mexicana'*, and *G. artemisiae* populations, carefully chosen to represent the widest genetic diversity known (Europe, The Americas). It appears that all tests are not reliable when compared to published results. Overall, *G. pallida* populations were correctly identified, even if some RFLP patterns were lacking (South American populations). But when considering *G. rostochiensis* populations, several tests gave false negative or positive results. Lack of amplification or observed at a wrong size respectively explain false negatives (especially for one Bolivian population) and positives (confusion with other species). Most of the *G. tabacum* populations were often confused with PCN and the '*G. mexicana*' populations usually identified as *G. pallida.* These results highlight the need of both a deep validation of molecular tools before use for official analysis and further scientific investigations regarding the taxonomic status of particular populations (*G. 'mexicana'* and some *G. sp* populations). At present, common operating procedures for detection and identification of regulated nematodes are discussed at a regional or international level. Thus we propose that the development of new molecular tools should include an obligatory validation process on a defined species set and the definition of limit of use before adoption as a reference test.

Root-knot Nematodes Virulent on Tomato with the Resistance Gene *Mi-1*

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The *Mi-1* gene in tomato confers effective resistance against several species of root-knot nematode. However, populations of nematodes that can reproduce on tomato with *Mi-1* have been noted in many locations in the world, including several instances in processing tomato fields in California. We have isolated a strain of *Meloidogyne javanica* that can reproduce on tomato with *Mi-1* from a culture of an avirulent strain after selection in the greenhouse. DNA blots and AFLP analysis indicated that the two nematode strains are closely related. cDNA AFLP studies identified a gene, *Cg-1,* that is present in the avirulent strain, but not in the virulent strain. *Cg-1* is a member of a small gene family with one or more copies missing in the virulent strain compared to the avirulent strain. The longest open reading frame is 32 amino acids and initiates at the fourth AUG in the predicted transcript. When *M. javanica* J2 of the *Mi-1*-avirulent strain were soaked in double-stranded RNA corresponding to part of the predicted *Cg-1* transcript, they produced progeny that were virulent on tomato carrying the *Mi-1* gene strongly suggesting that *Cg-1* is required in the nematode for *Mi-1*-mediated resistance. Molecular characterization of virulent tomato isolates from different locations in California indicates multiple origins of virulence but also suggest that farming practices may be contributing to the spread.

Selection of Virulent Populations of *Meloidogyne javanica* by Repeated Cultivation of *Mi* Resistance Gene Tomato Rootstocks in the Field

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The use of tomato rootstocks carrying the *Mi* resistance gene has been proposed as a non-chemical alternative to soil fumigation. However, rootstock responses to *Meloidogyne* spp range from highly resistant to susceptible. To determine if repeated cultivation of resistant tomato rootstocks selected for resistant breaking populations able to circumvent the resistance response, field trials were conducted in a plastic house artificially infested with an avirulent population of *M. javanica* for three consecutive years. Treatments included an experimental rootstock cv. PG76, a commercial rootstock cv. Brigeor, and the resistant cultivar cv. Monika and susceptible cv. Durinta as controls for reference. Rootstock cv. PG76 responded as highly resistant (reproduction index = 7%) after the first cropping cycle (3.4 generations), showed intermediated resistance (RI = 33%) after the second one (6.7 generations), and became fully susceptible (RI = 94%) after the third one (10 generations). Rootstock cv. Brigeor showed an intermediate resistance level (RI = 41%) after the third cropping cycle. Resistant cv. Monika retained a high level of relative resistance that was not circumvented after three cropping cycles (RI = 25%). At the end of the 3-year-study, the frequency of plants with gall ratings ≥ 5 was 52% of the plants of rootstock cv. PG76, 43% of rootstock cv. Brigeor, 25% of resistant Monika, and 95% of the plants of susceptible cv. Durinta. The virulence of the population from plots with rootstock PG76 exposed to the selection pressure of the *Mi* resistance gene in the field was confirmed in pot tests. The population in plots with susceptible cv. Durinta remained avirulent. Repeated cultivation of resistant rootstocks selected a virulent population from an avirulent one under agronomic conditions. The genetic background of the resistant rootstocks along with the frequency of cropping cycles was the main driving forces selecting for virulence.

Tomato Innate Immunity to Root-knot Nematodes

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Responses of resistant (*Mi-1/Mi-1*) and susceptible (*mi-1/mi-1*) tomato (*Solanum lycopersicum*) to root-knot nematode (*Meloidogyne spp.)* infection were monitored using cDNA microarrays and the role of salicylic acid (SA) and jasmonic acid (JA) defense signaling was evaluated during these interactions. Array analysis was used to compare transcript profiles in compatible and incompatible interactions of tomato roots 24h after nematode infection. The *jai1* and *def1* tomato mutant, altered in JA perception and signaling, respectively, and tomato transgenic line *NahG,* altered in SA signaling were evaluated in the presence or absence of the root-knot nematode resistance gene *Mi-1*. The array analysis identified 1497 and 750 genes differentially regulated in the incompatible and compatible interactions, respectively. Of the differentially regulated genes, 37% were specific to the incompatible interactions. *NahG* affected neither *Mi-1* resistance nor basal defenses to root-knot nematodes. However, *jai1* reduced tomato susceptibility to root-knot nematodes, while not affecting *Mi-1* resistance. In contrast, the *def1* mutant did not affect root-knot nematode susceptibility. These results indicate that JA-dependent signaling does not play a role in *Mi-1*-mediated defense but an intact JA signaling pathway is required for tomato susceptibility to root-knot nematodes. In addition, low levels of SA might be sufficient for basal and *Mi-1* resistance to root-knot-nematodes.

Early Resistance Responses of Coffee (*Coffea arabica*) to Root-knot Nematode (*Meloidogyne* spp.) Infection

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Root-knot nematodes (*Meloidogyne* sp.) are major pests damaging the cash crop coffee culture (*Coffea arabica*) in Latin America. *C. arabica* varieties resistant to *M. exigua* or *M. incognita* were identified and resistant coffee roots exhibited a typical hypersensitive response (HR). To understand physiological and molecular mechanisms underlying coffee resistance responses to *Meloidogyne* spp., we undertook a genomic approach based on the construction of substractive (SSH) cDNA libraries. SSH libraries were generated from pools of total RNA obtained from excised root tips 2 and 4 days after inoculation with *M. exigua.* Functional annotation of the unigene set showed that 30% of the ESTs encoded putative homologues of known resistance- and defence-related proteins. In addition, half of the ESTs unigene set represented novel coffee genes and 35% of the annoted ESTs did not share significant similarity to plant protein database entries. Real-time quantitative RT-PCR expression analyses of 115 genes from several functional categories were monitored during *M. exigua* infection time-courses of resistant and susceptible coffee varieties. A higher number of genes exhibited expression changes in the susceptible variety than in the resistant variety (60 and 40 %, respectively) and a significant number were activated in the susceptible variety (37%). In HR-exhibiting plants, only 17% of genes were up-regulated, displaying transient regulation over 5-days time course experiments. A comparative study of the coffee gene regulation during resistance to *M. incognita* is currently ongoing to select for common *Meloidogyne* resistance reponses-candidate genes. Functional analysis of candidate genes in *Agrobacterium rhizogenes*-transformed roots assays will reveal new insights into plant resistance responses to root-knot nematodes.

Virulence of *Globodera pallida* in Relation to Resistance in Potato

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Globodera pallida is the most damaging nematode pest to UK agriculture and occurs in many potato growing regions worldwide. A number of sources of resistance to *G. pallida* have been identified in the Commonwealth Potato Collection (CPC) and are used in the potato breeding program at SCRI. High level resistance from *Solanum tuberosum* spp. *andigena* CPC2802 has been introgressed into potato cultivar Vales Everest and the reproduction of many isolates of *G. pallida* is greatly reduced on it. Markers are being developed to assist with the introgression of resistance from CPC2802 and *S. vernei* and new sources of resistance from the CPC are also being assessed.

Repeated reproduction of *G. pallida* populations on potato clones with resistance derived from *Solanum vernei* or CPC2802 leads to selection for increased virulence which is specific to the source of resistance used. Comparisons of several expressed sequences from populations of *G. pallida* with differing levels of reproductive ability on clones derived from *S. vernei* have revealed a relationship between sequence polymorphisms in chorismate mutase, and increased virulence.

Session Thirty-six – New Technologies for Plant Nematode Control

Convenors: Daguang Cai & Rodrigo Rodrigues-Kabana

Innovations in Nematode Management on Turf in the USA

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The state of Florida in the United States has over 2.5 million ha of turfgrass which brings in over $10 billion US to the state economy annually. Turfgrass is used on lawns, golf courses, athletic fields, sod farms, parks, and other venues. Plant-parasitic nematodes are among the most important pests on turfgrasses in Florida, however effective nematicides are not available for most turf uses. Fenamiphos has been the most widely used turfgrass nematicide in the U.S. for over thirty years, but as of 2006 it is longer being manufactured in the U.S. due to environmental concerns. Over the past eight years a great deal of research effort in Florida has sought to find innovative ways to manage plant-parasitic nematodes on turfgrass in the absence of fenamiphos. These efforts include: a) new uses of older nematicides such as 1,3-dichloropropene, b) development of new nematicides based on chemicals and plant-derivatives such as furfural, mustard-bran, and sodium azide, c) new nematicides based on novel chemistries, d) biological control organisms such as *Pasteuria* spp. and *Paecilomyces lilacinus*. These efforts have met with varying degrees of success from complete failure to registration and industry acceptance of commercial products. Experimental results from field trials with numerous management tactics will be presented and future directions will be discussed.

From Suppressive Soils to Suppressive Plants: The Role of Endophytes in Plant Driven Management of *Radopholus similis*.

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A number of nematode antagonistic fungi have been isolated from banana and plantain roots and corms over the past two decades. Among them are a number of non-pathogenic *Fusarium oxysporum* and *Trichoderma atroviride* isolates recovered in Guatemala and Costa Rica from banana and plantain roots in soils identified as nematode suppressive. The identified inducing agents of the observed suppression are nematode antagonistic endophytic fungi. Because the designation of the soils as being suppressive is misleading, the term *in-planta* suppressiveness was coined. *In vivo* screening tests with isolates from these fields identified fungi that significantly reduced the number of *Radopholus similis* in roots between 72 and 85%. Re-isolation of inoculated fungi from roots confirmed the endophytic nature of the fungi. Subsequent greenhouse and field studies have proven that by inoculating selected endophytes onto tissue culture banana plants, nematode penetration and reproduction in these plants is reduced and plant growth as well as yield is promoted. In other words, the plants become resistant to nematode attack. Multiple inoculations of endophytes increase both nematode control and plant growth promotion effects. Endophytic fungi that colonize plant roots are perfectly placed to act as biological control agents of nematodes, as they are present in the same tissues as those where nematode attack occurs. Conversely to mycorrhizal fungi, which are also present in plant roots, the mutualistic endophytes isolated from banana, are not obligate symbionts, and can therefore be produced in industrial fermentors. In addition, with endophytes, there is no need for repeated soil inundative applications, as only the rhizoplane of transplants is treated prior to field planting. Large scale inoculations of transplants have already been successfully carried out. The results of long years of research demonstrate the future potential of *R. similis* management through *in-planta* suppressive *Musa* plants.

Using natural Resistance Mechanisms for Plant Nematode Control

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Using natural resistance mechanisms for plant nematode control is a major focus of our recent research. To identify genes involved in the *Hs1*$^{pro-1}$ mediated resistance response in sugar beet, the cDNA-AFLP technique and ATH1 GeneChips were used for comparative transcriptome analyses. A group of germin-like genes were identified whose expression is specifically upregulated upon nematode infection in both resistant beet and *Hs1*$^{pro-1}$ transgenic *Arabidopsis* plants, suggesting their role in the *Hs1*$^{pro-1}$-mediated nematode resistance response. To investigate its potential for plant nematode control, we transformed susceptible beet roots and *Arabidopsis* plants with the gene *BvGLP-1* that shows homology to oxalate oxidase-like genes and is functionally hypothesized to be involved in hydrogen peroxide generation in plant cells. Two gene expression constructs were generated, of which pAM-BvGLP-1 with 35S-promoter for a constitutive over-expression and pBin-BvGLP-1 with *Hs1*-promoter for a feeding-cell specific expression. Transgenic beet roots and transgenic *Arabidopsis* plants were used for nematode infection experiments in which non-transgenic beet roots and *Arabidopsis* plants served as controls. As a result, either transgenic beet roots or *Arabidopsis* plants expressing the gene showed strong anti-nematode effect. The regular development of nematode was inhibited resulting in significant reduction in the number of developed female nematodes. The oxidase oxalate activity in transgenic plants was determined by biochemical and histochemical assays showing a correlation with the anti-nematode activity. However, a constitutive overexpression of the gene in plant cells resulted in a lower regeneration rate and strong stagnation of the growth of transgenic beet roots and *Arabidopsis* plants, which obviously suffered from an excessive hydrogen peroxide stress. This result strongly suggests that an enhanced oxalate oxidase activity in nematode feeding-cells at the early developing stage represents a key mechanism of the *Hs1*$^{pro-1}$-mediated nematode resistance, which can be effectively used for genetic engineering of plant nematode resistance.

Soil Biodisinfection as an Alternative to Soil Fumigants

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Spain was the fourth country in the consumption of methyl bromide (MB) in 1995. A total of 4,191 t of MB was applied as a soil fumigant to 8,988 ha of various crops, mainly strawberry (33%), pepper (29%), cucurbits (9%), and cut flower (9%). Among the non-chemical alternatives, biofumigation and biosolarization are outstanding, as is soil-less cultivation, crop rotation, the use of resistant varieties and grafting, which are in Spain an effective means of control when included in an integrated crop management system (ICM). The alternatives cost less, are equally effective as MB, and do not pose problems in their application. The critical use of MB for the year 2008 in Spain is 200 t only for strawberry nurseries. MB will be eliminated as soil fumigant in the EU in 2009. In Spain, this reduction is due to the development of biofumigation and biosolarization as alternatives in soil biodisinfection, for both nematodes and fungi present in crop remains. The biofumigation has been applied to the process where volatile toxic gases are released in the process of biodescomposition of organic amendments, plant roots, and tissues and where such gases manage diseases, nematodes, and weeds. Biofumigant materials can be solids and liquids, for that reason it is proposed the term soil *biodisinfection***.** The biodisinfection of soils is also effective in the control of weeds, increasing soil fertility and soil structure through the use of manures and crop remains, applying ecological criteria in crop production based on local resources. The results about the use of this alternative in extensive open fields and protected crops are demonstrated.

Bio-fumigation Potential of Kava and Wild Kava for Managing Root-knot Nematodes

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Effect of kava (*Piper methysticum* Forst) and wild kava (*Piper aduncum* L.) on the root-knot nematode, *Meloidogyne incognita* and host plant tomato was studied. Kava root powder, kava peelings, kava kosa, kava stem, kava leaves and wild kava leaves were used as soil additives in pot trials at 2% and 4% concentrations with pre-planting degradation periods of 0, 1 and 2 weeks. All the tested materials were able to reduce the number of galls when compared to the control. Generally, higher concentration of the additives without degradation produced greater gall suppression. Kava powder, kava peelings and kava kosa caused maximum gall suppression but also had adverse effect on plant growth. Kava stem, kava leaves and wild kava leaves on the other hand enhanced plant growth but had relatively lesser gall suppression. Kava is not suitable as soil amendment at the tested dosage but can be exploited for isolation of bionematicidal compounds whereas wild kava has the potential to be used as a biofumigant. Further trials can be carried out using wild kava in combination with a stronger gall suppressive and cost effective material such as kava kosa.

Session Thirty-seven – Precision Agriculture, Information Technology and Nematode Control

Convenors: Terrence Kirkpatrick & Charles Overstreet

The Need for More Efficient Management of Nematodes in Cotton

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Cotton is the most important natural fiber, representing almost 40% of the world fibre market. The crop is grown in over 100 countries, with China, the USA, Pakistan, Uzbekistan, and Egypt accounting for about three-fourths of the world output. Cotton yield per hectare has tripled since the mid-1940s, due to high yield potential and improved pest resistance in modern cultivars. Genetic engineering has recently yielded insect and herbicide-resistant cultivars that allow more efficient insect and weed control, but similar improvements have not been made with regard to plant-parasitic nematode management. The root-knot nematode, *Meloidogyne incognita*, is the most widespread economic nematode of cotton, and has been reported on cotton in most world production areas. In addition to root-knot nematodes, *Rotylelnchus reniformis*, *Hoplolaimus* spp., *Belonolaimus longicaudatus*, and *Pratylenchus* spp. are significant pathogens of cotton in many parts of the world. Nematode management in cotton can be difficult because of the absence of effective resistant cultivars, the impracticability of crop rotation, and the difficulty of detection and quantification of nematode problems. Although nematode populations are rarely uniformly distributed within fields, current strategies for nematicide use involve whole-field applications prior to or at the time of planting. Unfortunately, nematicides that are used in cotton are either only marginally effective, or they are expensive and pose certain environmental and health risks. In the face of expanding nematode problems in many regions and declining profit margins, more efficient and environmentally appropriate strategies for nematicide application will be vital.

Site-specific Technology: An Introduction and Investigation of its Use for Management in Louisiana, USA

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The last decade has seen radical changes in production agriculture technology and equipment that is now available. Some of these new technologies include yield monitors for either cotton and grain crops which measures exact yield across an entire field, aerial imagery from either satellites or airplanes that can be taken during the growing season, and hardware and software for very precise placement of nematicides within a field. Our research in Louisiana has focused on identifying areas in a field where nematodes such as *Meloidogyne incognita* or *Rotylenchulus reniformis* occur and cause significant yield loss. Predominate soils in Louisiana where cotton is produced are alluvial soils deposited by the Mississippi River. These soils are highly variable in soil texture and often range from a sandy loam to clay within the same field. The use of a Veris 3100 Soil EC Mapping System measures apparent soil electrical conductivity and has proven to be an effective surrogate for soil texture in our soils (R^2 = 0.89 for clay). Fields can then be divided into a series of texture zones based on the EC_a. Since *Meloidogyne incognita* prefers a sandy soil, fields can fairly easily be separated into zones where this nematode occurs. *Rotylenchulus reniform* has proven to be much more difficult to isolate by texture in a field. Verification strips (6-16 rows of 1,3-D dichloropropene applied at 28.1 l/ha and injected 30 cm beneath the row and a similar number of untreated rows) have been applied to eight test fields to measure the nematicide response across various management zones. Positive response to the nematicide has consistently been in the soil textures with the lowest EC_a indicating its usefulness in dividing fields into management zones.

Practical Site-specific Nematicide Delivery on Cotton Farms in the Mid-South USA

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Cotton growers in the southern U.S. routinely include the application of either 1,3-dichloropropene (Telone II) or aldicarb (Temik) in their farming operations for nematode management. The most economically important nematodes in cotton are *Meloidogyne incognita* and *Rotylenchulus reniformis*. Nematicide applications are made at a single rate on a whole-field basis, and decisions to apply these chemicals are usually based on assay of a single composite soil sample arbitrarily collected from each field. However, nematode distributions and associate damage are not typically uniform in most fields. Soil texture is a key factor influencing the reproductive success and damage potential of these nematode species and on the crop. An understanding of soil textural changes within individual fields could allow more efficient sampling for nematode detection and quantification and provide a platform for determining strategies for site-specific nematicide placement. In field investigations (2005 & 2007), mobile soil electrical conductivity (EC) meters (Veris Technologies) were used to estimate and map site-specific soil textural variations within a production field in Northeast Arkansas. The soil EC maps were used to identify soil textural regions (zones) that were sampled individually for nematodes. Nematode population densities from each zone were then compared with known damage threshold values, and prescription application maps for site-specific placement of 1,3-dichloropropene were developed. Site-Specific applications of 1,3-dichloropropene were compared to an untreated control and a single 3 gal/acre rate of 1,3-dichloropropene. Results in this field indicated that cotton yield was comparable in zones receiving site-specific nematicide application to that where a single rate was applied, but 37 to 42 percent less chemical was applied with the site-specific approach. Both approaches resulted in significantly greater yield than where no nematicide was applied.

Considering Field Physical Characteristics in Assessing Risk and Delineating Nematode Management Zones

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Site-specific management (SSM) of nematodes requires identifying factors affecting nematode distribution, nematode population density, and nematode-induced yield losses, and then using that information to predict where nematode management will cost-effectively reduce yield loss. Using cotton (*Gossypium hirsutum*) as a model system, we accomplished this by 1) using multiple regression analysis to evaluate the relationship between cotton yield, soil physical and chemical properties, and southern root-knot nematode (RKN, *Meloidogyne incognita*) population density to identify factors most strongly affecting yield; 2) using the most important factors affecting yield to create a logistic regression model which predicts the probability of yield loss due to RKN (assess risk); and 3) translating the model predicting the probability of yield loss into field maps which delineate areas with different levels of probability (risk) of yield loss which can then be used as management zones (MZ) for SSM. The factors most useful for delineating MZ were soil electrical conductivity (EC, a proxy for soil texture), elevation, slope, and normalized difference vegetation index (NDVI), though soil EC was the single most important factor. Models created from multiple fields in 2005 and 2006 were used to create a MZ map for a field in 2007. Nematicide treatments were randomized and replicated in each MZ, and nematicides were shown to have a much greater effect in MZ predicted to have the greatest risk for loss to RKN. Though the most expensive nematicide treatments generally led to the greatest yield increases in all MZ, they were the most cost-effective treatments only in MZ identified as higher risk for yield loss from RKN; in lower risk MZ, less expensive treatments were the most cost-effective. Therefore, MZ based on field physical characteristics will allow SSM of nematodes in some fields.

Cost Effectiveness of Precision Nematode Management

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Precision nematode management systems can be cost efficient in a number of interlocking ways. First they can apply nematicides in a more precise manner than older gravity flow material. Systems developed at Clemson University can apply 1,3-dichloropene with a maximum absolute error rate of 6.7% and an average overall error rate of -2.1%. A newly developed aldicarb applicator can operate with measurement errors of -3 to 4.2 with a mean error of 1.1%. Aldicarb application error rates on older equipment can exceed 10%. Traditional grid sampling can be replaced with nematode distribution maps based on models relating nematode distribution to soil types determined using a soil electrical conductivity (EC) meter. Instead of taking, processing and paying for 10 to 20 samples per hectare a single pass with an EC meter can map an entire field for the cost of collecting and processing 2 to 3 traditional samples. By combining EC based maps with site specific application techniques precision nematicide application can be cost effective. An example of site specific application is a test conducted in 2002 in South Carolina. In a 10 acre field infested with *H. columbus* utilizing map based variable rate aldicarb applications based on % sand generated using an EC meter resulted in a 5% higher yield and 34% lower nematicide usage compared to a single rate application. Variable rate applications of 1,3-dicloropropene also increased yield 5% while decreasing nematicide usage 78% compared to a single rate application.

Projects are currently underway to create lower cost application systems that are also simpler to operate.

Occurrence of Entomopathogenic Nematodes in Different Agroecosystems and their Potential in the Management of Diamondback Moth

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Diamondback moth (DBM), *Plutella xylostella,* L. is one of the most destructive pests to brassicaceous crops and has developed resistance to the commonly used chemicals. This study was set out to determine the occurrence of entomopathogenic nematodes (EPNs) in different agroecosystems and assaying their potential as biocontrol agents in the management of DBM. Soil samples were taken from a planted forest, pasture, a coffee field, and a vegetable garden. EPNs were isolated from the soil using *Galleria mellonella* as the bait insect. Laboratory bioassays were conducted to determine the lethal time fifty (LT50), which is time till 50% lethality, of the EPN isolates to DBM larvae by leaf disc bioassay method. Five isolates of EPNs namely: *Heterorhabditis indica, Steinernema karii*, *Steinernema wesieri*, *Steinernema* sp., and *Heterorhabditis* sp. were used. The frequency of occurrence of EPNs was lowest, 27%, in the soil from vegetable garden, followed by forest soil, 33%. Soil from the pasture ecosystem had a moderate frequency of occurrence of EPNs, 50%. EPNs were most frequently isolated from a coffee field, which is rated as a moderately disturbed ecosystem, 77%. The LT50 of *S. karii, H. indica* and *S. wesieri* was 38.1, 20.27 and 23.8 hours, respectively. *H. indica, S. karii*, *S. wesieri*, *Steinernema* sp., and *Heterorhabditis* sp. caused 96.0%, 93.3%, 92.0%, 88.0% and 86.7% mortality in the DBM larvae within 72 hours, respectively. This study has demonstrated that the frequency of occurrence of EPNs is different in various agroecosystems. This study has also showed that EPNs have a great potential that may be exploited along with other suitable strategies in integrated management of DBM.

Plant-parasitic Nematodes Associated with Weeds in Developing Agriculture, with Special Reference to Root-knot Nematodes

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Plant-parasitic nematodes are ubiquitous, soil-borne pests that cause significant damage to a wide range of agricultural crops. Since a variety of weeds that occur in small-scale farming systems often serve as reservoirs for these parasites, this study focussed on the association between plant-parasitic nematodes and weeds. A nematode survey was conducted at 44 sites located in the eastern (Eastern Cape, Limpopo, KwaZulu-Natal and Mpumalanga provinces) and western (Northern Cape province) resource-poor farming regions of South Africa. Thirty-seven weed species and 33 genera were identified as hosts of plant-parasitic nematodes during this survey, which differed substantially with regard to their frequency of occurrence in the two regions. *Cynodon dactylon* had the highest frequency of occurrence in both regions. With regard to plant-parasitic nematodes, 20 species and 12 genera were reported for the first time in South Africa to parasitise weeds. Root-knot nematodes (*Meloidogyne* spp.), followed by *Pratylenchus zeae*, *Helicotylenchus dihystera* and *Rotylenchus unisex* were generally the predominant endo- and semi-endoparasites extracted both from root and soil samples in the two regions. *Meloidogyne* species identified by means of molecular techniques were *M. javanica* and *M. hapla*, with the latter species generally being predominant at some sites in the eastern region. *M. javanica* was predominant at some sites located in both the eastern and western regions. Weeds identified during this study that maintain plant-parasitic nematodes, particularly root-knot nematodes, could have a negative impact on crop production when they are not eradicated timely and effectively. This problem is of particular significance in resource-poor, subsistence-farming systems where literacy and knowledge levels are low.

A Successful Mass Production *in vitro* Entomopathogenic Nematodes, *Steinernema carpocapsae* (All Strain) for Control of Plant Pests in South Sumatra

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Insect pests have limited agriculture productivity world-wide especially in South Sumatra, Indonesia. On the recent days the use of pesticide to control insect pests has been known to cause human health and environment problems. The entomopathogenic nematode have received much attention in recent years because of their biological control potential against pest insects in agriculture. However, the wide use the nematode is still hampered by inefficient mass production especially *in vivo*. The objectives of this study were to culture the nematodes, *Steinernema carpocapsae* (All Strain) *in vitro* and to evaluate the effectivity againts some insect pests.

The result showed that soya flour gives the best Ijs production at 27 oC temperature and 350.000 Ijs inoculums with number of IJs 447596/gram after 14 days. The effectivity against *S. litura* showed that the lethal dose (LD_{50}) was Ijs 34 (25,8 ± 42,6) pro insect and lethal time (LT_{50}) was 36 (30 ± 50) jam. The nematode was be able to reduce the population of *Darna trima* and *Setora nites* on palm oil with LD_{50} 25 (18,6 ± 64,1). The entomopathogenic nematodes has been succesfull *in vitro culture* and it is potential to use against many pest insects in Indonesia.

Soybean Cyst Nematode, *Heterodera glycines*, in Iran

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, found in most soybean growing regions in the world, is considered the most economically damaging pathogen of soybean worldwide. A survey conducted in the northern provinces Mazandaran and Golestan, the main soybean producing areas in Iran, revealed SCN was wide spread in different localities of these regions. Of the 55 and 88 soil samples collected from soybean fields in Mazandaran and Golestan provinces, 10 (18.8%) and 23 (26%) samples were infested with *H. glycines,* respectively. The population densities of J2 and eggs ranged from 500-60,000 and 500≥100,000 per 250 ml soil. These population densities of *H. glycines* are likely responsible for some soybean yield reductions. The initial observations showed that the length of SCN first life cycle takes 30-34 days in early-planted fields (late May and early June), while in late-planted (early July), the white females are visible 20 days after planting, it seems several generations could be expected in a single growing season under field conditions in northern Iran. Race or HG Type tests conducted on 16 field populations. Race 3 or HG Type 0 was found to be the most commonly-occurring race in Iran with 93.75% frequency, while race 6 or HG Type 7 was found in 6.25% of tested populations. Most populations of *H. glycines* race 3 parasitized PI 88788 and PI 548316 indicator lines. Eight most commonly used soybean cultivars were tested against *H. glycines* race 3, seven, Sepideh, Sahar (Pershing), Gorgan 3, Williams, JK (Sari), BP (Telar) and Hill, had high female indices and were considered susceptible to race 3. Only the cultivar DPX showed low female indices and was resistant to race 3.

Study of Nematodes Associated with Saffron (*Crocus sativus* L.) in Iran

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Iran is a major world producer of saffron. Saffron, the world's most expensive spice, is derived from the stigmas of the saffron (*Crocus sativus* L.), a member of the Iridaceae. Commercial and traditional uses for saffron are many and varied, most are used as a spice in cooking; As a medicine, it is regarded to have sedative, antispasmodic, expectorant and aphrodisiac properties. In order to identify nematodes associated with saffron in Iran, 450 soil samples were collected during Autumn 2007. Soils were loamy or sandy-loamy in texture. Moving nematodes were extracted using whitehead tray method. Nematodes were identified at genus level and mounted for identification at species level. In this study, the most dominant plant parasitic nematodes found in the samples were *Filenchus* sp.(5.6%), *Geocenamus* sp.(39.5%), *Helicotylenchus* spp.(65.7%), *Tylenchorhynchus* sp.(35.2%) and *Tylenchus* sp.(3.1%); Fungivour nematodes contained in the samples were *Aphelenchoides* sp.(9.3%) and *Aphelenchus* sp.(41.1%); Bacterivour nematodes of the samples were *Acrobeloides* sp.(25.5%), *Acrobeles* sp.(13.2%) and *Cephalobus* sp.(2.5%) and dorilaimid nematodes(42.5%). Identification at species level is being studied currently.

Session Thirty-nine – Ecology and Biogeography of Entomopathogenic Nematodes

Convenors: Sergei Spiridonov & Larry Duncan

Intraspecific Groups in Steinernematid Species: Analysis of ITS rDNA Haplotypes

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Steinernematid species are characterized by different ranges of geographical distribution. *Steinernema feltiae* is reported from a number of temperate countries, but can be also found in tropical and subtropical habitats (isolations described from Middle East, Africa, South America, Australia), *Steinernema kraussei* was found throughout Holarctic region both in North America and Eurasia, but never in tropics or subtropics. The intraspecific structure of such widespread steinernematid species is interesting both as basic knowledge about EPN populations and as an indicator of genetic diversity of steinernematids, which is the source of potentially useful traits for biocontrol and biotechnology. The intraspecific structure of *Steinernema feltiae* and *S. kraussei* is studied through the phylogenetic analysis of ITS1+5.8S+ITS2 rDNA sequences for 42 strains. Obtained data are analysed with different phylogenetic methods (neighbor-joining, ML=maximum likelihood, BI=Bayesian inference). Maximum parsimony analysis with gaps treated as 'fifth base' was also used. Few intraspecific groups with high levels of bootstrap support are present both in ML and BI topologies. The clade of 6 cultures of *S. kraussei* isolated in Eastern Siberia and Sakhalin is strongly supported in BI tree. Some European strains of *S. kraussei* are forming well supported group. *Steinernema feltiae* strains with inserts in ITS1 rDNA are forming stable group in all topologies and always representing terminal clade in *S. feltiae* phylogram. The majority of these strains originate from Western and Northern Europe. Asian isolates of *S. feltiae* are occupying basal positions in the phylogram for this species. The insertion of 10 additional nucleotides in ITS1 rDNA of this species can be considered as unique evolutionary event, which happened probably somewhere in Europe. BI analysis demonstrated also another strongly supported clade inside *S. feltiae* consisting of two strains: from Armenia and Israel. RFBR support 08-04-00209a and 07-04-9005 Viet_a.

Habitat Quality as a Determinant of Entomopathogenic Nematode Distribution and Behaviour

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Entomopathogenic nematodes (EPNs) are widely distributed in nature. They are known to play and important role in regulating soil food-webs and are sold worldwide as biological control agents for broad range of insect pests. Natural populations of many species show marked habitat preferences, and this is usually attributed to distributions of susceptible hosts, although there is little published evidence to support this hypothesis. Here, we argue that habitat quality may determine natural distribution of EPNs and present laboratory and field data investigating survival and host finding of two EPN species, *Heterorhabditis megidis* and *Steinernema carpocapsae* in two very different media, sand and peat. In all experiments using peat *S. carpocapsae* out performed *H. megidis* whereas the opposite was true in sand. Possible reasons for these differences will be discussed.

Diversity and Distribution of EPNs in South America

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South America is a continent covering an area of 17,819,00 sq km, composed of 12 countries and 3 major territories, with all types of vegetation and climates. In spite of all its richness, the studies on entomopathogenic nematodes (EPNs) are still scarce and limited to few countries and labs. During our talk will review the history of EPNs in South America, and its landmarks, from Lauro Travassos to nowadays. We will point out all the surveys promoted in the continent, the species found, and the new ones described. Also, we will infer about the morphometric differences in species found in South America compared to the same ones found in other continents. There is an entire continent to survey and study in front of us, all we need is talented people to do it. The search for new strains is especially important growers are willing to use EPNs as biological control agents, since native nematodes are already adapted to local climate and soil.

Metapopulation Biology of EntomopathogenicNnematodes

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Metapopulation biology is concerned with the dynamic consequences of migration among local populations and the conditions of regional persistence of species with unstable local populations. Thus, a metapopulation is defined as a population of unstable local populations, inhabiting discrete habitat patches. One of the hallmarks of metapopulations is the appearance and disappearance of subpopulations from habitat patches as a result of frequent extinction and recolonization. We found evidence of metapopulation dynamics in an endemic population of *H. bacteriophora* in a vegetable production area in North Central Ohio. A total of 143 sites were sampled once a month from June to August in 2005 and 2006 from six distinct habitats located at approximately 2-3 miles apart: vegetables (20 samples), row crops (20), residential lawns (21), unmanaged crop 'grassy borders' (44), shrubs (18) and forested wetlands (20). At each sampling time, each site was located with a Global Positioning System (GPS) and 10 soil cores (2.5 cm diameter, 25cm depth) were randomly collected from a 1m^2 area. Cores were combined to form a composite sample and stored at 5°C until EPNs were extracted using a modified insect baiting technique. We analyzed the changes in nematode abundance for frequencies of extinction and recovery. Both temporal and spatial pattern of extinction and recovery are apparent in these data, consistent with metapopulation dynamics. We also investigated genetic variation in *H. bacteriophora* collected from the above sites using the mitochondrial gene, cytrochrome oxidase subunit *(cox1)*. We calculated *cox1* DNA sequence based pairwise distances between nematodes from different sites using maximum composite likelihood substitution model and found significant genetic variation. We also determined relationships between different *cox1* haplotypes for the 22 nematode isolates. The spanning tree showed the differences among haplotypes represented as number

Do Natural Enemies Regulate Entomopathogenic Nematode Spatial Patterns?

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Entomopathogenic nematodes (EPN) have been shown to be important natural enemies of *Diaprepes abbreviatus*, a major weevil pest of citrus in Florida and the Caribbean Basin, and recently introduced into Texas and California. In different regions of Florida where endemic EPN species diversity and predation of weevil larvae are high, the insect is a minor pest; whereas, the weevil can cause growers to abandon citriculture in regions with fewer species and little predation by EPN. Accordingly, we are studying biotic and abiotic factors that regulate spatial patterns of EPN across the Florida citrus industry. Nematophagous fungi (NF) have been shown to respond in a density dependent manner when EPN emerge in high numbers from insect cadavers and when EPN are added to soil as an augmentation biocontrol tactic. Predation rates by NF also vary depending on the species combinations of NF and EPN, suggesting the possibility that some EPN species may have a competitive advantage in habitats that favor particular NF. Among the EPN endemic in Florida citrus orchards, the numbers of *Steinernema diaprepesi* and *S. glaseri* were unaffected by three species of *Arthrobotrys* (trapping fungi) in soil bioassays, whereas numbers of *Heterorhabditis indica, H. zealandica* and *S. riobrave* were reduced significantly. In contrast, two endoparasitic fungi (*Catenaria* sp. and *Myzocytium* sp.) whose zoospores require free water to locate and infect nematodes, had no effect on numbers of *H. indica*, but preyed heavily on the other four EPN species. *H. indica* is frequently the dominant species detected in parts of Florida with poorly drained soils and high water tables. Ongoing research is characterizing the spatial patterns of NF in Florida to better understand their habitat requirements and their potential to affect EPN communities.

Next Generation Sequencing and its Application to Model Organisms

Grimmond, S.

Australia

The Impact of Next-generation Sequencing Technologies on Parasitic Nematode Genomics

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The application of genomics to study parasites from the phylum Nematoda has utilized mainly the Expressed Sequence Tags (ESTs) based approaches. Following the completion of the *C. elegans* genome sequence, the Genome Center (GC) at Washington University submitted over 500,000 expressed sequence tags (ESTs) from 36 nematode species, using the conventional Sanger sequencing. Hence, genomic approaches are revolutionizing molecular parasitology and the characterization of most genes now begins with their appearance in sequence databases. However, due to the proliferation of large-scale sequencing projects in recent years, several new sequencing technologies (so called 'next-generation' or 'massively parallel') are becoming available and are already having a significant impact on genomics and genetics in general. There are several dozen parasitic nematode whole genome projects underway at the GC and other labs that will be completed in 3-5 years. The parasitic nematode genomic projects at the GC also include sampling of the transcriptome through massively parallel sequencing. Therefore, with so much emerging data our key focus is on organizing this information in a manner that allows for the rapid comparison of gene-content across species in the phylum. We will discuss how well the old and the new sequencing technology complement each other and their pros and cons inferred from the analysis of the available Sanger ESTs and the newly generated 2.25 million 454/Roche cDNAs from parasitic nematodes.

Novel Approaches to Analyze Gene Expression during Pathogenesis: The Second Generation Comes of Age

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Rapid advances in genomic technologies and our ability to analyze and interpret the data are revolutionizing detailed studies of parasitic nematodes. There are at least 10 whole genome sequencing projects currently ongoing, and countless laboratories are performing expression analysis. Metabolomic and proteomic approaches can be applied to these systems as well, and this combination provides a powerful platform for cell biology. Also during this period, a number of key host species have been/are being sequenced. Recently, we completed a comprehensive transcriptional analysis of the tomato-root-knot nematode parasitic interaction using microarray approaches. We performed these studies on both the susceptible and resistant interactions. In the susceptible interactions, large suites of genes had altered expression patterns over time, mostly up-regulated. In some cases, however, specific genes or groups of genes were substantially repressed, suggesting a role in the alteration of the normal plant developmental patterns. In the resistant response, many typical resistance response genes were activated, but the data also revealed a role for a glycosyltransferase in the response to nematodes. Although conditioned by the classical R-gene Mi, resistance to nematodes appears to be manifested slightly differently than to other pathogens. New second-generation DNA sequencers, such as the Roche 454 FLX platform and the Solexa platforms, have revolutionized our abilities to both sequence and perform transcriptional analysis. These platforms enable extremely high-throughput analyses at a fraction of the cost of the first generation technologies, and in a much shorter time-period. We have begun applying these approaches at the Genomic Sciences Laboratory at NC State, to great success. These new technologies promise to rapidly expand our abilities to analyze many host nematode interactions. With the advent of fully sequenced reference genomes, it becomes possible to perform 2X skims of related parasite genomes, and to assemble these.

Distinctive Mitochondrial Genome Features of the Heteroderid Nematodes: Multipartite Structure and Extensive Poly(T) Variation within Protein-Coding Genes

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In virtually all animal mitochondrial genomes characterized to date, all mitochondrial genes are found on a single, circular molecule. However, in *Globodera pallida*, the mitochondrial genes are distributed on multiple circles, with individual circles having overlapping subsets of these mitochondrial genes. We are currently investigating the evolutionary origin of multipartism in the heteroderid nematodes. *G. rostochiensis* is very similar to *G. pallida*, with mitochondrial genes distributed on multiple circles, each about 8 kb in size. We have made initial investigations of the structure of the mitochondrial genome of *Heterodera glycines* and *H. cynodontis*. Although we lack complete data, we find no evidence for multipartism in *Heterodera* nematodes. This suggests that multipartism arose during the evolution of the Punctoderinae. We are currently investigating the mitochondrial genome of *Betulodera* to narrow down the origin of this unusual mitochondrial feature. During these investigations, we have noticed another novel mitochondrial feature, one that is similarly very rare amongst animals. Many protein-coding genes in *Globodera* have long poly(T) stretches, as many as 20 bases long. Further, when multiple copies of these genes are sequenced from the same cyst, we see variation in the number of Ts, with some representing frame-shift mutations that would result in the production of highly divergent proteins. The most common form is usually not a frameshift mutation, but frameshift versions can represent a significant proportion of the versions present. cDNA analyses and examination of EST databases indicate that these versions are not necessarily corrected, as cDNA and EST copies retain the frameshifts. This mitochondrial feature appears most pronounced in the *Globodera* mitochondrial genome, but also in the *Heterodera*. We also have preliminary evidence of T-variation in the mitochondrial ND4 gene of *Meloidogyne javanica*. Thus poly(T) variation is present in a broader evolutionary range of nematodes than is multipartism.

Connecting Genetics and Genomics in the Plant Parasite *Meloidogyne hapla*

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Focus on a model species has been crucial for rapidly advancing understanding of major groups of organisms. The root-knot nematode *Meloidogyne hapla* has several features that make it an excellent candidate to be a genetic and genomic model for plant parasitic nematodes. Not only is it an economically important and widespread pathogen, but it has a reproductive mode that allows controlled production of both parthenogenic and out-crossed progeny. This ability is highly advantageous for genetic analysis. We have developed inbred strains of this species and identified DNA markers that allow us to distinguish these strains. We carried out sexual crosses and took advantage of the parthenogenetic reproduction to produce 183 F2 lines segregating for DNA markers and pathogenicity traits. We monitored DNA marker segregation to produce a genetic linkage map with 280 markers. Traits for differences in pathogenicity, behavior, and attraction to specific hosts are segregating in the F2 lines and are being added to the genetic map. The DNA sequence has been determined for one of the parental *M. hapla* strains used in this genetic study (Opperman et al., this meeting) providing us with the needed resources to produce an integrated genetic and physical map. To this end we are determining the sequence of our DNA markers. These coordinated efforts will lead to molecular identification of key traits in the complex interaction between root-knot nematodes and their hosts. The anticipated resources should establish *M. hapla* as an important model system for future studies on nematode parasitism by our group and others and, also, as a resource for those groups investigating other nematodes, including the model nematode *Caenorhabditis elegans*.

Session Forty-one – Biological Control and Ecology of Nematode Antagonists

Convenors: Brian Kerry & Gregory Noel

Top-down Control of Nematodes in Natural Ecosystems

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Plant-feeding nematodes play a role in structuring the plant community composition in natural ecosystems. However, little is known on factors that regulate nematode abundance in nature. I will discuss results of a series of studies on factors that regulate the plant-feeding nematodes of the dune grass *Ammophila arenaria*. This grass is attacked by ectoparasitic, migratory endo- and sedentary endoparasitic nematodes. Each of these nematode species is regulated by several mechanisms, including bottom-up, horizontal and top-down control. For example, the sedentary endoparasite *Heterodera arenaria* is bottom-up controlled by the host plant *A. arenaria*. The presence of other endoparasitic nematodes regulated the timing of root attack by *Meloidogyne maritima*, whereas arbuscular mycorrhizal fungi (AMF) controlled numbers of *Pratylenchus penetrans*. Addition of microorganisms decreased the numbers of the ectoparasite *Tylenchorhynchus ventralis*. All in all, these combinations of control mechanisms resulted into a complicated protection of *A. arenaria* against plant-feeding nematodes.

I will also discuss nematode control in other natural systems. In a study on mid-succession grassland species, inoculation of soil microorganisms reduced the numbers of plant-feeding and plant-associated nematodes. Interestingly, the nematodes influenced aboveground interactions between plants, aphids and aphid parasitoids. Nematode addition reduced aphid offspring production independent of microorganism inoculation. Aphid populations were lowest in microcosms with combined nematode and microorganism inoculations. In contrast, parasitoid mortality and the proportion of males were significantly lower in microcosms with combined nematode and microorganism inoculations.

In another study on mid-succession grassland species, addition of nematodes reduced the biomass of grasses and increased the biomass of non-legume forbs. Addition of microorganisms and earthworms did not significantly affect numbers of plant-feeding nematodes. However, earthworm addition increased the tolerance of grasses to nematodes. We conclude that nematode control in nature depends on a variety of top-down, horizontal and bottom-up controls.

Bacteria as Natural Enemies of Plant-parasitic Nematodes

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Sustainable farming seeks to prevent soil erosion and contamination of groundwater and air. In order for sustainable production systems to become viable, control of economically important pests must be accomplished with minimal or no applications of pesticides. Management of nematodes may include crop rotation and genetic resistance, but often requires application of nematicides. Genetic resistance is not available for many crops, and when available, may not be durable. Development of biologically based alternative methods of nematode management for integration into sustainable crop production is needed. This paper will discuss biological control of nematodes with bacteria. Endophytic bacteria such as *Rhizobium etli* associated with induced resistance, rhizosphere inhabiting, toxin producing ectoparasitic bacteria such as *Pseudomonas synxantha*, and the endoparasitic bacteria, *Pasteuria* spp., will be considered.

Factors in Soil Suppressiveness of a Disease Complex

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Sudden death syndrome (SDS) of soybean, caused by *Fusarium virguliforme*, is synergistically increased by the presence of *Heterodera glycines*. These two pathogens combined account for the highest yield losses in soybean in the U.S. In Indiana, soils suppressive to this disease complex have been discovered under soybean monoculture in infestation trials with *F. virguliforme* and *H. glycines* in the majority of long-term trials. Concomitantly, foliar SDS symptoms and population densities of *H. glycines* were increased in preseason-fumigated plots compared to non-treated plots. This was novel because soil suppressiveness often is limited to pathogens of a single group of microbes, e.g., prokaryotes, basidiomycetes, hyphomycetes, or plant-parasitic nematodes. Objectives of this project were to determine how this suppressiveness develops and how it is maintained. In several trials, the effects of factorial infestation patterns with the pathogens on the development of suppressiveness were determined. In one trial, none of various cereal cover crops had negative effects on soil suppression, and plots remained in the same status of suppressiveness when bioassayed with soybean. Data from this project suggest that suppressiveness against the soil-borne SDS disease complex spontaneously develops under different conditions and that it withstands disturbances and thus will be useful for sustainable disease management.

Ecology of *Hirsutella rhossiliensis* and *H. minnesotensis* and their Biocontrol Potential against Plant-parasitic Nematodes

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Hirsutella rhossiliensis (Hr) and *H. minnesotensis* (Hm) are spore-producing hyphomycetes that parasitize vermiform nematodes. Hr has been observed on a number of species of nematodes throughout the world. Hm has been detected on the soybean cyst nematode (SCN) in the USA and China, and parasitizes all 15 species representing plant-parasitic, entomopathogenic, bacterial-feeding, and fungal-feeding nematodes in laboratory tests. Although the fungi can parasitize a wide range of nematodes, they are detected only on one or few species of nematodes in a particular field, indicating they have certain degree of specificity in parasitism. In some fields they parasitize a high percentage of a species of plant-parasitic nematode, and may be partially responsible for suppression of nematode population densities. Both fungi exhibit density-dependent parasitism; spatial and temporal changes of percentage of nematodes parasitized correlate positively with host nematode population density. Although the two fungi can grow on artificial media, they have little saprophytic ability in natural field soils. A number of abiotic factors associated with their parasitism of nematodes have been studied. For example, optimum temperature for parasitism of nematodes by Hr is around 25°C, and optimum pH for growth and sporulation of both fungi on agar is about 6.2. Percentage of SCN second-stage juveniles (J2) parasitized by the fungi is correlated negatively with soil pH and positively with sand. Tillage has little effect on percentage of SCN J2 parasitized by the fungi in Minnesota, USA, although laboratory study shows that disturbance of soil reduces their infectivity. The effectiveness of biocontrol using the two fungi has been evaluated on a number of nematodes in greenhouse and fields. Both fungi effectively lowered population densities of cyst and root-knot nematodes in greenhouse studies. In field experiments, both fungi reduced nematode population densities and increased crop yields sometimes, but not always.

Ecology of *Pochonia chlamydosporia* in the Rhizosphere

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Pochonia chlamydosporia is a facultative parasite of a range of nematode pests and has been widely studied as a biological control agent. It has two developmental phases of growth in the rhizosphere feeding initially on the exudates from plant roots before contact with cyst and root knot nematodes induces parasitic development and the colonisation and destruction of their eggs. The fungus exists as different biotypes based on their host preference, which affects their population dynamics on the roots and the parasitism of nematode eggs. Using selective primers and a range of PCR-based methods, competition between fungal biotypes in the rhizosphere and their selection during the saprotrophic and parasitic phases has been studied. The nutrition of the fungus has a marked effect on parasitic activity and there is no simple relationship between the abundance of the fungus in the rhizosphere and its efficacy as a biological control agent. The factors affecting the transition from saprotroph to parasite are poorly understood but the regulation of some enzymes involved in the infection process is known to be affected by nutrient levels in the environment. In *in vitro* bioassays, there appears to be a fitness cost for virulence in that virulent biotypes are less rapid colonises of soil. However, in longer-term trials in pots, slower growing virulent isolates parasitise more eggs and a number of factors appear to be important in determining the host parasite relationships of this fungus. Understanding these interactions will help develop more robust biological control strategies.

Session Forty-two – Root-knot Nematodes

Convenors: Janete Brito & Regina Carneiro

Root-knot Nematode Problems of Greenhouse Crops in Korea

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Root-knot nematode (RKN) problems are more severe in horticultural crops under greenhouse than in open field crops in Korea. The RKN damage is enhanced by consecutive monoculture for year-round cash crop production. *Meloidogyne incognita*, *M. arenaria*, *M. hapla*, *M. javanica*, *M. cruciani* and *M. hispanica* are the six species of RKNs reported in Korea. Recent studies on the RKN distribution in Korea suggested a presence of another species which showed unknown enzyme phenotypes of malate dehydrogenase and esterase. Surveys conducted since the 1980's to date revealed an interesting change in trend of dominant species in greenhouse soils from *M. incognita* in 1980's to *M. arenaria* in 2000's. Another observation is that *M. javanica*, a tropical species is frequently found in greenhouses in the temperate area located at 37°N. The major RKN species causing damages in open field crops, vegetables and medicinal plants is *M. hapla*. The most serious case of RKN problem is in the production of fruit vegetables, especially oriental melon under greenhouse conditions. Oriental melon is one of the most important cash crops in Korea produced year-round under the forcing culture system. Some greenhouses are consecutively used for over 10 to 20 years, thus contributing to the high density of RKNs. Due to the short period between harvesting and planting, it is not feasible to develop an effective RKN management scheme. Various research approaches are underway to control RKNs; screening of resistant varieties, cultural methods, and biological control using *Pateuria* spp. Integrated RKN management strategy is needed for environment-friendly and sustainable agricultural system.

Root-knot Nematode Species and Diseases in China

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Root-knot nematode is a most important plant parasitic nematode group in China. The species and diseases caused by the root-knot nematodes are outlined here. As in other countries, *Meloidogyne incognita*, *M.javanica*, *M.arenaria* and *M.hapla* are the most common and important. Some species described are from rice, fruit tree, potato, and forest plants. The distribution and importance of *M.enterolobii* are discussed. The root-knot nematode diseases on vegetable, fruit tree, tobacco and rice are very serious, and management measures include the application of nematicides, resistance cultivars, agricultural practice and biological control. (Supported by Ministry of Agriculture in China, Project No. nyhyzx07-050)

Root-Knot Nematodes of Economic Importance in Egypt

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Root-knot nematodes (*Meloidogyne* spp.) have been recognized as major pests in Egypt, causing yield losses to many crops of economic relevance. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the most frequent species and occur in most governorates. *Meloidogyne hapla* has been reported only in certain areas in the Delta region and Nubaria north of Egypt in sugarbeat and peanut. Survey data involving 5,000 samples from agronomic crops, vegetables and fruit trees from various cultivated areas in Egypt indicated that these root-knot nematodes are associated with galls, stunting and poor growth in 65% of the sampled crops. Affected crops by these pests included cucumber, pea, pepper, squash and tomato among the vegetables, and cotton, corn, peanut, sunflower and strawberry among the other crops. Fruit-trees damaged by these root-knot nematodes are banana, peach and grapevine. *Meloidogyne incognita* is rarely found in date palm. *Meloidogyne artiellia*, which is a major damaging nematode to leguminous crops in North Africa, has not been detected in Egypt. Most new cultivated land in the desert North of Egypt, which is cultivated mainly with banana plantation, fruit trees especially peach, sweet pepper, ornamentals, strawberry, and grapevines are infested with different species of root-knot nematodes causing reduction in crop growth and productivity at different levels according to the nematode species and the infested crops. The new valley in upper Egypt is heavily infested with *M. incognita,* especially in cucurbitaceae producing areas. Studies aiming at a more accurate determination of the identity of root-knot nematodes in Egypt are in progress using morphological and molecular methods.

Two Emerging Species of Root-Knot Nematodes in Florida, USA: *Meloidogyne mayaguensis* and *M. floridensis*

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Meloidogyne mayaguensis was first reported in the United States in 2002 and *M. floridensis* was first described in Florida in 2004. *M. mayaguensis* is reported in 25 counties in Florida and *M. floridensis* in five. These nematode species are of great concern because of their ability to overcome root-knot nematode resistance genes. *M. mayaguensis* is capable of overcoming the resistance of *Mi-1*, *N*, *Rk*, and *Tabasco* genes in tomato, bell pepper, cowpea, and sweet pepper, respectively, and unidentified genes in soybean cultivars. *M. floridensis* reproduces well on the root-knot nematode resistant peach rootstocks Flordaguard, Nemaguard, Nemared and Okinawa. Reported hosts of *M. mayaguensis* include: (i) vegetables − *Brassica oleracea* (broccoli, cabbage, mustard), *Citrullus lanatus*, *Curcubita pepo*, *Ipomoea batatas* and *Solanum melongena*; (ii) ornamentals − *Ajuga reptans*, *Amaranthus tricolor*, *Brugmansia* spp., *Buddleja davidii*, *Callistemon citrinus*, *C. viminalis*, *Caryopteris clandonensis*, *Clerodendrum ugandense*, *Gardenia* sp., *Hibiscus grandiflorus*, *Lantana camara*, *L. montevidensis*, *Myrica cerifera*, *Petunia* sp., *Plectranthus scuetellarioides*, *Salix sepulcralis*, *Solandra maxima*, *Tecomaria capensis*, *Tibouchina x* compacta, *Tibouchina x elegans* and *Washingtonia* spp.; (iii) cover crops − *Canavalia ensiformis* and *Vicia sativa*; (iv) herbs − *Ocimum basilicum*; (v) fruit trees − *Psidium guajava* and *Carica papaya*; (vi) trees − *Paulownia elongate* and *P. tormentosa*; and (vii) weeds − *Amaranthus retroflexus*, *Bidens pilosa*, *Brassica kaber*, *Eclipta prostrata*, *Fatua vilosa*, *Panicum* sp., *Poinsettia cyathophora*, *Portulaca oleracea*, *Senna obtusifolia* and *S. americanum*. Reported hosts of *M. floridensis* include: (i) vegetables − *C. lanatus*, *Cucumis sativus, C. pepo*, *S. melongena*, *Phaseolus vulgaris* and *S. esculentum*; (ii) ornamentals − *Antirrhinum majus*, *Dracena sp.*, *Hibiscus* sp., *Impatiens wallerana*, *Verbena hybrida*; (iii) cover crops − *Brassica napis* and *Trifolium incarnatum*; (iv) herbs - *Anethum graveolens* and *O. basilicum*; and (v) weeds − *Emilia sonchifolia*, *Ipomoea quamoclit* and *Nasturtium officinalis.* Reported hosts of both nematodes include: *Abutilon theophrasti*, *Amaranthus retroflexus, A. spinosus*, *Cucumis anguria*, *Cnidoscolus stimulosus*, *Dichondra repens*, *I. Violacea*, *I. triloba*, *Leonotis nepetaefolia* and *Phytolacca americana*. Some isolates of *Pasteuria penetranas* were compatible with *M. floridensis* but incompatible with *M. mayaguensis*.

Meloidogyne mayaguensis and *M. ethiopica,* the Major Root-knot Nematodes Parasitizing Guava and Grapevine in Central and South America

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The worst nematode problem affecting guava is that created by *Meloidogyne* spp., which recognized AS A limiting factor in commercial guava production in Central and South America. Considering the difficulty of identifying *Meloidogyne mayaguensis* only by the perineal pattern, this species has been misidentified in different regions around the world. Severely infected trees are associated with general chlorosis, nutrient deficiency symptoms increase rapidly, reduced flowering and fruiting, culminating in the death of the plants. This nematode is making cultivation of guava in the infested areas unfeasible, causing serious economic problems to growers and the economy of the region. Recently, a survey made in Chile using esterase isozyme indicated the wide distribution of *M. ethiopica* in about 80 % of samples on grapevine, kiwi and tomato. The nematode occurred over a range of 1,000 Km, including all nurseries sampled. Contaminated nurseries have resulted in serious infestation by *M. ethiopica* in vineyards in various places in Chile and the introduction of this nematode in Rio Grande do Sul State, Brazil on kiwi seedlings. *M. ethiopica* is a highly aggressive species in these more sensitive grape varieties (Chardonnay, Sauvignon Blanc and Pinot) causing significant mortality rates, or in the best of cases, rendering its host plant virtually non-productive. In this presentation we will focus on distribution, hosts, damage, source of resistance and antagonistic plants to be used in management strategies to control these two nematodes.

SESSION FORTY-THREE – MARINE AND FRESH WATER NEMATODES

CONVENORS: JULIA ZOGRAF & ZHI NAN ZHANG

Some Genera and Species of the Enoplids (Enoplida: Thoracostomopsinae) from the Sea of Okhotsk and the Sea of Japan

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The subfamily Thoracostomopsinae is a diverse group of large nematodes - predators that feed on different invertebrates. Many species of the thoracostomopsids discovered in the last centuries were improperly described. This report deals with the comparative anatomy of the head and male reproductive system of the marine Thoracostomopsinae based on a collection of free-living marine nematodes of the East of the Sea of Japan and the Sea of Ochotsk of Russia. Detailed description of ten species belonging to genera *Oxyonchus, Enoplolaimus, Mesacanthion, Epacanthion, Enoploides,* and *Paramesacanthion* is given. Seven out ten species are named as new. Careful examination using methods of confocal (CM), light (LM), and scanning electron microscopy (SEM) allowed us to uncover a number of morphological characters to which little attention has been paid by former researchers. The usage of methods of CM and SEM also permitted us to revise the phylogenetic significance of some morphological features including labial region and stoma as well the structure of male copulatory apparatus.

Environmental Factors Affecting Nematode Community Structure in the Changjiang Estuary and its Adjacent Waters

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A total of 21 meiofauna groups was collected from 18 stations in the Changjiang River estuary and its adjacent waters. Meiofauna abundance ranged from 76±44 ind.10cm^{-2} to 5510±2497 ind.10cm^{-2}. Nematodes dominated the samples at all stations, ranging from 61.3% to 96.8% of total meiofauna. A total of 263 nematode species or morpho-species belonging to 119 genera and 29 families was identified at the studied stations. Xyalidae (34.8%), Comesomatidae (16.9%), Linhomoeidae (14.8%), Axonolaimidae (6.7%) and Chromadoridae (4.6%) were the most abundant nematode families at all stations, accounting for 77.8% of the total nematode fauna. The most abundant genera were *Daptonema*, *Cobbia*, *Sabatieria*, *Dorylaimopsis* and *Terschellingia*, accounting for 50.0%. The most abundant species were *Cobbia* sp1, *Dorylaimopsis rabalaisi*, *Daptonema* sp3, *Filitonchus* sp2, *Parodontophora marina*, *Microlaimus* sp2, *Daptonema* sp5, *Cobbia* sp3, *Axonolaimus* sp1 and *Spilophorella* sp2, accounting for 52.8%.

The area exhibited studied high nematode abundance and high species biodiversity. Measurements of environmental factors were made, including grain size, salinity, temperature, sediment organic matter content, Chl-a and Pheo-a. Different combinations of environmental variables are responsible for the meiofauna and nematode communities' structures. However, BIOENV results indicate that water depth, salinity, Chl-a, Pheo-a and silt-clay content were more closely linked to variation in meiofauna (mainly, nematode) community structure in the studied area. Among these, water depth, salinity and Pheo-a were most responsible for nematode assemblage discrimination in the studied area.

Free-living Marine Nematode Communities from Patagonian Coastal Lagoons

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This paper describes a baseline study on nematode biodiversity from three different latitudes (Península Valdes, 43ºS; San Julián, 49ºS and Río Gallegos, 51ºS in Chubut and Santa Cruz provinces) contrasting coastal upper littoral marine areas with saline coastal lagoons in Patagonia. The lagoons vary in salinity from hypersaline to almost fresh water. The objective of the study was to give the first inventory of the fauna of these habitats, in particular a detailed study of the dominant taxa the free-living marine nematodes.

Fitfteen lagoons and three upper littoral coastal areas have been sampled at the three latitudes. The samples have been fixed in 5% formalin with rose Bengal in the field. The geographical position (GPS), salinity and granulometry, have been taken. Nematodes were extracted using the elutriation/decantation/LUDOX TM method and specimens mounted on slides.

The Nematodes found were identified up to the lowest taxonomy level possible, and photographs were taken using a Photomicroscope. For nematode identifications, the keys of Platt & Warwick and Warwick et al. were used. The differences in nematode species composition between latitudes and environments were analysed by multivariate methods.

The results indicate that salinity and elevation above sea level seem to be the main environmental variables that explain the complex distribution found. Lagoons near the sea have similar nematode fauna to coastal littoral sites, and inland lagoons have a particularly extreme nematode fauna associated with the salinity regimes. Detailed information about species found plus dominant genera at each latitude will be presented.

This project was supported by Royal Society of London, UK and CONICET, Argentina.

Seasonal Fluctuations in Size Spectra and Composition of the Microphytobentos and Nematode Communities in the East of the Sea Of Japan Intertidal Sandflat

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The results of qualitative and quantitative investigation carried out on the composition, density, and distribution of microphytobentos and meiofauna in sandy sediments of the intertidal zone of the East of the Sea of Japan (Biological Station 'Vostok' 42°52′ N, 132°44′E). Temporal dynamics of the nematode community and microphytobenthos were observed from May 2006 to May 2008. Generally, 5 (dinoflagellates, diatoms, chrysophytes, eugleophytes, cryptophytes) taxa of microphytobentos and 11 taxa of metazoan meiofauna were found. Nematodes (20 species) were dominant comprising 70 – 90% of the community. Seasonal variation was pronounced for the different nematode groups with the peak of abundance in spring, summer or autumn and minimum abundance in winter and it were likely caused by temporal food changes as well as temperature and salinity changes. Particular emphasis was given to the reproduction, life history, and feeding strategy of marine nematodes (*Metadesmolaimus sp.*, *Lauratonema juncta*, *Microlaimus sp.* and *Enoplolaimus sp.*). The dominant species were noted to be in reproductive state throughout the year whereas the less dominant had restricted breeding periods and are well spaced. Aspects of the feeding ecology dominant species were considered. The study deals with the possibility of trophic link between microphytobentos and nematodes in fine sandy sediments. Analysis of nematode intestine revealed small numbers of ingested microalgae.

Study of Nematodes in the Family Tripylidae, Oerley 1880 from New Zealand

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Nematodes of the family Tripylidae, Oerley 1880 mainly occur in freshwaters and soils. The genera *Tripyla* Bastian, 1865 (= *Promononchus* Micoletzky, 1923, *Paratripyla* Brzeski, 1963), *Tripylella* and *Tripylina* Brzeski, 1964 are included in thi`s family. To date, there are eighteen valid species in *Tripyla*, one species in *Tripylella* and six species in *Tripylina*. Only *Tripyla affinis* de Man, 1880 and *Tripylina stramenti* (Yeates, 1972) Tsalolikhin, 1983 have been reported from New Zealand. Since March 2007, the nematodes in the family Tripylidae were investigated in the Waitakere Ranges Regional Park, Coromandel Range, Rotorua, Cambridge, Hamilton regions and the Arthurs Pass National Park in New Zealand. A total of 80 soil and litter mixture samples from native forests have been examined. On morphological grounds (e.g. nematode body length, width; inner, outer labial and cephalic sensillae shape and length; the number of cervical setae and its distance from anterior end; the shape and position of the dorsal tooth, amphid; female vulval position and the structure of reproductive system, tail length, width and shape, etc.) some seven species of Tripylidae have so far been discriminated.

SESSION FORTY-FOUR – GENE KNOCK-DOWN APPROACHES IN NEMATODE RESEARCH

CONVENORS: GODELIEVE GHEYSEN & RALF SOMMER

Genetic and Transgenic Approaches in the Nematode Model *Pristionchus pacificus*

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Pristionchus pacificus has been established as a model system in evolutionary biology, in particular in evolutionary developmental biology (evo-devo). *P. pacificus* shares with *Caenorhabditis elegans*many technical features starting form self-fertilizing propagation with the spontaneous generation of males, which allows the use of forward genetic technology. Under laboratory conditions, the life cycle of *P. pacificus* is 3-4 days (20° C) on *Escherichia coli* as food source. The genome of *P. pacificus* has recently been sequenced and is substantially larger than the one of *C. elegans* or *Brugia malayi*. Because studies on the regulation of vulva development revealed many important differences between *P. pacificus* and *C. elegans*, we have started to establish a comprehensive tool kit for functional studies in these nematodes. In the talk, I will provide an overview on the state of reverse genetics by RNAi and morpholino studies, forward genetics, genomics and DNA-mediated transformation.

Gene Silencing and Neuromuscular Signalling in Plant Parasitic Nematodes (PPNs)

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PPNs represent a serious threat to sustainable crops, accounting for more than $US 150 billion in annual agricultural loss worldwide. We have discovered that neuronally-expressed genes in both *Meloidogyne incognita* and *Globodera pallida* infective juveniles (J2s) are particularly susceptible to RNAi, and that silencing can be induced through simple soaking procedures, resulting in the profound inhibition of nematode motility. One class of these neuronal targets is the FMRFamide-like peptides (FLPs), a large family of neuropeptides intimately involved in the control and regulation of neuromuscular function; unusually diverse and abundant in the phylum Nematoda. Many of the drugs used to control nematode parasites do so through disruption of neuromuscular function, and as the neuropeptide complement is extraordinarily enriched, and conserved among nematodes, we argue that neuronal signalling processes harbour significant potential as targets for novel *in planta* control strategies. However, as specificity concerns become realized in a range of model organisms, we now demonstrate an atypical response to dsRNA in a plant parasitic nematode for the first time. Negative control dsRNAs, that shared no significant regions of homology with identifiable nematode targets, were created from a range of plant, and non-plant-derived constructs, and used to soak the worms. Although the control dsRNAs had no observable affect on *G. pallida* J2 behaviour, they induced significant phenotypic changes in *M. incognita* J2s.

Transgene Driven RNAi for Cell Specific Knock-down of Gene Function in Targeted *C. elegans* Neurons

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The nematode *C. elegans* has become an important model for understanding how genes influence behavior. However, in this organism the available approaches for identifying the neuron(s) where the function of a gene is required for a given behavioral trait are time consuming and restricted to non essential genes for which mutants are available. We have described a simple reverse genetics approach for reducing, in chosen *C. elegans* neurons, the function of genes (Esposito *et al.,* 2007). The method is based on the expression, under cell specific promoters, of sense and antisense RNA corresponding to a gene of interest. By targeting the genes *osm-10*, *osm-6* and the Green Fluorescent Protein gene, *gfp*, we showed that this approach leads to efficient, heritable and cell autonomous knock-downs of gene function, even in neurons usually refractory to classic RNA interference (RNAi). By targeting the essential and ubiquitously expressed gene, *gpb-1*, which encodes a G protein □ subunit, we identified for the first time two distinct sets of neurons in which the function of *gpb-1* is required to regulate two distinct behavior: egg-laying and avoidance of repellents. The cell specific knock-downs obtained with this approach provide information that is complementary to that provided by the cell specific rescue of loss of function mutations and represents a useful new tool for dissecting the role that genes play in selected neurons. We are now extending the use of this method to approach questions of neuronal development and to study the neuronal function of essential genes.

Wnt Signals and Frizzled Activity Regulate Anterior-posterior Neuronal Polarity in *C. elegans*

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Neurons are highly polarized cells with distinct functional domains such as axons and dendrites. The polarity of a developing neuron determines the precise exit point of its axon as well as the initial trajectory of axon outgrowth. We used *C. elegans* PLM mechansosensory neurons to understand how anterior-posterior neuronal polarity is achieved.

PLM (left and right) is a bipolar neuron whose cell body is located in the lumbar ganglia. Two processes extend in opposite directions from the cell body. A long anterior process extends to the midbody region; a short posterior process extends toward the tail. We used a GFP reporter transgene to follow the trajectories of PLM processes, and to identify mutants in which antero-posterior neuronal polarity was compromised.

We found that LIN-17/Frizzled and LIN-44/Wnt regulate the polarity of the PLM neuron. In *lin-17* and *lin-44* mutant animals the PLM anterior process was much shorter than the wild-type while the posterior process was much longer (n>100). This inversion of polarity was visible in the embryo soon after PLM was born. In wild-type embryos, PLM extended the main process with the growth cone anteriorly. In *lin-17* and *lin-44* mutants, the main process with the growth cone extended posteriorly. The *lin-17* defect was rescued by a transgene expressing LIN-17 selectively in PLM and few other neurons, suggesting that LIN-17 acts cell-autonomously in PLM (n>100). By tagging LIN-17 cDNA with the red fluorescent protein mRFP1, we found that this receptor was asymmetrically localized on the PLM posterior process (n=20). We propose that Wnt and Frizzled genes are essential determinants for proper orientation of neuronal polarity.

Understanding Root-knot Nematode Development using RNAi

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We and others have recently shown that the host-generated dsRNA can efficiently trigger RNAi, and thus can disrupt gene function, in plant-parasitic nematodes[1-4]. Our laboratory currently uses this technology to identify genes that are essential for nematode development. As an initial step, we focused on two groups of genes from the root-knot nematode, *Meloidogyne incognita*: 1) potential homologs of *Caenorhabditis elegans* genes involved in gut development, and 2) genes expressed in secretory glands[5]. So far, we have tested eleven genes – three from the former and eight from the latter. Tobacco plants expressing dsRNA of all the eleven genes show significant resistance to root-knot infection. Time course analysis revealed disappearance of the corresponding endogenous mRNA as early as five days post-inoculation. Upon microscopic examination, we found the nematodes lacking secretory gland mRNAs arrested at various larval stages. Involvement of secreted proteins in larval development is intriguing. Perhaps the development of these organisms requires continuous interaction with the host, and some of the secreted proteins are part of such an interaction.

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Session Forty-five – Industrial Production of Biological Control Agents Involving Nematodes

Convenors: Ralf-Udo Ehlers & Richou Han

Mass Production of Entomopathogenic Nematodes: Production Technology and Costs Define Potential Markets

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Entomopathogenic nematodes (EPN) can be produced *in vivo* and *in vitro*. *In vivo* culturing involves high costs in labour making this method unsuitable for markets in industrialised countries. *In vitro* culturing is possible on solid and liquid media. Major quantities of EPN nowadays come from liquid culture. Production technology has been significantly improved during the past decade due to a better understanding of the life cycle of EPN. Major targets for process improvement are the reduction of process time, increase of dauer juvenile density and quality. After mass production nematodes are harvested by centrifugation, sifting or passage through hydrocyclons. Formulation aims at prolongation of shelf life and preservation of dauer juvenile quality. The short shelf life of EPN is the major bottleneck for sales in larger markets. Genetic and technical improvements are measures to enhance shelf life and quality. Production costs are related to potential future markets for EPN application.

Functional Significance of Symbiotic *Xenorhabdus* and *Photorhabdus* Bacteria in Nematode Reproduction

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Entomopathogenic *Steinernema* and *Heterorhabditis* nematodes, associated with their *Xenorhabdus* and *Photorhabdus* symbiotic bacteria are used as a commercial bioinsecticide for many economically important insect pests. The interaction between these nematodes and their symbiotic bacteria during reproduction is very important in the commercial production of this bioinsecticide. Functional significance of these symbiotic bacteria in nematode reproduction has been explored at least in the four levels: food signals, nutrients, trans-specific toxins, and bacterial colonization. Food signals are the most important materials produced by the symbiotic bacteria. After invasion of an insect host, the IJ exits from the infective stage into a feeding stage as a response to yet unknown signals in the hemolymph of the insect. The symbiotic bacteria produce such food signals in artificial media which also trigger the recovery of the IJs. One significant character of the symbiosis between entomopathogenic nematodes and their bacteria is the nutritional dependence of the nematodes on their bacterial associates. Nutrient suitability of a symbiotic bacterial isolate is also important for heterologous nematode strains if no toxic effect from the bacteria on the nematode is observed. It was observed that incompatible bacterial isolate usually show toxic effect on the non-symbiotic nematode (trans-specific activity). Attempts were made to identify the genes involved in the trans-specific nematicidal activity of *P. luminescens* LN2 for *H. bacteriophora* H06. Bacterial colonization in the intestines of IJs is an important process in the symbiosis. Significant differences were reported in the ability of the nematodes to retain the bacterial cells in the intestines of the IJs. Genes required for nematode colonization have been identified.

Comparative Analysis of the Biology of *S. capocapsae* and *S. feltiae* in Liquid Culture

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Steinernema capocapsae and *S. feltiae* are biological control agents produced in monoxenic liquid culture. Their symbiotic bacteria *Xenorhabdus* spp. were cultured prior to inoculation of dauer juveniles (DJs). The DJs recover from the arrested stage and develop to adults. The influence of the DJ and bacterial density on the recovery and development of the nematode population was investigated. A higher bacterial density significantly increased the induction of DJ recovery. Variable DJ inoculum densities ranging from 1,000 to 10,000 DJs/ml at constant bacterial density had no impact on recovery. However, recovery of *S. carpocapsae* was variable among experimental batches, ranging from 56 to 80%. In contrast, recovery of *S. feltiae* was less variable reached approximately 90% in all batches. The fecundity of parental females was reduced at higher nematode inoculum density. The first generation progeny juveniles of both species developed directly to DJ at higher inoculum density, while the progeny in lower inoculum density continued development to another reproductive cycle. According to the relation of recovery and fecundity, the optimal inoculums density is 5,000 DJs/ml for *S. capocapsae*, and 6,000 DJs/ml for *S. feltiae*. In order to define the optimal process temperature, DJs of both species, *S. carpocapsae* and *S. feltiae* were inoculated at 4,000 DJs/ml and incubated at 25°, 27°, 29° and 31°C for *S. carpocapsae*, and 20°, 23°, 25° and 27°C for *S. feltiae*. Recovery was constant among populations incubated at different temperatures. However, a delay of growth was observed for *S. feltiae* at 20°C. The fecundity of both species was suppressed at highest temperatures. At the highest temperature, the DJ yield of both species reached only half of the density recorded in cultures at lower temperature on 15 dpi.

Stability Issues: Maintenance of Beneficial Traits in Entomopathogenic Nematodes

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A number of beneficial traits such as virulence, reproductive potential, and environmental tolerance are key factors in determining an organism's ability to produce high levels of efficacy in biological control. Deterioration or loss of beneficial traits during laboratory or industrial culture production is detrimental to biocontrol efficacy. During in vivo production, both partners in the nematode-bacterium complex can undergo change, which contributes to reduction in beneficial traits. The nematode's bacterial symbiont also deteriorates when repeatedly cultured in vitro. Changes in nematode beneficial traits may be due to genetic or non-genetic (e.g. nutritional) causes. Methods to deter trait deterioration include maintenance or improvement of media, creation of selected inbred lines, or improved cryopreservation techniques.

Mass Production of the Rhabditid Slug Parasite *Phasmarhabditis hermaphrodita*

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The rhabditid nematode *Phasmarhabditis hermaphrodita* is a slug parasitic nematode that is sold in several European countries as a biological control agent for slugs under the tradename Nemaslug®. Mass production is achieved using fermenter technology developed for entomopathogenic nematodes (EPNs). However, the biology of *P. hermaphrodita* is very different from EPNs posing some challenges for mass production. We review the biology of *P. hermaphrodita*, its various life-cycles and its relationship with bacteria and discuss the challenges posed in mass-production and possible solutions.

SESSION FORTY-SIX – CEREAL AND POTATO CYST NEMATODES

CONVENORS: SUE TURNER & JAVIER FRANCO PONCE

An Overview of the Status of Cereal Cyst Nematodes on Wheat and their Management through Host Plant Resistance

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On wheat three main species are documented to be the most economically important: *Heterodera avenae*, *H. filipjevi* and *H. latipons* which belong to the cereal cyst nematode complex. *H. avenae* is the most widely distributed species being found in less temperate regions throughout the world. *H. latipons* is essentially only Mediterranean in distribution, however, has also been found in northern Europe and Iran. *H. filipjevi*, formerly know as Gotland strain of *H. avenae* appears to be found in more continental climates but also Greece and India.

Yield losses caused by these three species on wheat range from 15-96% in various studies conducted around the world. Greater yield losses are found where drought conditions prevail in sandy soils, and with wheat monoculture cropping systems, which are commonly practised through North Africa and Western Asia.

Although rotation with non-cereals offers an effective control method, in many countries this option is limited. The most economically and environmentally sustainable option is the use of host genetic resistance in wheat. However, CCN is complex with several species and pathotypes within species. To date nine single dominant genes known as *Cre* genes have been identified in wheat and its wild relatives (*Aegilops spp.)*, however their effectiveness is dependent the species and pathotypes prevailing in a given region. Effective control through host genetic resistance has been achieved in Australia with the release of commercially resistant cultivars. Work in many other countries has also identified new sources of resistance and effective genes, and some of these have been deployed in breeding programs (eg Raj1 in India). A newly formed initiative known as the International Cereal Cyst Nematode Initiative (ICCNI) has been established in 2007 with CIMMYT, several leading ARIs and NARs partners including Turkey and China to work collectively on greater understanding and sustainable control of CCN on wheat.

Cereal Cyst Nematodes: A Threat and Challenge to Food Safety in China

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Wheat is a major food staple in China who produces the largest volume of wheat (120Mt/year) with average yields of 4t/ha. Recent research has identified a widespread distribution and high population density of the Cereal Cyst Nematode (*Heterodera spp.).* New survey data in 2006-2007 in the Northwest China (Gansu, Shanxi, Ningxia and Qinghai) has found CCN in all provinces (58% of 168 soil and root samples). With this new finding CCN (*H. avenae*) has now been reported from 11 provinces in China also including Hubei, Henan, Hebei, Beijing suburb, Inner Mongolia, Anhui, Shandong. The populations of CCN in these drier northern provinces such as Qinghai were 14-37 eggs/g soil, with an average density of 29 eggs/ g soil. The CCN incidence in the higher rainfall central provinces of Hubei, Beijing, Hebei and Henan is high ranging from, 64-98%, with the range of density in these 4 provinces ranging from 0.6-50 eggs/g soil, with an average 5 eggs/g soil. Overall in China the population density is much greater compared to other countries where CCN has been reported to cause economic damage in wheat.

Preliminary yield loss trials in 2005-06 and 2006-07, using Aldicarb 15G® (22.5kg/ha) which provided around 60% control of CCN revealed the losses on common Chinese wheat cultivars to range from 0.42%-17.34% in Hebei and 10-40% in Henan.

Molecular characterization of 150 selected populations from Qinghai, Inner Mongolia, Henan, Hebei using RFLP digest of the amplified rDNA-ITS region revealed a close relationship to “type B” of *H. avenae*. One hundred and fifty ITS amplification products were cloned and sequences were lodged in the GeneBank, to study the phylogeny and relationships between CCN populations. The CCN National research network cooperation with CIMMYT International, Australia (SARDI) and Belgium has been established. Work is underway to investigate the biology, economic losses, resistance, pathotype, and management methods for CCN, and China is a key member of the recently formed International Cereal Cyst Nematode Initiative (ICCNI).

Progress in the Quantification of Potato Cyst Nematodes

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The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* occur in many potato growing regions world-wide and their widespread occurrence is of increasing concern to growers. Monitoring and detecting their presence in seed and ware land is of importance to limit their spread and for deployment of appropriate management strategies. To improve the efficiency of detection and to enable quantification of the two species, a quantitative PCR assay has been developed. The assay has been used with DNA extracted from three types of samples; pure cysts, soil 'floats' and soil to determine sensitivity and specificity parameters. The application of the assay to an experiment to determine how the two species of PCN compete on potato genotypes with differing degrees of resistance is described. The use of the assay for the detection and quantification of the two PCN species in typical statutory samples is demonstrated. In addition the potential to use the assay for soil samples will be discussed.

The Potential for Management of Potato Cyst Nematodes with Bio-fumigants and Bio-stimulants

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In recent years the control of potato cyst nematodes (*Globodera rostochiensis, G. pallida*) (PCN) have been heavily reliant on synthetic nematicides, resulting in good marketable potato yields, but not necessarily reducing PCN levels. Following the withdrawal of many nematicides a number of plant-based products are now becoming available for agricultural use with claims to either kill a range of plant pests or to act as a plant bio-stimulant, enabling crops to better tolerate pest attacks.

Greenhouse pot trials with green manure products, claimed to possess bio-fumigant properties against plant parasitic nematodes, evaluated the effect of their incorporation on PCN development. Blended mustard (*Brassica* spp.) and rocket (*Eruca sativa*) plants were incorporated into PCN infested soil together with a potato tuber. PCN multiplication and residual contents were assessed at plant senescence. The *Eruca sativa* treatment showed differences between the two PCN species, with both cyst reproduction and viable contents of *G. pallida* being lower than untreated controls; whereas the effects on *G. rostochiensis* were not so apparent. The *Brassica* spp. treatment resulted in more variation within both PCN species and may have been affected by exceptionally high greenhouse temperatures.

Field trials evaluated the effect of additional plant-based bio-fumigants/stimulants on *G. pallida* population levels and resulting potato yields and quality. Two formulations contained seaweed bio-stimulants, and one bio-fumigant mustard and chilli pepper extracts. These were compared with a fumigant nematicide and untreated controls. The effect of *G. pallida* on growing potato crops was assessed by recording haulm lengths and weights. All assessments indicated that the nematicide and the mustard/chilli pepper formulation treatments gave most protection to plants and were more effective at reducing PCN levels.

Preliminary trials indicate that these plant-based products have the potential to accelerate PCN decline within an integrated management system once optimum treatment regimes are further clarified.

An Update on the New Discovery of *Globodera pallida* in the USA

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Idaho is the largest potato producer in the United States, growing about one-third of the country's potato production (12.5 billion pounds), which earned farmers about US$700 million and was worth about US$2 billion to the state. In 2006 cyst nematodes were discovered in tare dirt at a potato (*Solanum tuberosum*) processing facility in eastern Idaho. The morphology of cysts and second-stage juveniles, together with molecular analyses, established the identity of the species as the pale cyst nematode *Globodera pallida* (Stone, 1973) Behrens, 1975. Discovery of *G. pallida* in Idaho was significant to potato producers and exporters since it can attack the potato roots and reduce yields by up to 80% and severely restrict trade. Early discovery of PCN in Idaho provided the opportunity to implement an efficient management / elimination program that could minimize future potato production costs and enhances product quality and marketability. Though PCN is widely distributed in many potato-growing regions throughout the world, its infestation in Idaho appears to be isolated. Additional surveillance programs have been initiated to contain further spread of the pest to neighboring fields within the vicinity. Regulations were implemented to restrict the movement of plants and soil with appropriate sanitation procedures for equipment used on the regulated field for the spread of this nematode. Appropriate crop rotation and the use of certified seed and nematicides are an effective and practical strategy to enhance PCN decline rates. This, together with regular sampling to monitor PCN viability levels in the infested regions, is the basis for the eradication program now in place.

This PCN discovery is an example of how industry and government can come together to establish a co-ordinated management program to deal effectively with this newly introduced legislated pest.

SESSION FORTY-SEVEN – NEMATODE BARCODING, BIODIVERSITY AND ECOLOGY
CONVENORS: ANTOINETTE SWART & TOM POWERS

A New *Globodera* Cyst from South Africa

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Nematode cysts from potato fields in the Sandveld area in South Africa were collected during a delimiting survey for the presence of potato cyst nematodes in the region. Morphological and molecular analysis of the cysts confirmed the presence of *G. rostochiensis,* as well as an unknown *Globodera* species in the samples. The rDNA Internal Transcribed Spacer Region of the nematodes were amplified by PCR and sequenced. The sequences obtained from some populations were almost identical to *G. rostochiensis* sequences obtained from Genbank, while sequences from other populations were not similar to either *G. rostochiensis* or *G. pallida.*, containing 38 point mutations and 12 insertions when compared to *G. rostochiensis.* Morphologically the cysts of the unknown *Globodera* sp. varies in colour from yellow to brown and are subspherical with a protruding neck. The vulval region is circumfenestrate and in 50% of the cysts small or large cuticular thickenings (bullae or vulval bodies) are present around the vulval basin, sometimes stretching to the anus and beyond. In some aspects this species resemble *G. rostochiensis* (presence in potato fields and some aspects of the morphology of the J2 and cyst), *G. pallida* (presence in potato fields and aspects of the morphology of the J2 and cyst), *G. achilleae* (stylet of J2 and morphology of the cyst) and *G. millefolii* (morphology of the cyst, especially the presence of bullae). The unknown cysts showed no signs of hatching when incubated in potato root exudate at 20°C. Inoculation of germinating potato tubers with larvae from the unknown *Globodera* sp. and incubation at 20°C did not result in any reproduction after 14 weeks. Low numbers of these cysts were found in the soil and no damage was reported in this area. A number of indigenous plants and weeds were screened as possible hosts of the cyst.

Connecting MOTUs and Morphology: Results from a Neotropical Rainforest

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In March of 2005 nematode collections were made at La Selva Biological Research Station, located near the Caribbean coast of Costa Rica. The objective was to determine the nematode species diversity in the humid rainforest and to explore the possibility that nematode communities were stratified among soil, litter, and understory habitats. An 18S ribosomal barcode was used to generate 167 molecular operational taxonomic units (MOTUs) from 360 individual nematodes. The MOTUs displayed strong stratification among the three habitats and a neighbor-joining tree indicated that MOTUs represented a taxonomically broad cross-section of the phylum. Only six of the MOTUs identically matched sequences deposited in GenBank. In March 2007 we returned to La Selva to attempt to connect the MOTUs with morphological assessments of the specimens. Not surprisingly these collections generated more MOTUs and species identity was not easily recovered. A subset of the MOTUs from the Criconematoidea will be discussed to illustrate the process of linking the morphological and molecular data sets.

Genetic Variability of *Xiphinema Index*, the Nematode Vector of *Grapevine Fanleaf Virus* (Gflv), Inferred by Microsatellites Loci and Mitochondrial Sequences

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Xiphinema index is a migratory ectoparasitic nematode and the vector of *Grapevine fanleaf virus* (GFLV) causing vine degeneration and considerable yield losses. This nematode, introduced from the Middle East and worldwide distributed, reproduces by meiotic parthenogenesis. A better knowledge of its genetic variability will provide important insights for the development of alternative control strategies to nematicides. Mitochondrial markers (mainly from the Cytochrome B gene) were developed for a phylogeographical approach at the world scale. The weak genetic divergence (~1%) between faraway populations did not bring conclusive information on the native area and suggests that only a limited part of the native genetic diversity has been introduced into the different vine regions. Nevertheless, Western populations (California, Chile, Spain, France) belong to the same haplotype and thus might share a same introduction origin. By contrast Eastern populations show a higher diversity in agreement with the hypothesis of the Eastern nematode origin. Eight microsatellite markers were developed for a complementary approach of population genetics, mainly focused among and within populations at the regional scale of the Bordeaux vineyard. Results confirm the parthenogenetic status of *X. index* but also suggest a few events of sexual reproduction. The genetic structure of populations is characterized by a weak differentiation between vineyards worldwide, which favors the hypothesis of a same origin of introduction. By contrast, we observed a strong differentiation between nematodes from different grapevine fields of the same vineyard, as well as between nematodes from different sampling points of a same grapevine field. This high genetic diversity observed in the Bordeaux vineyard suggests multiple introduction events in this region. Moreover, we did not observe any isolation by distance at both the scales of the Bordeaux region and the grapevine field. Taken together these results strengthen the hypothesis of limited active and marked passive dispersals of the nematode (through human activities and grapevine replants).

From Microscope to Molecule: Initial Work at Developing Diagnostic Tools for Plant Parasitic Nematodes of Sugarcane

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Many crops are attacked simultaneously by a combination of a number of nematode species and genera. Conventionally their identification has been by means of morphological characteristics. However, recent advances in molecular biological techniques enable the development of newer, potentially more powerful, means of identification. This work reports on: (1) the use of the rDNA internal transcribed spacer 1 (ITS1) region to discriminate between nematodes commonly found associated with sugarcane. The ITS1 amplification fragments varied in size, from 400 bp (*Meloidogyne javanica*) to 1200 bp (*Longidorus pisi*, *Paratrichodorus minor*, *Xiphinema elongatum*, *Xiphinema zulu*). Discrimination between certain species was possible by size differentiation of the ITS1 amplification products. However this type of discrimination also has potential pitfalls, such as when different genera (*Longidorus* and *Xiphinema*) and different species (*Scutellonema africanum, S. brachyurus* and *S. truncatum*) have similar size fragments. Discrimination with nematode universal primers also has limitations when multiple taxa compete for reagents and primers in the same PCR reaction; (2) the use of sequence data to study the genetic diversity of these nematodes. It was found that variation between specimens belonging to the same species was low for *Helicotylenchus dihystera, P. lobatus, P. sacchari, S. brachyurus* and *X. elongatum*. Conversely, specimens of *M. javanica* and *Pratylenchus zeae* exhibited greater sequence variation. Variation was usually greater between species than within species and consistent specific clusters were identified; some variation between individuals belonging to the same population was observed; (3) the use of sequence data to design species-specific reverse primers that could be used in conventional- and quantitative PCR assays. Real-time PCR with SYBR Green I dye enabled discrimination between *M. javanica* and *P. zeae* or *X. elongatum* by melting peak analyses. Due to competition between amplicons, multiplex reactions were not feasible. Real-time PCR enabled quantification of the numbers of these three species in nematode samples extracted from the field, with a significant positive correlation between real-time PCR and counts performed with microscopy. Altogether, these results provide a good base for further development of molecular tools for diagnostic and ecological studies.

Changes in Soil Nematode Assemblages under Low Tillage, Mulched Vegetable Production Systems in Queensland

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Vegetable production requires high use of inputs and frequent soil disturbance to maximise production. There are concerns that this type of production system is having a negative impact on the physical, chemical and biological status of the soils. An alternative system, that reduces tillage operations and grows a green manure crop for use as weed suppressive mulch, is being investigated commercially. However, growers are unsure if the low tillage-mulch system is having the desired affect on soil properties. Nematodes are integrators of soil conditions that respond quickly to changes in the food supply or environmental condition and can be used as indicators of the biological condition of the soil.

Vegetable farms at three locations, Gympie, Bowen and Burdekin, were used in a study where a low tillage-mulch system was compared to nearby conventional vegetable production. Nematodes were extracted from composite soil samples by placing 200 g of soil on a basket supporting a single ply tissue in water for 48 hours. Nematodes were identified using morphological characters and nematode community assemblage identified and indices calculated.

A greater proportion of plant-parasitic nematodes and a lower proportion of bacteriavores and fungivores were found in the low-till mulch system compared to conventional production systems. However, nematode community analyses showed that the low-till-mulch system had greater structure index, a measure of the number of trophic layers in the soil food web and the potential for regulation by predators than the conventional soil.

The results demonstrated that low tillage-mulch production systems resulted in different soil nematode assemblage compared to conventional vegetable production systems. Further work is required to determine if the changes in soil nematode assemblages are indicative of long term sustainability of the low till-mulch production system.

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SESSION FORTY-EIGHT – EXPLOITING RNA INTERFERENCE IN PLANTS AND NEMATODES

CONVENORS: CARLA DE GIORGI & ROSANE CURTIS

Host Delivered RNAi: An Effective Strategy to Control Plant Parasitic Nematodes

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Root-knot nematodes (*Meloidogyne* spp.) are obligate, sedentary endoparasites that infect many plant species causing large economic losses worldwide. They have the ability to infect hundreds of plant species, which often lack natural resistance. Available nematicides are being banned due to their toxicity or ozone-depleting properties and alternative control strategies are urgently required. We have produced transgenic tobacco (*Nicotiana tabacum*) plants expressing different dsRNA hairpin structures targeting a root-knot nematode (*Meloidogyne javanica*) putative transcription factor, *MjTis11*. We provide evidence that *MjTis11* was consistently silenced in nematodes feeding on the roots of transgenic plants. The observed silencing was specific for *MjTis11*, with other sequence-unrelated genes being unaffected in the nematodes. Those transgenic plants able to induce silencing of *MjTis11*, also showed the presence of small interfering RNAs. Even though down-regulation of *MjTis11* did not result in a lethal phenotype, this study demonstrates the feasibility of silencing root-knot nematode genes by expressing dsRNA in the host plant. Host Delivered RNA Interference-triggered (HD-RNAi) silencing of parasite genes provides a novel disease resistance strategy with wide biotechnological applications. The potential of HD-RNAi is not restricted to parasitic nematodes but could be adapted to control other plant-feeding pests.

Transcriptome Analysis of Syncytia Induced by the Cyst Nematode *Heterodera schachtii* in *Arabidopsis* and Role of Myo-Inositol Oxygenase Genes for its Induction and Maintenance

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Arabidopsis thaliana is a host for the sugar beet cyst nematode *Heterodera schachtii*. Juvenile nematodes invade the roots and induce the development of a syncytium which functions as a feeding site for the nematode. We report here the transcriptome of syncytia induced in the roots of Arabidopsis. Microaspiration was employed to harvest pure syncytium material which was then used to prepare RNA for hybridization to Affymetrix GeneChips. Initial data analysis showed that gene expression in syncytia at 5 and 15 days after infection was not much different and both time points were therefore compared together to control roots. Out of a total of 21,138 genes, 18.4% (3,893) had a higher expression level and 15.8%

(3,338) had a lower expression level in syncytia as compared to control roots with a multiple-testing corrected False Discovery Rate below 5%. A Gene Ontology (GO) analysis of up- and downregulated genes showed that categories related to high metabolic activity were preferentially upregulated. A principal component analysis was applied to compare the transcriptome of syncytia with the transcriptome of different Arabidopsis organs obtained by the AtGenExpress project. This analysis revealed that syncytia are transcriptionally clearly distinct from roots (and all other organs) and thus form a new organ inside the root.

Among the most strongly upregulated genes in syncytia were MIOX4 and MIOX5, coding for myo-inositol oxygenase. In plants, UDP-glucuronic acid is synthesized by the oxidation of UDP-glucose by UDP-glucose dehydrogenase. However a second pathway has been described and involves the oxidation of free myo inositol by myo-inositol oxygenase. In Arabidopsis, myo-inositol oxygenase is encoded by four genes (MIOX1, MIOX2, MIOX4, MIOX5), MIOX3 being a pseudogene. We have started a project to analyze the role of the MIOX gene family in the development and function of syncytia.

Silencing of Plant Cell Cycle Genes Inhibits Nematode Development

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Inhibitor studies showed that activation of the cell cycle is important for development of nematode feeding sites (NFS). The aim of this study was to silence expression of cell cycle genes to interfere with NFS development.

First, the *AtCDKA;1* gene was chosen for its importance throughout the cell cycle in combination with the nematode-induced AtWRKY23 promoter. Transgenic *A. thaliana* lines containing inverted repeats of the *AtCDKA;1* gene and with reduced *AtCDKA;1* expression in roots, leaves and galls were analysed in more detail. When the lines were infected with the root-knot nematode *Meloidogyne incognita*, significantly fewer galls and eggmasses developed on the roots of the transgenic compared to wild type plants. Infection of the *AtCDKA;1* silenced lines with *Heterodera schachtii* resulted in a significantly lower number of cysts compared to the controls. The S266 and S306 lines showed no phenotypic aberrations in root morphology and analysis at different time points after infection demonstrated that the number of penetrating nematodes was the same but fewer nematodes could develop to maturity in the silenced lines. In conclusion, our results demonstrate that silencing of *CDKA;1* can be used as strategy to produce transgenic plants less sensitive to sedentary plant-parasitic nematodes.

Second, the *AtCCS52* gene was choosen for its importance in endoreduplication. Transgenic *A. thaliana* lines with hairpin constructs of the *AtCCS52* gene in combination with different promoters were generated and several lines with reduced *AtCCS52* expression level and lower ploidy were shown to be also less susceptible to nematode infection compared to the controls.

Identifying Members of *Mi-1*-mediated Signaling Pathway using Virus-induced Gene Silencing

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The tomato *Mi-1* gene encodes a protein with nucleotide-binding and leucine-rich repeat motifs and mediates resistance to three root-knot nematodes (*Meloidogyne arenaria, M. incognita, M. javanica*), potato aphids (*Macrosyphum euphobiae*) and whiteflies (*Bemisia tabaci*). *Mi-1*-mediated resistance is characterized, in part, by the hypersensitive response (HR), a form of programmed cell death. Using virus-induced gene silencing (VIGS) we have functionally assessed the role of many genes during *Mi-1*-mediated defense in both *Nicotiana benthamiana* and tomato. In *N. benthamiana*, a large-scale VIGS screen was performed by targeting homologs of tobacco genes induced following TMV infection or treatment with plant defense chemical elicitors. The silenced plants were then assayed by transient expression of a constitutively active form of *Mi-1,* known as *Mi-DS4*, and phenotyped for attenuation of *MiDS4*-mediated cell death. In tomato, candidate genes involved in plant defense or *Mi-1*-mediated defense were silenced, and resulting lines assayed for attenuation of *Mi-1*-mediated aphid or nematode resistance. From these VIGS screens, several genes that play a role in *Mi-1*-mediated defense have been identified. Both novel genes and those previously associated with plant defense against other plant pathogens were isolated.

SESSION FORTY-NINE – COMMERCIAL PRODUCTS AND PROCEDURES FOR SOIL DISINFESTATION AND NEMATODES CONTROL

CONVENORS: NANCY KOKALIS-BURELLE & INGA ZASADA

Chemical Alternatives to Methyl Bromide for Field-Grown Ornamental Crop Production in Florida

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Identification of alternatives to methyl bromide for field-grown ornamental crops in the U.S. is particularly challenging because of the need to control rogues (off-varieties), limited availability of labeled materials, difficulty gluing wide sheets of virtually impermeable films for broadcast fumigation, and the proximity of occupied structures to production areas. Numerous commercial and experimental field trials were conducted under a range of nematode, weed, and pathogen pressures in southeastern and south central Florida to evaluate broadcast applications of Midas™ (iodomethane:chloropicrin 50:50), and Paladin™ (dimthyldisulfide (DMDS): chloropicrin 79:21). Crops included snapdragon (*Antirrhinum majus*), celosia (*Celosia argentea* var. cristata), sunflower (*Helianthus annuus*), delphinium (*Delphinium elatum*), and caladium (*Caladium hortulanum*). Overall, both Midas and Paladin provided nematode and weed control comparable to methyl bromide (MeBr) with no significant phytotoxicity observed in any of the crops tested. Immediately following treatment, both compounds reduced *Meloidogyne* spp. juveniles in soil as well as MeBr, and recolonization often occurred more slowly in Midas treatments compared to MeBr. However, under subtropical conditions and high nematode pressure, parasitic nematodes rebounded to similar levels in all treatments by 10 weeks after fumigation. Interestingly, Midas and Paladin allowed for faster recolonization of soil by microbivorous nematodes than MeBr. In caladium, a long-season crop (10 months), no differences in nematode control were observed among the three fumigants by the end of the season. However, Paladin resulted in lower total yield than MeBr. Application of Midas and Paladin requires more oversight and precision because these compounds are much less volatile than MeBr, and therefore do not move as well through the soil. Future research on chemical alternatives for this industry will focus on three new compounds, citroxin, ethanedinitrile, and 2-bromoethanol, which have demonstrated potential for control of *Meloidogyne* spp., weeds, and pathogens.

Field Studies using *in vitro* Produced *Pasteuria* Endospores to Control Sting Nematodes on Turf

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The sting nematode, *Belonolaimus longicaudatus*, causes extensive damage to turf on golf course and athletic fields. The few remaining nematicides used to control this problem are heavily restricted in their use and expensive. *Pasteuria* species parasitizing *B. longicaudatus* have been observed in most of the Southeastern United States and undoubtedly are ubiquitous parasites of this nematode. Studies have demonstrated that high soil densities of *Pasteuria* endospores in the soil can suppress sting nematode populations. *In vitro* produced *Pasteuria* sp. endospores, cultured from infected *B. longicaudatus*, were tested for efficacy against sting nematodes in field tests. The objectives of these experiments were to determine the time of year, formulation and rate of application necessary to give the optimum control of sting nematode on turf. Seven sites were chosen on golf courses and athletic fields located in south and central Florida. Time of application was November when sting nematode populations begin to increase. Rates of applications included 5 $x10^{10}$; 2.5 x 10^9; $5x10^9$; or 0 endospores applied to 1.5- m^2 plots, replicated 5 times. Soil samples were taken at 15 days after treatment and at three 30-day intervals thereafter. Data collected included number of sting nematodes alive and dead and percent of nematodes encumbered with *Pasteuria* endospores in $100cm^3$ of soil, turf health, and root growth. The initial 15 day soil sample data showed a significant ($P<0.05$) decrease in nematode populations at two sites in central Florida but at two sites in south Florida, using a different spore batch, sting populations were not reduced.

Possible Mechanisms Decreasing the Damage on Radish by the Root-lesion Nematode in a Soil Amended with Okara and Coffee Compost

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Damage by the root-lesion nematode (*Pratylenchus penetrans*) on radish is a serious problem in Japan. The Kanagawa Agricultural Technology Center has been conducting a field study to test the effect of a compost consisting of okara (the residue of Tofu production) and coffee extraction residue since 1996 and found that the damage was consistently lower in the okara and coffee compost (3 t ha^{-1}) amended soil (OC-soil) than a soil (CM-soil) with cow manure compost (8 t ha^{-1}). In this study, mechanisms of the OC-soil was estimated by comparing the number of *P. penetrans* and total nematodes, community structures of nematodes, fungi and bacteria among the OC-soil, CM-soil and a soil amended with chemical fertilizers in 2006 to 2007. The number of total nematodes was consistently high in the OC-soil during the monitoring period. The number of *P. penetrans* was almost the same between the OC- and CM-soils before radish cultivation both in 2006 and 2007. Nematode community structure analyzed by PCR-DGGE was different among the three soils both in 2006 and 2007. Specific bands were detected in the OC-soil and nearest to be *Prismatolaimus intermedius* and *Distolabrellus veechi*. While community structures of bacteria and fungi were not different in the non-rhizosphere soil, bacterial community structure in the radish root was different between the OC- and CM-soils both in 2006 and 2007. From these results, the suppression mechanisms of damage on radish by the root-lesion nematode in the OC-soil might be related to enhanced numbers of free-living nematodes and bacteria community colonizing the radish roots.

Putting Waste to Work: Commercially Available Waste Products for Nematode Control

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Approximately 1 billion tons of organic and inorganic by-products are generated each year in the United States. Some of these waste products are available commercially and it would be desirable to incorporate them into plant-parasitic nematode management programs. While the concept of utilizing waste products (e.g., composts, biosolids) to manage plant-parasitic nematodes is not new, the widespread implementation of these products has still not been realized. Lack of implementation as a pest management strategy is due to several reasons, including product inconsistency; availability and cost. Research should strive to understand the complex processes responsible for nematode suppression by these products and identify environments in which they can be utilized. Research conducted on a biosolid amendment for plant-parasitic nematode control highlights the need to understand the mechanism responsible for nematode suppression and how the environment and management practices can be manipulated to improve the effectiveness and reliability of waste products as plant-parasitic nematode management strategies.

Seed Treatment Nematicide Alternative for Cotton

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Avicta Complete Cotton was introduced as a new seed treatment for nematode, insect and disease management in cotton by Syngenta Crop Protection in 2006. Azoxystrobin, Fludioxonil, Mefenoxam, Thiamethoxam, and Abamectin are the active ingredients layered on the seed. The nematode species targeted in cotton production include the reniform, *Rotylenchulus reniformis,* the root-knot, *Meloidogyne incognita*, and the Columbia lance, *Hoplolaimus Columbus* nematodes. Avicta Complete Cotton has been examined by University or Extension scientists in 15 states across the southeast and mid south cotton production regions of the United States. All cotton seed treatment nematicide tests were planted in field locations with a history of nematode infestations. Plot design, size, number of replications, and cotton variety varied by researcher participant. Nematode population development was determined at various intervals through the season and cotton yields were collected on all tests. In 2006 and 2007, from 185 university trials Avicta Complete Cotton produced yields similar to the current standard nematicide Temik 15G (aldicarb) in 80% of the trials. Avicta and Temik 15 G increased cotton yields over the insecticide plus fungicide treated controls in 75 and 63 % of the trials, respectively over all nematode species. In north Alabama Avicta increased yields in 3 of 4 years with an average of 232 kg seed cotton /ha. Temik 15G increased cotton yields in 2 of the 4 years. However, the combination treatment of Avicta Complete Cotton and Temik 15 G increased yields in all years with a 487 kg/ha increase. Avicta Complete Cotton offers the cotton grower an alternative option for nematode management their production system.

SESSION FIFTY – RENIFORM, LESION AND FALSE ROOT-KNOT NEMATODES

CONVENORS: AN MACGUIDWIN & MARCELO DOUCET

Nacobbus aberrans Thorne, 1935) Thorne & Allen, 1944 in Argentina

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In Argentina, the false root-knot nematode is represented by populations with origin in cultivated and uncultivated areas. The sites where the species is found are located in very diverse geographical areas that are subjected to highly different conditions and climates. There seems to be some correlation between each one of these populations and their geographical origin as well as the host on which they multiply. Clear differences among populations have been observed so far through comparisons that considered diverse parameters. Thus, for example, based on morphometrical characters, grouping of populations raised on the same host have been detected. Furthermore, a genetic structure analysis showed that populations tended to group together according to their original host, revealing a certain degree of similarity among populations originated in the same host. When considering the evaluation of the reproductive capacity of populations multiplied on different plants under controlled conditions, different behaviours were observed. Such differences were recorded both among populations for a single host and among plants for a single population. These studies confirm the existence of biological entities of different agricultural importance within the species that would show preferences for certain hosts. The great variability that *N. aberrans* shows in relation to its host/s indicates the need for detecting an accurate differential host test that will be useful to establish suitable management strategies and to gain knowledge on associations between populations.

Basic Biology of *Rotylenchulus reniformis* on Cotton

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Basic biological questions of *Rotylenchulus reniformis* currently being evaluated at Auburn University include: 1) the potential vertical, horizontal and temporal movement of this nematode under irrigated and non-irrigated field conditions; 2) the effect of enhanced seedling plant vigor on nematode numbers; 3) the identification of nemaphagous fungi pathogenic to *R. reniformis*; and 4) the evaluation of FAME analysis for identification of *R. reniformis.* Vertical movement of both *R. reniformis* was observed to migrate from the initial 2.5 cm depth placement to a depth of 91cm in irrigated and non-irrigated tests. Movement of *R. reniformis* males was affected by irrigation, with males in the irrigated test migrating twice the distance of males in the non-irrigated test. Populations of vermiform females and juveniles were higher in the inoculated row while higher populations of males were found dispersed in adjacent rows. Nitrogen and Nitrogen plus Phosphorus starter fertilizer applications applied to cotton at planting produced fewer *R. reniformis* per gram of root in greenhouse evaluations as compared to the control. Fungi found associated with vermiform *R. reniformis* life stages and eggs across the state include: *Arthrobotrys dactyloides*, *Paecilomyces lilacinus, Fusarium oxysporum* and *Dactylaria brachophaga.* Pathogenicity test indicate some isolates reduce *R. reniformis* in greenhouse conditions. Fatty acid prints have been identified for *R. reniformis* and *M. incognita.* Eleven fatty acids have been found to be significant to distinguishing among *R. reniformis* from *M. incognita.* Six of these fatty acids are observed in a much greater concentration in *R. reniformis* than *M. incognita.* These results indicate that *R. reniformis* and *M. incognita* can be demarcated by FAME analysis.

Reniform Nematode and Cotton Production in the USA

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During the past two decades, reniform nematode (*Rotylenchulus reniformis*) has emerged as a major pest problem in upland cotton, *Gossypium hirsutum* L., in the mid-South and Southeast regions of the USA Losses from this pest have been estimated at over one billion (U.S. dollars) during the past 10 years. There is clear evidence that this nematode is still spreading and has certainly increased in the both incidence and severity in a number of states. Although a number of other crops are susceptible, incidence and severity appears to be greatest on cotton. Yield losses from this nematode have been reported as high as 60% in some fields but typically average between 10-20%. Currently, there are not any cultivars of upland cotton that are planted the USA that have any level of resistance against this nematode. The primary methods that are utilized to manage this pest are crop rotation and nematicides. Corn is the most widely planted rotational crop for managing reniform in the mid-South with the addition of peanuts in the Southeast. The predominate nematicide until recently was aldicarb. Seed treatment nematicides including abamectin and thiodicarb have recently seen increased use during the past several years. An older fumigant, 1,3-Dichloropropene, has seen increased usage during the past several years particularly in the area of site-specific nematicide application. This technology is now being extensively evaluated for use against the reniform nematode in cotton in both regions of the USA Fields can be delineated into management zones based on soil texture utilizing apparent soil electrical conductivity (EC_a), population densities of reniform nematode, aerial imagery collected during the cropping cycle, or simply where the crop responds to nematicide application. Reniform nematode remains a serious threat to the cotton industry in the USA

***Pratylenchus* and *Radolpholus*: Ecological and Evolutionary Relationships Based on Morphology, Genetics, Host and Life History Characteristics**

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The nematode genera *Pratylenchus* and *Radopholus* are, by any measures, closely-related in terms of evolution and ecology, with both traditionally classified in the same family, and both living as migratory internal root parasites. However, there are considerable differences in some characteristics, such as in life where sexual dimorphism occurs in *Radopholus* but not *Pratylenchus*, in the development of the reproductive system in to be didelphic in *Radopholus*, but prodelphic in *Pratylenchus*, and in the dorso-ventral position of the oesophageal glands. Furthermore, there are many species of *Radopholus* in Australia, but only one apparently native *Pratylenchus* species. In terms of genetics, the two genera evolved separately in according to a large-scale analysis of a few representatives of each genus within the entire suborder Tylenchina. More detailed analyses of the same gene, but in many more populations and species within each genus suggests that the two genera evolved closely with each other. In terms of hosts, both have wide host ranges, but there are some differences between genera. What is going on? Barring some form of reticulate evolution, not all lines of evidence can be correct.

In this presentation, all lines of evidence are evaluated, and the relationships between the two genera clarified. The implications of these evolutionary relationships for attempts to manage the damaging pests in both genera are discussed.

PLENARY SESSION FIFTY-ONE – FUTURE TRENDS IN NEMATOLOGY SCIENCE: IMPORTANCE AND OPPORTUNITIES

CONVENORS: VIVIEN VANSTONE & VIRGINIA FERRIS

Let's Go Exploring

Ferris, V.

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During the past 50 years, paradigm shifts have occurred in all of biology (including nematology) that were unthinkable to one who began her present job in 1965. The entire field of information technology flourished; the discovery of the double helix in 1953 was followed by tumultuous progress in molecular biology including the discovery of restriction endonucleases, DNA cloning, PCR, automated DNA sequencing, large arrays, high throughput molecular analysis, genetic transformation, distributed computer hardware and amazing new software. It is likely that future paradigm shifts will occur at an even more rapid pace, and that nematologists who are young today will see themselves transformed even more rapidly than I saw myself adapt and change during my career. Several areas in which I foresee great progress have been discussion topics at this Congress. Let's explore together a few of these, which might include: trends in phylogeny, evolution and classification of nematodes; exploitation of information about nematode genomes; bioinformatics and data mining; radical changes in nematode diagnostic methods; the future of molecular barcoding; a better understanding of biodiversity; new methods for doing ecological research; and radical changes in the kinds of research programs undertaken by future graduate students in nematology.

What Different Fields of Nematology Can Learn from Each Other

Pastor, C.

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During the Plenary Session, a mental representation diagram related to knowledge about Nematology will be developed. The objective will be to speak about the differences in levels of learning in the various Nematology fields, and to try to relate those levels of learning to the our experiences during the 5ICN week. After gathering ideas, we will attempt to show them as a synthesis of the Congress.

Rounding Up and Pointing the Way Forward

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This presentation will focus on some of the research highlights of the conference and attempt to integrate the various approaches to examine the direction and likely progress that the science of Nematology will take in the near future. The usefulness of such conferences from a scientific and, of course, a social standpoint will be examined, with the author's experience of other meetings being central to the analysis. The author's opinion about the long-term changes in other aspects, such as journals, publications, teaching and funding will also be presented.

POSTER PRESENTATIONS

TOPIC ONE – EVOLUTION, PHYLOGENY AND CLASSIFICATION

Nematode Fauna of a Biotope Pond Sediment Compared with Surrounding Marsh and Rice Paddy Fields in the Kanto District, Japan

Araki, M.

National Institute for Agro-Environmental Sciences

The function of agricultural land in conserving the environment and biodiversity has been widely noticed recently and it is important to understand the nematode biodiversity in paddy fields, which is the most common land use in Japanese arable land, as well as the damp environments surrounding them. Imamura (1931) gave a splendid list of nematodes in rice paddies in Tokyo, Kanto district. However, little work has been done since then to investigate the nematode fauna of rice paddy. The situation is the same in wetlands, ponds or rivers in agricultural ecosystems. I had the opportunity to investigate nematode fauna in a biotope pond, marsh and rice paddy in Koibuchi College of Agriculture and Nutrition in Ibaraki Prefecture, Kanto district in 2006-2007. The biotope consisting of the pond and a marsh planted with Manchurian wild rice (*Zizania latifolia*) was created on the paddy field in 2004. Surrounding rice paddy has been cultivated in a conventional or organic way. Pond sediments were taken on October 2007 and soil samples were taken on June 2006 after rice-planting and on January and October 2007 from fallow paddy. Nematodes were extracted from the samples by sieving followed by modified Baermann technique using trays. The major nematode genus in pond sediments was *Tripyla,* and family Rhabditidae, genera *Plectus*, *Chronogaster*, *Rhabdolaimus*, *Prismatolaimus*, *Ironus*, *Mononchus* and *Dorylaimus* etc. were detected. The nematode fauna of the conventional rice paddy is similar to that of pond sediments, but the most common genera in conventional rice paddy were *Rhabdolaimus* and *Dorylaimus*. *Chronogaster* was one of the most abundant genera in the organic rice paddy though genus *Rhabdolaimus* was also very common. The marsh bore few nematodes as well as the pond sediments characterized by genus *Plectus*. It seems that pond sediments and rice paddy have different nematode fauna.

Evolution of Feeding Types and Plant-Parasitism in Tylenchina (Nematoda: Rhabditida): Different Constraints Compared to Feeding-independent Structures

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Tylenchina are a morphologically and functionally diverse group of nematode species that range from free-living bacteriovores, over transitory grazing root-hair feeders to highly specialized plant-parasites with complex host associations. We performed phylogenetic analyses of small subunit rDNA sequences from 97 species including an analysis that account for the RNA secondary structure in the models of evolution. The present study confirms the sister relationship of the bacteriovore Cephalobidae with the predominantly plant-parasitic Tylenchomorpha. All analyses appoint the fungal-feeding Aphelenchidae and Aphelenchoididae as being polyphyletic but the morphology based hypothesis of their monophyly could not be significantly rejected. Within the Tylenchomorpha, the families that exclusively parasitize higher plants are joined in a single clade. However, only the monophyletic position of the (super)families Hoplolaimidae and Criconematoidea were supported; Anguinidae Tylenchidae, Belonolaimidae and Pratylenchidae appeared to be paraphyletic or polyphyletic. Parsimony and likelihood ancestral state reconstruction revealed that burrowing endoparasitism and sedentary endoparasitism each evolved respectively at least six and at least three times independently, mostly from migratory ectoparasitic ancestors. Only root-knot nematodes have evolved from burrowing endoparasitic nematodes. Traditional classifications are partially misled by this convergent evolution of feeding type and associated morphology. Contrastingly, mapping attributes of the gonoduct cellular architecture, including newly obtained data of 18 species belonging to the Aphelenchoidea, Criconematoidea, Anguinidae and Panagrolaimidae, revealed a broad congruence of the gonoduct characters and the molecular phylogenetic hypothesis. Yet, the proliferation of uterus cells has evolved multiple times, associated with derived endoparasitic feeding specialization and resulting reproduction mode. Ancestral state reconstruction further revealed that the gonoduct of the morphologically and ecologically dissimilar tylenchid and cephalobid nematodes evolved from a common ancestor.

Molecular Differences between *Radopholus similis* Isolates in Australia

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Radopholus similis is one of the top ten most destructive plant-parasitic nematodes in the world. In banana, control measures such as crop rotation, resistant cultivars, soil amendments and chemical control can reduce nematode levels. Currently, nematicides are used, which are potentially hazardous to the workers and the environment. Furthermore, their efficacy being reduced due to enhanced biodegradation. In Australia, the nematode susceptible cultivar Cavendish, constitutes 95% of the industry with the remaining 5% consisting of Lady Finger, Goldfinger and a few other cultivars. Studies have shown that different isolates may differ in pathogenicity on different banana cultivars.

Cobon and Pattison (2003), conducted glasshouse trials using several cultivars of *Musa* spp. (banana) and showed that different Australian isolates had varying levels of pathogenicity. Therefore, we intend to investigate *R. similis* isolates using molecular analysis in an attempt to identify genetic groupings that might reflect different levels of pathogenicity. Previous phylogenetic studies will be supplemented with further work using the internal transcribed spacer region (ITS) of the nuclear rRNA to determine evolutionary relationships of haplotypes within *R. similis*. If pathogenicity is linked to known genotypes, knowledge of which isolate occurs in a region might assist in the selection of resistant banana cultivars and eventually reduce the need for nematicide applications.

Based on the preliminary results of the ITS sequences, it appears that *R. similis* is not native to Australia. All Australian isolates sequenced (n=10; Queensland and northern New South Wales) had an identical haplotype, which is nested within a cluster of haplotypes originating mainly from Asia. For the pathogenicity test, the preliminary result on several cultivars of banana shows that isolates from Pimpama are more aggressive than other isolates. We are currently using Amplified Fragment Length Polymorphisms (AFLP) to assess whether there is variation among isolates of *R. similis* from different regions.

Comparison of the 28S Gene D2/D3 Expansion Segments of Stem Nematode (*Ditylenchus dipsaci*) and Potato Rot Nematode (*Ditylenchus destructor*)

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The Stem Nematode (*Ditylenchus dipsaci*) is the major pest of many economically important crops. Effective management of this pest is complicated by existence of populations with different host preferences. Some populations are specialized on a few host plant species; others are able to attack plant species from all sorts of families. Another problem is the ability of *Ditylenchus dipsaci* to survive on infested plant debris for several years. The relative and morphologically similar species *Ditylenchus destructor* is much less dangerous pest. The aim of this work was to characterize variable 2D and 3D segments of the 28S gene of the *Ditylenchus dipsaci* and *Ditylenchus destructor* nematodes and to assess differences between sequences of the both species. After isolation the DNA was amplified with the use of universal primers derived from the genome of *Caenorhabditis elegans*. Fragment of the length of approximately 784bp and 779bp respectively was obtained in this manner; this fragment was used for ligation into pTZ57R/T plasmid and transformation of competent cells of *Escherichia coli* Dh5α. Plasmids were selected, isolated and cloned fragment sequenced. The comparison of *Ditylenchus dipsaci* and *Ditylenchus destructor* populations revealed extensive level of variability; the overall value of alignment score was only 78. Comparison with NCBI database using BLAST algorithm revealed high sequence similarity of *Ditylenchus dipsaci* D2/D3 segments with corresponding segments of *Subanguina radicicola* nematode. The research was supported by the Ministry of Agriculture of the Czech Republic; project numbers Mze-0002700603 and MSM 6046070901.

Survey and Characterization of Root-knot nematode (*Meloidogyne spp.*) in Kiwi (*Actinidia deliciosa*) in the Extreme South of Brazil

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Meloidogyne ethiopica has been associated with damage in kiwi (*Actinidia deliciosa*) in South region of Brazil. In Chile, this nematode also attacks grapevine plants and causes decline in yield quality and production of kiwi fruits. Considering the importance of *M. ethiopica* to grape and kiwi in Brazil, a survey of root-knot nematode in kiwi orchards and nurseries was carried out in the Rio Grande do Sul State. Forty four populations of *Meloidogyne* spp. obtained from kiwi root samples were characterized biochemically by esterase isoenzime. The *Meloidogyne* sp. atypical populations were also characterized morphologically. *M. arenaria* Est. A2 (Rm: 1.20, 1.30) was the most frequent species and occurred in 66.65% of the analyzed samples. *M. ethiopica* presenting phenotype Est. E3 (Rm: 0.92, 1.15, 1.23) was detected in kiwi orchards and nurseries occurring at 16,6% of the samples, in association with others species. *M. javanica* Est. J3 (Rm: 1.00, 1.21, 1.30), *M. hapla* Est. H1 (Rm: 1.17) and *M. incognita* Est. I1 (Rm: 1.05) and I2 (Rm: 1.03, 1.10) were also identified in 29.9%, 16.66%, 3.33%, and 9.79% of the samples, respectively. Moreover, only one *Meloidogyne* atypical population (3,3%) was detected in this survey (Est. L3; Rm: 1.00, 1.16, 1.32), but it was not possible to identified this species by perineal patterns.

Characterization of *Longidorus poessneckensis* from the Czech Republic

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Longidorus poessneckensis (Nematoda: Dorylaimida) was found in the rhizosphere of *Quercus* at Cerne Voderady, Czech Republic. Until now *L. poessneckensis* has been recorded only from Central Europe (Austria, Czech Republic, Germany and Slovakia). Given the lack of knowledge of molecular and morphological features of *L. poessneckensis*, a comprehensive characterization of *L. poessneckensis* from the Czech Republic based on morphological and molecular characters was carried out. Also, the evolutionary relationship of *L. poessneckensis* with *L. macrosoma*, *L. helveticus* and *L. uroshis* was inferred by the characterization of sequences of gene encoding for the cytochrome c oxidase subunit I (*cox1*), second internal transcribed spacer (ITS2), small subunit (18S) and D2/D3 expansion segments of large subunit (28S). Phylogenetic relationship was inferred by using maximum likelihood and maximum parsimony methods. All multiple alignments yielded similar basic trees supporting the uniqueness of *L. poessneckensis* and the validity of the four *Longidorus* species identified using morphological characters. Phylogenetic analyses revealed that *L. poessneckensis* is more closely related to *L. macrosoma* and *L. helveticus* than to *L. uroshis*. (The work was supported by project QG60123 and MZe0002700603).

Mitochondrial DNA Frameshift Mutations in the Heteroderidae Nematodes: A New Phylogenetic Marker for the Cyst-forming Nematodes?

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Mitochondrial genomes (mtDNA) are used extensively in phylogenetics due to their sole maternal inheritance, apparent lack of recombination, and sequence conservation within a taxon. Typically, animal mtDNA have a highly compact gene arrangement with no introns, minimal non-coding sequence, and with intra-individual variation limited to infrequent silent mutations. However, the mtDNA of cyst-forming, plant-parasitic nematodes (Tylenchida: Heteroderidae) is unusual, with recombination apparent in the *Globodera* genus, as well as abundant intra-individual variation. Our research found that nematodes of the *Heterodera* and *Gobodera* genera have extensive variation in the length of long poly-thymidine tracts within protein-coding genes, such that the reading frame would not be maintained in all gene copies if they were coded for directly. Comparisons with expressed sequence tag (EST) data indicated that this variation was also present at the mRNA level. Further studies are required to determine whether these copies are translated or edited, and what effect they have on the physiology of the cyst nematodes.

Thus far we have observed poly-T variation only in cyst-forming nematodes, but not in their close relatives, the root knot nematodes (Tylenchida: Meloidogynidae). We therefore hypothesise that poly-T variation is a synapomorph for the Heteroderidae, and as such may provide a new phylogenetic character for the cyst-forming nematodes.

Symbiotic Association of a Monhysterid Nematode in Perianal Folds of Stinkpot Turtles

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The Monhysteridae is a family of free-living nematodes that is associated with fresh and marine benthic habitats. An examination of perianal folds of the stinkpot turtles, *Sternotherus odoratus,* from Virginia, USA, found several monhysterid nematodes. Morphological characters such as circular amphids, unstriated cuticle, single outstretched ovary, single testis, caudal glands, terminal spinnerette and the absence of a apophysis on the gubernaculum suggest close affinity to the Monhysteridae. Confocal microscope observations of the buccal morphology note distinct teeth and denticles on the extensions of the buccal wall and suggest that this is a new genus. The systematic position of this genus is compared to other commensal monhysterids. Affinities of this genus with *Gammarinema, Monhystrium* and *Tripylium* that are ectocommensals of crustaceans are discussed. The genus *Tridentulus* has denticles on the base of the stoma but lacks the buccal wall extensions. A monhysterid, *Odontobius ceti,* has also been described from baleen plates of whales. Stinkpot turtles feed on benthic fauna and may acquire the symbionts when sediment is disturbed. The significance and evolutionary aspects of the transition to commensalism of this genus and related adenophorean genera are discussed. The feeding behavior of stinkpot turtles that may facilitate acquisition of these commensal nematodes is discussed as a pathway to symbiosis.

TOPIC TWO – MORPHOLOGY AND DEVELOPMENT

Influence of Temperature on the Life Cycle of the Root-knot Nematode, *Meloidogyne hispanica*

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Global warming has the potential to influence the occurrence and distribution of plant diseases. *Meloidogyne hispanica* is a virulent pathogen of a variety of plant hosts and has been reported in Africa, Asia, Europe, North, Central and South America and Australia. Development and generation times of *M. hispanica* in susceptible tomato plants cv. Easypeel were studied at 15, 20, 25, 30 and 35ºC in growth chambers. Three-week-old seedlings were inoculated with 150 second-stage juveniles (J2). Three/seven days after inoculation (DAI), the seedlings were uprooted and the roots washed to remove all J2 outside the roots. They were then transplanted into Petri dishes with sterilized soil. Nematodes were dissected out from roots and their developmental stages and sex recorded daily for the period between 3-10 DAI; every two days for 10-30 DAI and every 5 days after 30 DAI until the completion of the life cycle. The life cycle (from J2 to J2) was shorter at 25 and 30ºC (34 and 26 days, respectively). At 20ºC, the life cycle was twice as long as that at 25ºC (70 days). At 15ºC, females were only found at 75 DAI and egg production was not observed until 80 DAI, this is probably related to the unfavourable conditions of plant growth at this temperature. Eggs were laid after 22 DAI at 35ºC, but no hatching occurred at this temperature indicating the upper temperature limit for life cycle completion. At higher temperatures (30 and 35ºC) development of J2 into females decreased slightly, but the number of males increased. Thus *M. hispanica* is most suited to soil temperatures around 25^{o}C and predicted climate change indicates that this nematode will spread in Southern Europe.

Developmental Biology of the White-tip Nematode *Aphelenchoides besseyi*

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The white-tip nematode *Aphelenchoides besseyi* is the causal agent of white tip disease of rice, and the disease occurrence and nematode distribution have been reported in almost every rice-growing region of the world. Although we have observed that this nematode has two sexes, male and female, it has been reported to have a strongly biased sex ratio or even to reproduce parthenogenetically. We have studied the early embryogenesis of *A .besseyi* HM-M (a strain isolated in Hiroshima, Japan; a gift from Dr. K. Togashi) from fertilization to the 4-cell stage under Nomarski optics and examined the chromosome structure by DAPI staining. After passing through the spermatheca, an oocyte is fertilized by sperm, the eggshell is formed, the egg is deposited, and the male and female pronuclei are reconstructed. The male pronucleus moves toward the female pronucleus, they meet and move to the center of the egg where they rotate 90° and fuse. The embryo then divides unequally to form an anterior and larger cell and a posterior and smaller cell.

The larger and smaller cells divide anterior-posteriorly and essentially at the same time, although the smaller cell seems to divide ahead more frequently than the larger one, to form the 4-cell stage. Because the embryo is very long and narrow (about 20 × 80 µm), the four blastomeres are initially arranged in tandem. At 30ºC, it takes about 28 hours from oviposition to hatching as an L2 juvenile, and about 70 hours from hatching to become an egg-laying, mature adult. The chromosome structure was observed by DAPI staining of germ cells or early embryos; a haploid sperm (N=3) fertilizes a haploid oocyte (N=3), and becomes a diploid embryo (2N=6). All chromosomes appear similar except for one that is much bigger than the others. Our preliminary results suggest that developmental studies of *A. besseyi* will reveal very interesting and unique characteristics of chromosome number and size, cell division patterns, and cellular arrangement during embryogenesis, as well as a mechanism of sex ratio determination.

In Vitro Hatching of *Heterodera filipjevi* in Laboratory and Winter Wheat Field Conditions in Turkey

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The Cereal Cyst Nematode *Heterodera filipjevi* (Madzhidow) is widely spread and causes damage in wheat production area in Turkey. In order to control this nematode it is essential to understand population development. For this purpose, *in vitro* hatching of *Heterodera filipjevi* (Madzhidow) was investigated under laboratory and field conditions. Five temperatures of 5, 10, 15, 20 and 25^{o}C were used in laboratory experiments. Experiments were carried out in micro plate dishes with 12 replications with 2 cysts in sterile water for each replication. Cysts were kept in initial temperatures for 2 months then all temperature treatments changed each other to see temperature change effect on hatching. Experiment was followed for one year. Total number of eggs and hatching percentage in each replication was calculated. Highest hatching percentages were obtained with 15, 10 and 5^{o}C treatments; 94.1, 91.9 and 75.2% respectively. Hatching at 20 and 25^{o}C treatments was low; being 21.9 and 18.6% respectively. Hatching significantly increased with the temperature change from 5^{o}C to 20^{o}C and from 10^{o}C to 20^{o}C at the rate of 48.5% and 42.4%, respectively. Hatching of same population was also followed under natural field temperature conditions from July (harvest time) for one year. Two cysts were placed into 1.5 ml eppendorf tubes being 12 tubes as replicates. Tubes are buried into soil at 20 cm depth with a temperature recorder. Juveniles hatching started at 17^{o}C in October, and hatching was negatively correlated with temperature (R^2= 0.37). On average 93.5% hatching was recorded. Hatching under field conditions confirmed the laboratory results. It is suggested that optimum juvenile hatching is below 20^{o}C and hatching increases with the change from lower temperatures to high temperatures.

Sperm Development in the Free-living Marine Nematode *Leptosomatides marinae* (Enoplida, Leptosomatidae)

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The leptosomatid *Deontostoma californicum* was the first free-living marine nematode which spermatozoa have been studied by electron microscope (Proc. Helminthol. Soc. Wash. 1973. 40: 30-36.). Recently we started a new study of male gametes in the leptosomatids using *Leptosomatides marinae* as the representative. The reconstitution of the nuclear envelope during the nuclear collapse of the spermatids and spermatozoa in *L. marinae* confirmed the unity of the enoplids by this plesiomorphic character (Russ. J. Nematol. 2006. 14: 119-125). The cytoplasmic events observed in spermatogenous cells of *L. marinae* are also of special interest. In the early spermatids the proliferation of rough endoplasmic reticulum (RER) and dictiosomes occurs. Two types of cytoplasm inclusions appear: fibrous vesicles bounded by double membrane and dense vesicles with internal system by microvillus-like projections. The latter are easily identified as 'membranous organelles' or MO, unique component of nematode sperm. In the late spermatids the fibrous vesicles were no longer detected; the numerous MO tend to be in close association with the nucleus and its nuclear envelope. Periphery of the cell is occupied by mitochondria and extensive synthetic apparatus (RER, dictiosomes). The immature spermatozoon has the central elongated nucleus surrounded by the electron-light halo. The periphery of the cell is occupied by numerous dense MO interspersed by occasional mitochondria. The surface of the cell is arranged as numerous parallel ridges filled with microtubule-like fibres. The spermatozoon development of *L. marinae* has the general enoplid character: absence of MO-FB complexes. Moreover, the FB (fibrous bodies, one more unique component of the nematode sperm) have not been detected as it was observed also in Anticomidae, Anoplostomatidae, and Tripyloididae. The alternative pattern of cytoplasmic events in the enoplids, separate development of FB and MO (Enoplidae, Oxystominidae, Oncholaimidae) is also unique for nematodes. The enoplid spermatozoa provide nematode phylogeny by distinct differential characters.

TOPIC THREE – NEMATODE PHYSIOLOGICAL AND ULTRA-STRUCTURAL ANALYSIS

Trichinella pseudospiralis : Muscle Phase Study after Modulation of the Host Response, using an Immunostimulant Drug

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Trichinella pseudospiralis a non encapsulating species of the genus ***Trichinella*** and the only species that infects both mammals and birds ,was not considered a potential pathogen of humans and domestic animals,however this pathogen has since been detected in domestic animals and humans in different locations around the world.

Infection with *Trichinella pseudospiralis* is accompanied by pronounced suppression of host inflammatory response.This study examines the effect of infection with this parasite on one major component of cell mediated immunity: delayed type hypersensitivity (DTH) reaction and the effect of administration of an immunostimulant drug **(**Levamisole) on host response to *Trichinella pseudospiralis* infection at different time intervals.Early administration of Levamisole restored DTH response to normal and histopathological examination of infected muscles revealed evident collagen deposition around the muscle larvae.

Scanning electron microscopic examination revealed multiple clear cystic formations on the surface of the diaphragm muscle. Such structures were seen evidently when the drug was given as early as the 6th day post infection.A membrane like structure was observed around *Trichinella pseudospiralis* larvae, a very interesting observation regarding this non encapsulating parasite.

A Homologue of the Human *dj-1* Gene is Upregulated in Response to Desiccation and Osmotic Stress in the Nematode *Aphelenchus avenae*

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Anhydrobiosis is a phenomenon whereby certain organisms including rotifers, tardigrades and numerous nematode species enter a state of suspended animation when subjected to drought conditions. These anhydrobiotic organisms can potentially survive in this desiccated state for indefinite time periods. The nematodes *Aphelenchus avenae* a free-living mycophagous nematode, and *Panagrolaimus superbus* a bacterial feeding nematode, both display anhydrobiotic capabilities. We screened an *A. avenae* EST dataset to identify genes upregulated upon desiccation and osmotic pressure and thirty distinct ESTs were isolated. One of these ESTs encoded a gene with homology to human *dj-1*, a gene which has attracted much attention recently due to its involvement in early onset familial Parkinson's disease (PD). We present here a phylogenetic analysis of the *dj-1* gene family. Numerous functions for the DJ-1 protein have been elucidated, these include: a positive regulator of the androgen receptor; an RNA binding protein and a transcriptional regulator. More recent studies have shown that human DJ-1 also acts as a redox sensitive molecular chaperone. Our data indicate a role for the DJ-1 protein in desiccation tolerance. We have successfully expressed the *A. avenae dj-1* gene and the *P. superbus dj-1* gene in the pET30 Ek/Lic expression system. Molecular characterisation of these recombinant proteins is ongoing. In humans, the DJ-1 protein is known to bind α-synuclein (implicated in PD pathology) and prevent its aggregation. Both the protective properties of recombinant *A. avenae* and *P. superbus* DJ-1 and the structural characteristics are being investigated in aggregation and chaperone assays using circular dichroism and FTIR techniques.

Bonifati, V. *et al.* (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. *Science* **299**, 256-259.

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Anhydrobiosis and Cryobiosis: Overlapping Adaptations in Nematodes of the Genus *Panagrolaimus*

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In dry Antarctic valleys the invertebrate fauna is predominantly comprised of rotifers, tardigrades and nematodes – all organisms capable of undergoing anhdyrobiosis. Nematodes are also the most abundant and diverse invertebrates in the maritime regions of the Antarctic. We show that Arctic and Antarctic species the nematode genus *Panagrolaimus* are members of an anhyrobiotic clade which also contains strains from temperate and continental regions of the world. Our data show that strongly anhyrobiotic strains of *Panagrolaimus* - both fast and slow dehydration strategists - when fully hydrated in water are capable of surviving freezing to -80 ^{0}C and that a linear relationship exists between anhydrobiotic capacity and freezing tolerance in this nematode genus. This ability to survive extreme freezing is not restricted to *Panagrolaimus* isolates from polar regions. We find that cold acclimation improves the freezing tolerance of *Pangrolaimus,* most notably those isolates which have poor cryotolerance when unacclimated. The freezing and thawing regimes used in this study give a strong indication that strongly desiccation tolerant strains of *Panagrolaimus* are adapted to survive intracellular ice formation. Our data are consistent with the hypothesis that adaptations which protect anhydrobiotic strains of *Panagrolaimus* from cellular dehydration overlap with adaptations which protect the nematodes during freezing.

Studies on Amino Acid Profile and its Biochemical Path of Dominant Phytonemas of Altitudinal Variant Environments

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Studies on the amino acid profile was conducted on *Hoplolaimus indicus, Helicotylenchus indicus, Hemicriconemoides mangiferae* and *Tylenchorhynchus bravilineatus* infesting *Eucalyptus globules, Pinus roxburghii, Prunus persica, Pyrus malus* and *Citrus aurentium* of two altitudinal variants states of India Uttarakhand (high altitude) and Uttar Presedh (plain)

Total 20 amino acids were detected from all the studied nematodes. Common amino acids in all the nematodes were detected as - arginine, aspartic acid, cysteine, cystine, 3,4-di hydroxyphenylalanine, glutamic acid, glycine, hydroxyproline, isoleucine, leucine, phenylalanine, proline, serine and tyrosine. Alanine and histidine were exclusively absent in *T. bravilineatus* whereas aminobutyric acid was absent in *T. bravilineatus* and *Hop. indicus* both. Ornithine and threonine were exclusively absent in *Hel. indicus*. Methionine was not detected from *Hop. indicus* and *Hel. indicus.* Least amino acids were detected from the nematodes of the higher altitudes whereas the larger numbers were detected at plain. The studied phytonematodes did not possess ornithine, glycine and threonine at higher altitudes. Presence of all the three amino acids revealed strong possibility of urea cycle in *Hop. indicus* and *T. brevilineatus*. Presence of aspartic acid, glutamic acid and histidine in *Hel. indicus* might be suggestive of their involvement in some biosynthetic activity and not in urea cycle. The maximum number of exceptionally different amino acids was found in *T. brevilineatus* at plain.

Transamination reactions, involving aminotransferases, are common system in many parasites for the interconversion of the amino acids e.g. α-ketogluterate to glutamic acid and pyruvate to alanine. Several amino acids can act as donor for both these reactions. Both these systems appear to be operating in *Hop. indicus, Hel. indicus* and *H. mangiferae* under different plant hosts in varied environments. The study also indicated that the healthier plants at high altitude do not permit the direct transfer of amino acids to parasitic phytonemas.

TOPIC FOUR – PLANT-PARASITIC NEMATODES: ROOTS

Occurrence of Rice-root Nematode, *Hischmaniella oryzae* among 11 Rice and10 Weed Selections

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The occurrence of rice-root nematode, *Hirschmaniella oryzae* in roots of 11 rice, *Oryza sativa,* selections and 10 weed species belonging to 7 families was recorded during 2006-07. Nematode density in composite root samples was determined by a Modified Sieving-Baermann Funnel technique. Nematode root populations varied greatly among rice selections and weeds. Ten rice cultivars including Super Basmati, Basmati-198, Basmati-2000, Basmati-370, Basmati-385, KSK-133, KSK-201, KSK-282, IR-6, and IR-9 exhibited nematode population levels in excess of the damage threshold level (5-30 nematodes per g of root). Only Basmati Pak supported nematode populations below the threshold level. The Basmati selections supported varying levels of nematodes. Basmati-370, Basmati-385, and Super Basmati supported significantly greater nematode populations than three other Basmati selections. Two IR selections supported the same number of nematodes but significantly fewer than KSK selections. Six weed species including *Echinochloa colona, E. glabrescens* (poaceae), *Chenopodium album* (chenopodiaceae), *Cyperus difformis, Rumex dentatus* (polygonaceae), and *Scripus maritimus* (cyperaceae), supported nematodes at levels similar to that recovered from rice roots. Four other weed species including *Coronopus didymus* (brassicaceae) *Marsilea minuta* (marsileaceae), *Paspalum distichum* (poaceae), and *Sphenoclea zeylanica* (campanulaceae) were consistently infected by the nematode but at levels reduced from those found in rice roots. This study indicates that rice-root nematode is able to infect all commercially grown cultivars and weeds common to Pakistani rice fields. These results further demonstrate that weed hosts act as a reservoir for over-wintering *H. oryzae.*

Pathogenicity and Management of *Pratylenchus penetrans* on Apple in Quebec, Canada

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In 2006, three 1-year dwarf-type apple rootstocks were exposed to field estimated densities of 0 (undetectable), 500 (intermediate), and 5000 (high) *P. penetrans* kg^{-1} under field microplot conditions in a split-plot design with eight repetitions. Based on shoot dry weights, growth of roostocks Bud-9, M-9, and M-26 was reduced by 30 and 36%, 23 and 25%, 14 and 29% when exposed the intermediate and high *P. penetrans* densities respectively. Trunk diameters were also reduced by 31% (Bud-9), 19% (M-9) and 15% (M-26) when exposed the high density and Bud-9 exhibited a 17% reduction under the intermediate density. From 2004 to 2006, a field experiment was performed to evaluate the potential of a 1- and 2-year cover cropping to management of *P. penetrans* densities and assess its incidence on the growth and production of apple transplants. Cover crops evaluated were forage pearl millet cv.

CFPM101, barley cv. Myriam, buckwheat, and a mixture of timothy-clover. As controls, apple transplant holes were field with pasteurized field soil or fumigation (metham sodium). After both 1- and 2-year cover cropping, pre-plant *P. penetrans* densities were lower under forage pearl millet than under the other cover crops. In the 1-year rotation plots, annual growth heights of M-26/Ginger Gold and Bud-9/Ginger Gold apple transplants were significantly reduced by 22 and 31% after timothy-cover, 31 and 13% after buckwheat, 12 and 21% after barley, 9 and 16% after forage pearl millet respectively when compared to pasteurized soil controls. In the 2-year rotation plots, annual growth heights of M-9/Cortland and M-26/Cortland apple transplants were significantly reduced by 43 and 44% after barley, 23 and 29% after buckwheat, 13 and 23% after timothy-cover, and increased by 7 and 8% after forage pearl millet respectively when compared to fumigated controls.

In 2005, number of fruits and total fresh weight of fruits produced by Bud-9/Ginger Gold apple transplants were significantly reduced by 23 and 24% after timothy-clover mixture, 32 and 31% after buckwheat, 22 and 24% after barley, 18 and 21% after forage pearl millet respectively when compared to control. In this same trial, productivity of M-26/Ginger Gold transplants were not significantly affected by the one-year rotation but a reduction of 8 and 12% in number of fruits and total fruit weights of respectively was observed in plots previously in barley which harboured the highest pre-plant *P. penetrans* densities.

Symptoms Description of Orange Trees Infected with *Pratylenchus jaehni*, Rootstock Resistance, Host Range and Development of Young Plants Inoculated

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Pratylenchus jaehni is the most aggressive nematode for the citrus crop in São Paulo State. In the present research the symptoms of orange plants infected by the nematode were described and documented. Also, the resistance of six rootstocks was evaluated, as well as the host range of the nematode crops and weeds. In microplots, at field conditions, it was studied the influence of the nematode in increasing levels of inoculum on the development of young plants of orange trees. The plants infected by the nematode show low foliar density, small leaves and fruits, smaller rate of growth and, under severe water stress they may die. Necrotic lesions on the roots, characteristic of the infection and death of feeder roots are observed. Among six rootstocks studied Rangpur lime was the only susceptible one to the nematode. Cleopatra tangerine, citrumelo Swingle, Sunki tangerine, *Poncirus trifoliata* and citrange Carrizo are resistant to the nematode. Among the plants species studied only the millet, soybean, maize and *Crotalaria juncea* are susceptible to the nematode. After 12 months from the inoculation of *P. jaehni* in young plants of orange trees `Valência', grafted on Rangpur lime, it were observed reductions of 22% in the height of the plants, 22.5% in the diameter of the stem, 20.4% in the diameter of the second branch and 43.7% and 52.5% of the canopy volume at 11 and 12 months, respectively, after the inoculation.

Survival of *Radopholus similis* (Cobb) in Volcanic Soils without Host-plant

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The burrowing nematode *Radopholus similis* is the most damaging nematode on bananas. To minimize applications of nematicide, cropping systems based on fallow, rotation crop, and clean planting material have been developed in the French West Indies. Here, we study soil survival of *R. similis* to optimize the benefit of the intercropping period and to increase the economical durability of banana cropping systems.

We monitored for six months in the laboratory the survival of calibrated populations of *R. similis* on nitisol and andosol, two soils derived from volcanic ashes and pumices. We studied different water potentials ranging from 0 to -700 kPa, on soil previously treated by frost or undisturbed. Mortality of adult *R. similis* follows a sigmoid decrease. In soils previously treated by frost, *R. similis* survive better in wet soils (-0,1 kPa) than in dry soils (-100 to -700 kPa). At the opposite, on undisturbed soils, *R. similis* survived better in dry soils. Half lives were two weeks inferior (from 27 to 37 days) for juveniles *R. similis* compared to adult females. Males survived from 40 to 71 days, significantly longer than females; one possible explanation is that males' energy is totally directed towards the reproduction and not for food foraging (males doesn't feed as they exhibit no functional stylet and digestive tractus). These results are consistent with the absence of anhydrobiosis strategy in *R. similis* compared to *Pratylenchus coffeae*. These results also suggest that *R. similis* survival depends not only of environmental conditions such as soil moisture and microbial activity, but also of their behaviour.

Water Dissemination of *Radopholus similis* (Cobb) on Nitisol in Martinique

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New cropping systems have been developed in the French West Indies that combine fallow or rotation crops with nematode-free vitro-plants to avoid the repeated applications of nematicides in banana fields. However, sometimes after only two to four years, the burrowing nematode *Radopholus similis* progressively reinfests banana fields and causes damages and economic losses again. Among different hypothesis for reinfestation, we studied the possibility that nematodes were disseminated by runoff and leached water from upstream infested fields. At the decimetre scale, we analyzed the dispersion of *R. similis* individuals on soil surface under a 1 m² rainfall simulator; water leaching of nematodes was also studied using soil cylinders apparatus in aspersion chambers. At the field scale, reinfestation of nematodes was monitored plant by plant. The experimental field was divided in plots surrounded or not by ditches. All studies were conducted on nitisol, which are representative of lowland banana fields in French West Indies. Results show that water runoff is likely to disseminate *R. similis* individuals over long distance on soil surface when soil moisture is already close to the field capacity. At the opposite, dissemination to soil depth of *R. similis* is limited: less than 8 % of the nematodes reached layers deeper than 10 cm after exceptional

rainfalls that represent several times the poral volume of the soil. A passive dissemination model could only explain partially the distance covered by nematode individuals and not the percentage of dead or non-active nematode which increases with covered distance or soil depth. It seems that *R. similis* have developed behaviour of escaping leaching. However, from a practical point of view, 50- to 80-cm deep ditches can efficiently prevent *R. similis* dissemination in banana fields.

Occurrence, Abundance and Distribution of Plant Parasitic Nematodes Associated with Sugarcane in Western Kenya

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A study was conducted in the four sugarcane growing zones of Western Province of Kenya to identify plant parasitic nematodes associated with the crop and determine factors influencing their occurrence and distribution. Soil was scooped from the sugarcane rhizospheres at a depth of 5-20 cm from which 200cm^3 was taken for analysis. Nematodes were extracted using the modified Baermann funnel technique and identified up to the genus level and enumerated. Plant parasitic nematodes belonging to 15 genera were recovered in sugarcane fields in Nzoia, Mumias, West Kenya and Busia sugar production zones in Kenya. Three genera namely *Pratylenchus*, *Scutellonema* and *Meloidogyne* were predominant with percentage densities of 21, 18 and 13, respectively. Soils in Nzoia Sugarcane Zone were more heavily infested with the parasites while the West Kenya Sugarcane zone was least infested.

Sandy clay soils were found to contain more parasitic nematodes than clay or clay loam soils. Nematode numbers were significantly different among the ecological zones from which samples were taken. Plant parasitic nematodes associated with sugarcane are widespread in western Kenya sugarcane zones and thus there is need to determine their effect on cane yields and quality.

Identification of Medicinal Hosts for Root-knot Nematodes in the North of Iran

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Iran is considered as a major source for medicinal plants in the world, because of historical backgrounds in medicinal identification and utilization to remedy human and animal diseases and the plants significant diversity. So, study on plants pathogens to control their damage is really important. During a survey conducting about distribution of root-knot nematodes(*Meloidogyne* spp.) in the north of Iran, root infection of 10 important species of medicinal plants: *Artemisia abrotanum* L.(Wormwood), *Capsicum annuum* L.(Red Pepper), *Lycopersicum esculentum* L.(Tomato), *Ocimum basilicum* L.(Basil), *Olea europaea* L.(Olive), *Oxalis corniculata* L.(Woodsorrel), *Punica granatum* L.(Pomegranate), *Rosa canina* L.(Rose), *Viola odorata* L.(Sweet Violet) and *V. tricolor* L.(Heartsease) were observed during 2006. Infected roots were collected and females were extracted from roots, then morphology of perineal patterns were studied and *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 and *M. javanica*(Treub, 1885) Chitwood, 1949 were identified. Galls induced by these two species occurred both individually and in clusters, varied in size, with evident distortions of entire root circumference in each host. Severe infection and damage was observed in basil and tomato by *M. incognita* and *M. javanica*, respectively.

Study of Nematodes Associated with Ornamental Foliage Plants in the North of Iran

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In order to identify nematodes associated with ornamental foliage plants in greenhouses in the north of Iran, soil and root samples of the most common ornamentals:(Aloe, Aluminium plant, Angel wings, Begonia, Chinese evergreen, Climbing philodendron, Croton, Desert privet, Devils ivy, Dumb cane, Dwarf banana, Elephant foot tree, Finger plant, Flaming dragon tree, Goose foot plant, Ivy tree, Madagascar dragon tree, Mother of thousands, Prayer plant, Rubber plant, Screw pine, Spider plant, Spineless yucca, Star fish, Sweetheart plant, Swiss cheese plant, Tiger begonia, Umbrella grass, Umbrella tree, Upright philodendron, Velvet plant and Weeping fig) were collected during 2004-2006. The samples were washed and the nematodes were extracted by sieving and centrifugal sugar-flotation technique. Then they were fixed and transferred to glycerin according to the Seinhorst modified method. The permanent slides were mounted and studied thoroughly. In this study 35 species of Tylenchida, 4 species of Rhabditida, 1 species of Dorylaimida were identified. The most important species were *Meloidogyne incognita*, *M. javanica*, *Pratylenchus neglectus* and *Scutellonema brachyurus*. The root lesion nematode, *P. neglectus* was found in 35% of soils and 5% of root samples, with the population density of 10-120/200 g soil.The root-knot nematodes, *Meloidogyne incognita* and *M. javanica* were the most dominant species with significant damage up to 100% in some samples. In 29% of cases both species were present in the same soil. *S. brachyurus* was found in 10% of soils and 6% of root samples, with the population density of 50-430/200 g soil.

Anatomical and Histological Alterations Induced by a Species of Plant-parasitic Nematode of the Genus *Hemicycliophora* in Celery (*Apium graveolens*) Roots from Argentina

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In order to characterize the anatomic and histological alterations in roots of celery (*Apium graveolens*) produced by the presence of a species of the genera *Hemicycliophora*, we evaluated the host-nematode relationship through exomorphological, anatomical and histological analyses. Plants grew in soil obtained from Pucará, Aconquija, Department of Andalgalá, province of Catamarca. Samples of both infected and healthy roots were processed for optical microscopy. The infected roots showed galls of different characteristics: small, simple galls with cylindrical shape, and larger, complex galls, with various shapes. On the surface of both types of galls, nematode specimens could be observed. It could also be observed that the small galls showed cellular hyperplasia affecting the pericycle which produced prominences of meristematic tissue. These prominences formed primordia of lateral roots. The vascular tissues were disorganized and displaced. In the complex galls the histological changes were similar to those observed for the simple galls. However, the complex galls were formed by numerous differentiated lateral roots, some of which were also modified, producing simple galls. The central body of complex galls was constituted by adult tissues. The changes induced by the nematode were associated to the presence of galls with an important proliferation of lateral roots. Their histological characteristics suggest that the simple galls represented a stage previous to their development into complex galls and the alterations of their vascular tissues probably affected their functionality.

Host-finding in Potato Cyst Nematodes

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Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* are stimulated to hatch from cysts within the second and fifth week after potatoes start to produce roots. Lipid reserves of potato cyst nematodes allow them to survive during ca. ten days in their search of a suitable host plant. We hypothesize that potato roots release chemicals that guide newly hatched nematodes to roots. The aim of this study is to identify the chemical cues and behavioural mechanisms that allow potato cyst nematodes to recognize and localize their host.

Attraction of nematodes to potato roots and root exudates is investigated in an agar-plate and a glass-tube bioassay. Nematode behaviour during different phases of the host-seeking process is described. Root exudates are obtained from potatoes grown in sand, in hydroponic and aeroponic systems. The hatching and attraction response of nematodes to roots, root exudates and fractions of root exudates from a susceptible potato variety are studied using these bioassays. In addition, first results of the chemical analysis of active exudates by GC-MS, HPLC-DAD, HPLC-MS are presented.

The Effect of Different Levels of Population Densities of *Heterodera filipjevi* (Madzhidov, 1981) Stelter, 1984 on Bread Wheat under Microplot Conditions

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The three species of cereal cyst nematodes *Heterodera avenae* Wollenweber, 1924, *H. filipjevi* (Madzhidov, 1981) Stelter, 1984 and *H. latipons* Franklin, 1969 are considered to be the most economically important species on wheat compared others in the *H. avenae* complex. *H. filipjevi* and *H. latipons* are widely distributed in Iran, while *H. avenae* has limited distribution on wheat. To determine the impact of different levels of population densities on wheat four levels of population densities of *H. filipjevi* 2.5, 5, 10 and 20 eggs and J2s /g^{-1} soil as initial populations were tested on wheat cultivar Sardari in microplot under natural field conditons arranged as a Completely Randomized Design replicated seven times. The results showed increasing the *H. filipjevi* initial population significantly reduced several growth parameters of wheat (including plant height, number of tillers, spike height, dry root weight, aerial dry weight, and grain yield) compared to untreated control. The final population increased with increasing initial population while the reproduction factor (RF – final population/initial population) reduced by increasing the initial population but it was still greater than one in all treatments. The regression analysis showed significant negative relationship between the initial populations and grain yield, and grain yield was reduced even at lowest population density and reached a maximum reduction of 47% at population density of 20 egg and J2s/ g^{-1} soil.

Destroying *Melodogyne chitwoodi* in Potato before Processing

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Columbia root-knot nematode (*Meloidogyne chitwoodi*, CRKN) infects potato tubers and causes quality defects such as galling of the external surface and brown spots surrounding the egg masses of adult females inside the tuber. Both symptoms are considered quality defects by the potato industry and can cause crop rejection. Furthermore, CRKN is considered a quarantined pest by several countries and infested tubers are not allowed entry. For example, in 2005 57 containers (approximately 1,140 MT) of chipping potatoes from the United States were denied entry into South Korea due to the presence of CRKN and the potatoes were returned. This resulted in lost revenue to the growers and a loss of product needed for processing potato chips in South Korea. Although CRKN is aggressively managed a probability exists that a small percentage of tubers may be lightly infested. An alternative to re-exportation of potatoes when low amounts of CRKN are detected is to destroy CRKN in infested potato tissues before processing. Several procedures were tested as destruction protocols for eliminating live CRKN from potato tissues by treating either whole tubers or peels, placing the tissues on a mist chamber and daily examining any recovered nematodes for viability. Exposure of CRKN–infested tissues to chlorine in the form of bleach at concentrations up to 25,000 ppm Cl was ineffective. Boiling potato peels and water

associated with peels for five minutes was effective but judged to be unacceptable to the industry. Heating potato peels and associated water to 60 ºC or above for one minute or longer was found to be an effective destruction protocol. Submersing whole tubers in water at 80 ºC for a minimum of three minutes before peeling was a reliable destruction protocol that would prevent escape of living CRKN through the peeling process.

Variability of the Reproductive Capacity of *Nacobbus aberrans* Populations from Argentina on the Pepper Cultivar 'California Wonder'

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Reproduction of seven Argentinean populations of *N. aberrans* on the pepper (*Capsicum annuum*) cultivar 'California wonder' was evaluated. Nematodes were from different localities of origin: Coronel Baigorria and Río Cuarto (province of Córdoba), Lisandro Olmos (Buenos Aires), El Pucará del Aconquija (Catamarca), Tunuyán (Mendoza), Lules and Tafí del Valle (Tucumán). All the populations were collected from soils cultivated with horticultural crops, except for the first one, which was naturally associated with a weed (*Chenopodium album*). The experiment was conducted in greenhouse conditions at a mean temperature of 21 °C; six replications per population were performed. One hundred active second-stage juveniles were inoculated per plant at planting. After 90 days, the Reproduction Factor (RF=final density/initial density) was calculated for each population. All the populations reproduced on pepper. However, three significantly different groups of populations were distinguished: a) Coronel Baigorria, Lules, El Pucará del Aconquija and Tafí del Valle (with RF ranging between 1.6-6.2); b) Río Cuarto and Lisandro Olmos (RF=25.1-31.4); c) Tunuyán (RF=47.7). The variability detected in the reproductive capacity of *N. aberrans* on a single host is useful for the formulation of alternative strategies to manage this nematode using resistant cultivars.

Biogeography of the False Root-knot Nematode (*Nacobbus* spp.) and its Major Crop Hosts

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Spatial separation of populations is one of the reasons for the variation in biography of this nematode. Such variation is detected in plant parasitic nematodes over both great and relatively small distances. Different organisms with the same ecological requirements can be sympatric only if the environment they inhabit is heterogeneous. This heterogeneity may be spatial or temporal but can also be provided by host plants. *Nacobbus* spp. are endemic to the American continent and distributed along two biogeographical regions (Palearctic and Nearctic). These regions are centres of origin of crops, including, amongst others, potato (*Solanum* spp.), tomato (*Lycopersicon* spp.), chilli pepper (*Capsicum* spp.) and beans (*Phaseolus* spp.) that were domesticated by ancient American civilizations in highly developed agricultural systems. The morphological and physiological variation of *Nacobbus* spp. populations along its geographical distribution range have been studied using morphometrics, physiological (i.e. response to a set of differential plants) and molecular approaches in order to identify species, ‘groups’ or races of the nematode. However, little attention has been paid to *Nacobbus* spp. biogeography, biology, host-nematode-environment relationships, or to explore co-evolution patterns with host plants and crops. To understand *Nacobbus* spp. speciation processes and their geographical distribution patterns, it is necessary to consider geological events, biological traits, and ecological conditions for both the nematodes and their native hosts as grown in the agro-ecosystems where nematodes have thrived with and without human interference. In this poster the biogeography for both host and parasite, and differences in the life cycle of the nematodes in different Solanaceae hosts, are examined for selected North and South American populations of the species *N. aberrans s.s*. and *N. bolivians*.

Investigation of a Model System to Develop Controls for Plant-parasitic Nematodes

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Plant-parasitic nematodes infect vast numbers of economically important crop plants causing severe ecological and economic losses worldwide costing a minimum of US$125 billion (2003) annually. Broad-acre control measures such as soil treatment or use of resistant cultivars are limited and increasingly ineffective. Other agronomic practices, such as annual crop rotation, are of limited utility. Thus, new options must be explored in order to regulate plant-parasitic nematode populations and reduce the ecological damage and economic losses.

As plant-parasitic nematodes have a slow-life cycle (1-3 months) and are obligate parasites, functional investigation in the laboratory is difficult. In this study, we are evaluating a model system for controlling parasitic nematodes using RNA interference (RNAi) technology. The model system comprises the fungal feeding nematode *Aphelenchus avenae* and *Botrytis cinerea.* This approach will mimic the biology of plant-parasitic nematodes and potentially allow for a quick turnover of experiments, thus allowing us to identify and validate effective nematode-specific target genes.

We identified 48 potential nematode-specific target genes by combining a database screen of reported *C. elegans* RNAi phenotypes and comparing nematode genomic and EST databases with those of plants and other non-target species. We have demonstrated that *A. avenae* ingests material directly from solution, as shown by FITC uptake, and that *in vitro* silencing of the gene *rpt-3* by dsRNA appears to reduce sensitivity to the acetylcholinesterase inhibitor aldicarb. *B. cinerea* has successfully been transformed with a GFP-expression construct and cloning strategies for an RNAi construct have been devised.

Community Structure of Plant-Parasitic Nematodes under Different Soil Type and Latitude in Soybean Fields

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Plant-parasitic nematodes are important as a potential yield limiting factor in agriculture production, they cause estimated annual crop losses $78 billion worldwide. Soybean is major economic crop in China, there are many genera of plant-parasitic nematodes in soybean rhizosphere. The test was conducted in major soybean production area of China in 2005 to confirm community structure, occurrence frequency and population density of plant-parasitic nematodes in soybean rhizosphere, which will offer data for assessment on agriculture soil health, control of soybean root diseases and study on soybean rhizosphere microbial ecosystems in the future. In total 86 soil samples were collected from soybean fields of 9 provinces (HeiLongjiang, Neimenggu, Jilin, Liaoning , Shandong, Shanxi, Heibei, Henan, Jiangsu), soil samples include 8 soil types (black soil, meadow soil, aquic brown soil, alkaline saline soil, brown soil, white puddled soil, sandy soil, aquic soil) from 32°05′N to 52°50′N. Baermann funnel technique was used to extracted nematodes from soil samples.

In total 13 genera and 6 species of plant-parasitic nematodes were identified, 8 genera and 4 species were first reported in soybean fields of HeiLongjiang Province. *Aphelenchus*, *Heterodera* and *Helicotylenchus* survive widely in soybean fields, the occurrence frequencies of them were 75%, 69.1% and 63.1%, respectively. There were 12 genera in black soil, 9 genera in meadow soil and 6 genera in brown soil. The result indicated that community structure of plant-parasitic nematodes were influenced by soil type in soybean field. Soil with high organic matter had better community structure and lower population density of plant-parasitic nematodes. Latitude had little effect on distribution of plant-parasitic nematodes range from 32°05′N to 52°50′N.

Crop Rotation to Replace Nematicide Treatments for Assessing Chickpea Varietal Tolerance to *Pratylenchus thornei*

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Field trials were conducted to determine if crop rotation would suppress soil populations of *Pratylenchus thornei* and eliminate the use of nematicde plots when assessing chickpea cultivars for tolerance. Assessing yield response to *P. thorne*i is difficult due to problems associated with nematicide penetration in the sub-soil. In year one, plot treatments consisted of canola, linseed, canaryseed, wheat and bare fallow, applied with or without aldicarb at 10kg/hectare in a three randomized block design. The following year the same plots were re-cropped with four chickpea cultivars and one intolerant wheat cultivar and aldicarb re-applied to the nematicide plots. Results from the pre-crops revealed wheat produced significantly higher nematode numbers throughout the soil profile than the other treatments. *P. thornei* populations peaked at depths of 45-60cm but nematicide was only effective down to depths of 30cm. Canola and fallow resulted in significantly lower populations of mycorrhizal spores than wheat. Canaryseed resulted in low nematode numbers in contrast to wheat and high mycorrhizal spores comparable to wheat so it was selected as the best control treatment for future use. All pre-crop treatments had similar moisture down to depths of 60cm. Chickpea yields on pre-cropped canary plots increased by 10-30% compared to yields on pre-cropped wheat plots. Nematicide on canary plots gave no additional yield of the following chickpea. Results demonstrated that using resistant canary and susceptible wheat plots is an effective alternative to plus nematicide and minus nematicide treatments when assessing tolerance in chickpea cultivars. This methodology has been successfully implemented in subsequent trials.

Molecular Markers for Resistance to Root-Knot Nematode on Egyptian Cotton

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Survey of nematodes in cotton fields at Fayoum Governorate indicated nine genera of plant-parasitic nematode associated with cotton root *Helicotylenchus, Hemicycliophora, Hoplolaimus, Meloidogyne, Paratylenchus, Pratylenchus, Rotylenchulus, Tylenchorhynchus* and *Xiphinema* The root-knot nematode (RKN) (*Meloidogyne* spp.) was widely distributed in the surveyed fields. Identification of RKN, *M. incognita* isolates revealed one race. Race 3 .The rates of nematode build-up gradually increased with increased inoculation level up to 2000 juveniles / plant but later a gradual drop in numbers occurred. Relative susceptibility of cotton cultivars subjected to infection by, *M. incognita* race 3, revealed that 2 were rated as highly susceptible, Giza 88 and Menoufi, 5 were rated as susceptible, Giza 70, Giza 83, Giza 85, Giza 89 and Giza 90 and 4 were rated as resistant, Giza45, Giza 80, Giza 86 and Giza 91 Plant growth in susceptible cultivars was significantly affected by nematode infection, whereas plants of resistant cotton cultivars were not affected. In the susceptible cotton Giza 83, the life-cycle of *M. incognita* race 3 durated about 25 days. In the resistant cotton Giza 86, it lasted about 45 days. Plant cortical layer, stellar regions and pericycle tissues changed by RKN infection. Also, similarity matrix based on RAPD-PCR analysis was 67.6%, between the two resistant cotton cultivars and was 60.6%, between the two highly susceptible and susceptible cotton cultivars. RAPD-PCR showed some molecular markers related to RKN resistant and susceptible plants. For instance, primer OP-A16 gave specific markers with MW of 260 bp for (Giza70), 950bp; 700bp and 300bp for (Giza 90) and 250bp for (Giza 80).

Distribution of Known and New Migratory Endoparasitic Nematodes on Wheat Production in the Isparta Province of Turkey

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Migratory endoparasitic nematodes, especially *Pratylenchus* species are considered economically important nematodes on cereals. A survey was conducted from 8 locations of Isparta taking 63 random soil samples in wheat fields around post flowering between May and June of 2007. Nematodes were extracted soil by using modified Baermann Funnel technique and samples examined under dissecting microscope and species identified using morphological characters. *Pratylenchus thornei*, *P. neglectus*, *P. scribneri* and *Pratylenchoides alkani* were identified by using morphlogical characters, and often these were found in mixed populations, and a single species were distributed in some locations. Of interesting not the species *Pratylenchoides alkani* was found in high populations in many samples and always in combination with *Pratylenchus. Pratylenchoides alkani* has not been reported on wheat, and this study would suggest it could be economically improtant.

The population dynamics of root lesion nematode were followed during one field season at three weekly intervals by taking from soil and root samples in different wheat cultivars (İkizce, Kızıltan, Gün 91, Mirzabey). Root lesion nematodes were well developed in all wheat cultivars, and all cultivars were considered susceptible, with possibly two generations over one season. The number of lesion nematode in the root over the season increased whilst soil number declined. This trend was reversed at the end of the season, presumably due to exiting of nematodes from roots into the soil. More work is being conducted to determine the economic importance of both lesion and *Praylenchoides* on wheat.

Survey for Root-lesion and Stunt Nematodes in the Northern Australian Grain Region

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The presence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in some parts of the northern grain region has been known since the 1960's and yield loss in wheat caused by *P. thornei* has been demonstrated since the late 1970's. However, the distribution of *P. thornei* was considered restricted to the Darling Downs in Queensland and to a few localities in northern NSW. Following the diagnosis of *P. thornei* in a wheat crop in a newer cropping area around Goondiwindi in 1996 a more extensive survey of wheat fields was conducted. Soil samples (0-30cm) collected mainly from under wheat crops were processed manually and nematodes extracted by the Whitehead tray method and enumerated under a compound microscope. Out of 795 fields tested from 1996 to 2002, *Pratylenchus thornei* occurred in 67%, *P. neglectus* in 32% (both species occurred together in 26%) and no *Pratylenchus* spp. were detected in 27%. *Merlinius brevidens* occurred in 73% of fields. Edaphic factors controlling the incidence of these nematodes were tested on 833 samples collected in 1996-7. All three nematode species had a broad pH range, namely from 6.5 to 9.5 for both *Pratylenchus* spp. and from 6.0 to 9.5 for *Merinlius brevidens.* All species were detected in soil samples ranging from <20 to 80% clay and from <20 to >80% sand. However, within this range maximum incidence of *P. thornei* was in finer textured soils than for *P. neglectus.* Soil organic carbon had no clearcut effect on the incidence of the species. The incidence of *P. neglectus* appeared to increase with increasing concentration of DTPA-extractable zinc and bicarbonate–extractable phosphorus in the soil. Observations indicate that *P. thornei* was spreading in the region in run-off water and in soil on farm machinery and increasing under intense cropping to wheat.

The Presence of Plant Parasitic Nematodes in Peanut Pods

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In South Africa peanut pod shells are commonly used as ground cover in horticulture. The presence of *Ditylenchus africanus* in peanut pod shells and its detrimental effect on peanut production in South Africa are well documented, prompting the authors to search for other plant parasitic nematode species which might also lurk in peanut pod shells. On examination of shells from farms in the Vaalharts Irrigation Scheme in the Northern Cape Province, we found *D. africanus*, *Meloidogyne* sp,., *Aphelenchoides arachidis* and *A. blastophthorus*. According to the South African Plant-Parasitic Nematode Survey (SAPPNS) database and literature, *Pratylenchus brachyurus, Tylenchorhynchus brevilineatus and Criconemoides ornatus* are also found on peanut shells. All these nematodes are risk species in the cultivation of peanuts but what is disconcerting for the home gardener, who uses peanut shells as mulch, is the large list of other host plants that they also attack. Most ornamental plants some turf grasses and vegetables such as tomato, beans, peppers, carrots, cucumbers, eggplant, are attacked by the root knot nematode, *Meloidogyne*. *Pratylenchus brachyurus* attacks among others apple, beans, citrus, grape, peach, pear, strawberry, rose, most vegetables and turf. *Criconemoides ornatus* affects potato, sweet potato and also ornamental plants. The effect of *D. africanus* and *A. arachidis* on plants other that peanut and cereals has never been established, but other legumes might be potential hosts for these nematodes. When adding compost or ground cover, containing peanut shells care must therefore be taken as there are currently no nematicides or soil fumigants available to home gardeners.

Burrowing Nematode (Radolpholus similis) on Ginger in Fiji

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Radopholus similis multiplies readily on ginger in Fiji and in situations where the nematode reaches high population densities, cavities form in rhizomes and affected tissues are discoloured. Recent surveys have shown that economic losses from *R. similis* are not as common as they were 20 years ago, largely because most Fijian ginger is now harvested about 5 months after planting rather than being grown to maturity. Another reason for a reduction in losses from *R. similis* is that immature ginger is usually grown in rotation with cassava and taro and high rates of poultry manure (15-20 t/ha) are applied before planting, and in this farming system, nematode populations are not high enough to damage the immature crop. If the nematode is introduced on infested planting material, small, shallow, sunken, water-soaked lesions may develop on immature rhizomes, but the damage is superficial and the crop is still marketable. *R. similis* is mainly a problem on crops that are held over to supply seed for the following season. On infested farms, the nematode continues to multiply as the season progresses and populations reach levels that may result in more than 50% of the seed ginger being rejected. Current work is focused on using tissue-culture to provide nematode-free planting material, encouraging growers to dip their seed in hot water using a well-established protocol (a temperature of 51ºC for 10 minutes), selecting fields to be retained for seed production on the basis of nematode counts taken at the time of immature harvest, and controlling volunteer ginger and weeds such as crowsfoot (*Eluisine indica*) that host the nematode during the non-ginger phase of the rotation.

Distribution of *Helicotylenchus multicinctus* and their Associated Natural Enemies in Banana Plantings in Hawaii

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Previously, the spiral nematode, *Helicotylenchus multicinctus*, was not listed as an important nematode pest of banana (*Musa* spp.) in Hawaii. The objectives of this study were to determine the distribution of spiral nematodes inhabiting banana fields in Hawaii and their natural enemies (e.g. predatory nematodes and nematode-trapping fungi). Twenty-seven banana farms were surveyed on six major Hawaiian islands. Spiral nematodes were detected in 17 out of 27 farms surveyed with densities of 220 to 1,427 nematodes per 250 cm^3 soil and 367 to 21,556 nematodes per 10 g dry root weight on banana cultivars with ploidy levels of AAA (‘Williams’, ‘Chinese Williams’, ‘Bluefields’, and ‘Valery’), AAB (‘Tall Brazilian’, ‘Santa Caterina’), ABB (‘Largo’, and ‘Ice Cream’), and BBB (‘Saba’). The survey revealed that *H. multicinctus* is more commonly found in banana than the burrowing nematode, *Radopholus similis*. Severely infected banana plants have poor root system and tended to topple. During this survey, several natural enemies of nematode pests were encountered. Omnivorous and predatory nematodes, which potentially prey on spiral nematodes, were found in 93% of the farms surveyed. Although not statistically different, organic farms had higher counts of omnivorous or predatory nematodes than many conventional banana plantings. Among the nematode-trapping fungal species observed, *Arthrobotrys oligospora*, *Monocosporium ellipsosporum*, and *M. eudermata* were the most commonly found nematode-trapping fungi.

Effects of *ced-9* Antisense Expression in Transgenic Tobacco Plants on *Meloidogyne incognita*

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As an alternative to using pesticides to control nematodes, we are exploring the possibility of using transgenic plants expressing nematode programmed cell death genes to control nematode infestation. We hypothesize that knocking down *ced-9*-like genes of plant parasitic nematodes using antisense RNA, will limit their proliferation and/or reproduction. Provided that there are similar *ced-9* sequences in parasitic nematodes, we predict that plants containing a reverse (antisense) *ced-9* gene would stimulate the programmed cell death pathway of parasitic nematodes, resulting in plant protection. We generated homozygous transgenic tobacco plants expressing either *ced-9-F* (*ced-9* gene clones in the sense orientation) or *ced-9-R* (*ced-9* gene cloned in the antisense orientation). Selected *ced-9-R* and *ced-9-F* transgenic tobacco lines, both expressing high levels of the transgene (as determined by competitive RT-PCR) and no other phenotypic effect, were tested for resistance to

Meloidogyne incognita (Root-Knot Nematode-RKN) by measuring gall formation, size of galls generated, and J2 hatching ability. The means of number of gall formation did not exhibit any statistical difference between transgenic and wild-type tobacco plants. Gall size was smaller, however, in transgenic *ced-9-R* or *ced-9-F* than in control plants. Furthermore, hatching ratios were low in *ced-9-R* transgenic plant lines, by approximately 50%, when compared to *ced-9-F* or control plants. Results from these experiments suggest that expression of either *ced-9-R* or *ced-9-F* genes in tobacco plants induced prevention of *M. incognita* proliferation. However, c*ed-9-F* expressing plants prevent the proliferation by limiting the size of galls formed, while *ced-9-R* expressing plants do so by both limiting the size of galls formed and by preventing embryo hatching. We speculate that the hatching prevention in the *ced-9-R* expressing plants is due to the action on a *ced-9* like sequence during embryogenesis of *M. incognita* taking place in the transgenic plant.

Histopathological Studies of Pomegranate Roots Infected by Root-Knot Nematode

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The present study reports the changes caused by root-knot nematode (*Meloidogyne incognita*) to Pomegranate (*Punica granatum*) in Sindh, Pakistan. Naturally infected roots collected from a depth of 5-30 cm from a Badin farm were cut into 0.5 and 1.00 cm long pieces, washed free of soil for 1 hour, fixed in F.A.A. and processed for histological study according to Sass (1964). Using a rotary microtome 10-12 µm thick sections were cut and stained with haemotoxylin and eosin. Photomicrographs were taken using an automatic photographic camera mounted on a research microscope Nickon Optiphot-2. It was observed that at some places the nematodes were responsible for the formation of several atypical cells, abnormal xylem, hyperplastic parenchyma and hypertrophy of tissue. Sections of root revealed the establishment of syncytium in extensively damaged endodermal cells. Cortical border appeared damaged at the site of penetration of the larvae. Metastatic cells were obvious in heavily infected portions of roots. Cell walls of altered pericycle became thicker due to presence of egg masses.

Topic Five – Plant-parasitic Nematodes: Seeds, Leaves and Stems

Seed- and Leaf-gall Nematode Infection of *Eragrostis* spp. Grasses in South Africa and Evaluation of a Management Strategy

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The presence of seed- and leaf-gall nematodes (*Subanguina* spp.) in seed of *Eragrostis* spp. grass is currently of great economic concern to the local grass seed industry. The adverse effect of these parasites on export as well as local markets since 2001 necessitated a survey in the *Eragrostis* production areas of South Africa. Furthermore, a mechanical, commercially viable technique for separating galled seed from uninfected seed was investigated. *Eragrostis* seed samples were obtained from 13 localities and soaked in water for 48 hours at 25º C for extraction of nematodes. To quantify the extent of seed- and leaf-gall nematode infestation, population density, frequency of occurrence (%) and prominence values (PV) were calculated. For identification purposes DNA fragments from these parasites were sequenced using several nematode primers. Separation of galled seeds from uninfected seeds was done for two separate nematode populations using three different aperture mesh sieves. Seed-and leaf-gall nematodes were identified as *S. weevilli*, with PV and population densities ranging from 0.1 to 205 in 1-g seed samples. With regard to sieving, the 250-µm mesh sieve contained a significantly lower number of seed- and leaf-gall nematode individuals as well as galls compared to the 600-µm and 500-µm mesh sieves for both populations. The unsieved control, however, maintained a significantly higher number of seed- and leaf-gall nematode individuals as well as galls than the three sieve treatments. Only one control strategy is not adequate, but an integrated management approach needs to be developed to ensure that grass consignments are free of seed- and gall leaf nematodes. Further research is in progress in an attempt to assist producers and the grass seed industry to address the nematode problem.

Efficacy of Abamectin as a Seed Dressing for Control of the Stem Nematode *Ditylenchus dipsaci* on Sugar Beet

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The stem nematode is an extremely damaging parasite of sugar beet in many growing areas of Europe. Control is complicated due to the lack of resistance and wide host range. Our research focused on the non-fumigant nematicide, Abamectin = avermectin B1a, produced by fermentation of the bacterium *Streptomyces avermitilis.* Abamectin is a very active nematicide with low toxicity to humans and the environment. We evaluated the efficacy of Abamectin as a seed treatment against *D. dipsaci* on sugar beet, *Beta vulgaris.* Greenhouse experiments showed that Abamectin treated seed protected the young seedlings of sugar beet from early *D. dipsaci* infection. The level of nematode penetration into the shoot of the seedlings of two cultivars, susceptible and moderately resistant to the stem nematode, was significantly reduced. There was no sign of phytotoxicity seen after treatment. *In vitro*

toxicity tests were conducted over 3, 9, 22 and 52 hours. Abamectin caused 40% paralysis within 3 hours of exposure to the nematode when compared to the check and reached 99.25% after 52 hours exposure. A high level of mortality was detected after exposure to Abamectin: 65 to 85% after 3 to 22 and 95% after 52 hours exposure, when placed in tap water. Abamectin seed treatment therefore would be a highly effective method of controlling the stem nematode on sugar beet.

Host Ranges of Four Populations of Stem and Bulb Nematode, *Ditylenchus dipsaci* in Iran

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D. dipsaci is widespread in alfalfa fields in Iran and the infection of garlic to *D. dipsaci* has been reported from a few regions in recent years. Four populations of *D. dipsaci* were collected from alfalfa and garlic plants in west, center, northwest and northeast respectively. The populations were cultured on monoxenic carrot disk to provide sufficient inoculation materials. The host ranges of two garlic populations were tested on garlic, onion, faba bean, common bean, sugar beet, pea and soybean. Alfalfa, *Melilotus* sp., common bean, clover, and sainfoin (*Onobrychis viciaefolia* Scop.) were tested with two alfalfa populations of *D. dipsaci*. The plants were inoculated at a rate of 100 *D. dipsaci* in 10µl of CMC (Carboxymethyl cellulose) suspension 1% and maintained in growth chamber at 20°C ± 2 for 16-hour light and 8-hours dark period, meanwhile high humidity was provided for all plants for one week. The plants were harvested after eight weeks (some after four weeks) and the aboveground parts were examined. The results showed both garlic populations were multiplied on all plants tested except on soybean, both populations highly multiplied on onion with reproduction factor (RF) of 30 and 128 for northwest and northeast populations, respectively, while the RF was 10 and 13 in garlic and 1-2 in faba bean, common bean, sugar beet and pea. The alfalfa populations showed high reproduction on common bean and the RF was 4 after four weeks, the RF on alfalfa and *Melilotus* sp. was 1-1.3 and for clover and sainfoin <1 after four weeks however, the population contained different developmental stages indicating multiplication of inoculated population. The existence of at least two races of *D. dipsaci* was confirmed in Iran, meanwhile the natural infestation of onion to *D. dipsaci* was revealed on onion bulb, stem and seed and it is a new record for Iran.

Infection of *Brachiaria brizantha* Seeds by *Aphelenchoides* Species, Efficacy of Irradiation and Chemical Treatment for Nematode Control

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With the objective of studying the infection processing of *Brachiaria brizantha* seeds by *Aphelenchoides* species, efficacy of irradiation and chemical treatment for nematode control, five experiments were carried at and laboratory and field conditions. To test the efficacy of chemical treatment, 1 kg of infected seeds containing 1,350 *Aphelenchoides* spp. by 10 g of seeds were treated with Abamectin at the doses of 6.0; 3.0 and 1.5 mL, Imidacloprid 12.0; 6.0 and 3.0 mL, Clotianidina 12.0; 6.0 and 3.0 mL, Tiodicarbe 30.0; 15.0 and 7.5 mL, Imidacloprid + Tiodicarbe 48.0; 24.0 and 12.0 mL and Carbofuran 50.0; 25.0 and 12.5 mL. Untreated seeds were used as controls. The experiment was carried out in the field, in Presidente Prudente County, SP and Tupaciguara, MG and in a greenhouse at UNESP Campus, in Jaboticabal. The seed irradiation was done using different doses of gamma-ray: 0; 500; 1000; 1500 e 2000 Gy, from ^{60}Co source at Agricultural Nuclear Energy Center, in Piracicaba, SP. Samples of 160 g of *Brachiaria brizantha* seeds were properly kept in regular paper bags and irradiated at the rate of 186.8 Gy/h. In another experiment, 10 g of *Brachiaria brizantha* seeds free from nematodes were placed on wet sand in gerbox and 10mL of a nematode suspension containing 300 specimens/mL was added. All the treatments used in this study had no efficacy for nematode control. Also, it was observed that the nematodes infect the seeds after they fall to the ground.

Nematode Galls on a Tiny Moss

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The minute Australian endemic moss *Stonea oleaginosa* was discovered by accident in 1969. It grows in semi-arid and arid areas of salt bush and mallee country across Southern Australia. In the original species description, it was reported that plants were found with abnormally elongated stems; this was attributed to infection by nematodes. Whilst examining recently collected specimens of *S. oleaginosa*, galls which resembled moss capsules were found in the apex of the plants which contained nematodes. Preliminary investigations indicate that the nematodes are an undescribed species of *Nothanguina*. The nematode galls are usually surrounded by female reproductive structures (archegonia), and it is postulated that the nematodes are modifying the archegonia and inhibiting fertilisation from occurring. Further research is required to obtain a comprehensive description of the nematodes, and a thorough understanding of their association with the biology of the moss. The relationship raises interesting questions regarding the evolutionary origins of the moss, as well as the origin and nature of the parasitic habit in the nematode family Anguinidae.

In vitro Cultivation of Stem Nematodes

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Biology of plant parasitic nematodes is often complicated to study. Elimination of negative factors is not always simple. The first presumption for successful study of nematodes is present of an adequate amount of viable nematodes. In vitro cultures are sufficient way to limit influence of external abiotic and biotic factors. Axenic culture of nematodes populations are possible and easy to store and transport. The aim of this work was to optimization of methods for axenization of stem nematode *Ditylenchus dipsaci*. In this case several procedures were used. In vitro cultures of whole plants and callus cultures were used for inoculation test. Model plants from host range of two bioraces of *Ditylenchus dipsaci* (chicory and garlic populations) were chosen. Essential factor for preparing of in vitro nematode cultures is sterilization of nematodes. Low mortality and successful sterilization of nematodes are the most important factors for in vitro nematode cultures optimization. Many different methods described in literature were tested. In our work using of $HgCl_2$ and streptomycin sulphate were tested with positive results. It is necessary to note that $HgCl_2$ is toxic substance but its use enables to obtain available nematodes for in vitro cultures in very short time period. Nematodes manipulation performed by pipetting and centrifugation increases sterilization efficiency as well. This work was supported by grant agency of agriculture research Ministry of Agriculture project QG50087 and by Ministry of Education, project MSM 6046070901.

Import of Pulse Grains and Risk of Introduction of Nematodes - Exotic to India

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Infected seeds, soil particles and plant debris as contaminants are important pathways for stem and bulb (*Ditylenchus dipsaci*), soybean cyst (*Heterodera glycines*) and pea cyst (*H. goettingiana*) nematodes through the import of soybean, pea and lentil harvested from infected fields. These nematodes are not known to occur in India and have been intercepted on several imported consignments at New Delhi. Examining ship-loads of consignments for nematode contamination by the available techniques and manpower, inefficient procedures to predict level of infestation, inappropriate control procedures, non-declaration of soil-free nature of the consignments on phytosanitary certificates, non- availability of pest-free areas/ nematodes distribution maps from the countries of export and non-specified tolerance levels in import permits of the importing countries are some of the difficulties faced by quarantine nematologists. Due to lack of symptoms, detection of nematode infestation in true seed is difficult. The *D. dipsaci* and cysts of *Heterodera* spp. can survive long under dry conditions during transportation and storage. They are difficult to eradicate or even effectively control once they are in the soil of the importing country. Very few nematologists work on seeds, nematode identification and distribution maps in the world. Nematodes along with other seed transmitted pests not reported to occur in the country are now critical in international trade, to meet phytosanitary requirements of WTO. This situation is adversely affecting not only

certain exporting countries but also putting large number of importing countries into either food shortages/ high product prices/ malnutrition or risk of unacceptable levels of introduction of alien nematode pests. Appropriate techniques for examination and removal of all soil particles and debris need to be developed for bulk consignments. Efficient mitigation measures, like commercial radiation procedures, hot air/ water equipments need to be tested on infested lots by the contaminated countries to boost their trade of agricultural produce/ seeds of host crops with minimal or acceptable level of risk to the importing countries.

Effect of Plant Essences on *D. dipsaci* Mortality

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Plant parasitic nematodes are the most meaningful pests of cultivated crops with disregarded importance. *Ditylenchus dipsaci* is the example of stem nematodes group that causes high yield losses. Host range of these phytoparasitic nematodes is wide and crop protection by traditional method, as crop rotation, is rather difficult. Worldwide used crop protection methods are usually based on chemical treatment. Within integrated pest management and environment protection the pesticides are reduced. New methods based on different principles are searched. In this case the treatment based on plant extracts and essences are preferred from this reason. In this work 50 plant essences and four plant extracts were used for in vitro mortality tests of *D. dipsaci*. Different concentration of plant essences and extracts were pipetted into cultivated plates. Nematode suspension (50 living nematodes per 25µl) was added into each variant of the tests. Mortality of nematodes was observed in different time periods by stereomicroscope. The best mortality effect was found in following plant essences: *Eugenia caryophyllata Thunb*, *Mentha arvensis*, *Ocimum basilicum*, *Origanum majorana*, *Thymus vulgaris, Datura stramonium*, *Origanum vulgare.* These received results of this work show possibility of using plant extracts and essences as nematicide treatment. This work was supported by grant agency of agriculture research Ministry of Agriculture project QG50087 and by Ministry of Education, project MSM 6046070901.

TOPIC SIX – ENTOMOPHILICS

Entomopathogenic Nematodes: Potential for their Use against Root-knot Nematodes

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Entomopathogenic nematodes [EPN]) have potential for biological control of insect pests. They are currently used for the control of soil and cryptic pests in several parts of the world. These nematodes can be easily mass-produced and applied using conventional spray equipment. They have a broad host range and are safe to the environment. These nematodes are soil-dwelling organisms, and are obligate parasites of insects. Species of EPN such *Steinernema glaseri*, *S. carpocapsae and Heterorhabditis megidis* have been successfully used to manage the insect populations below damaging level. Plant parasitic nematodes are generally controlled by synthetic chemicals, which are not environmentally safe. We have explored the use of EPN as alternate strategy to manage root-knot nematode, *Meloidogyne incognita* on tomato. Our preliminary investigation has demonstrated that the application of EPN tomato roots effectively reduced the invasion by second stage juvenile, their further development, and reproduction. The action of EPN probably involves the production of allelopathic chemicals by the symbiotic bacteria like *Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis*, residing inside EPN.

Steinernema glaseri and *Heterorhabditis. indica* at three levels of their densities (500, 1250 and 2500 individuals /pot) and at various time intervals (before, same time and after the application of root knot nematodes on tomato plants were applied in 80 ml pots. The entomopathogenic nematodes applied at 1250 and 2500/pot, 24 hr before, at the same time or 24h after, significantly reduced the invasion. Higher density of EPN was more effective. Both the EPN differed significantly with control in reducing the number of egg masses and females at all level of their application. There was no significant difference between time of application of EPN, i.e. before 24 hrs, at the same time, or after 24 h of root knot application.

Morphometric, Molecular and Biological Characterization of Spanish Native Steinernematid Strains and their Relationship with Bioassay to Assess their Activity and Sex-ratio

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Twenty-two native nematode entomopathogenic (EPN) strains were isolated from La Rioja soils (Northern Spain). The morphobiometric study rendered: 16 *Steinernema feltiae*, 4 *S. carpocapsae* and 2 *S. kraussei* strains. The genotype agrees with those described for *S. carpocapsae* and *S. kraussei*, and with *S. feltiae* A2 RFLP type. Statistical analysis was performed to study their morphometric similarity with respect to 3-5 reference strains, and their biological similarity was assessed through out penetration, migration and one-on-one bioassays using *Galleria mellonella* (Lepidoptera: Pyralidae) as host. Native strains showed morphometric intraspecific variation, with low morphometric similarity percentage. In penetration assay, mean values of larval mortality were 42.3-96.1%, 79.9-85.4% and 86.2% due to *S. feltiae, S. carpocapsae* and *S. kraussei*, respectively, with the IJs penetration being 1.9-15.4%, 2.3-5.3% and 7.0%, respectively, in the same order. These values, although slightly reduced in the sand column migration study, were agreed with those observed by other authors in the Mediterranean region whereas show higher differences with those of other geographic regions. The differences observed in both assays might be due to different searching behavior of each species in relationship with the intraspecific variations in IJs size. Female proportion of the three EPNs species from La Rioja developed into *G. mellonella* was significantly higher than the male one. Larval mortality values in the one-to-one assay were lower to those observed by other authors, being 0-21.7% for *S. feltiae*, 8.7-8.9% for *S. carpocapsae* and 6.3% for *S. kraussei*. Some strains very active in the penetration and migration assays showed 0% penetration in the one-to-one assay, suggesting that a part of the IJs population might not be infective. The results of this survey provide new knowledge of three steinernematid species and show the interest of their biological characterization to assess more accurately their activity.

Control of Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) using Entomopathogenic Nematodes in South African Apple and Pear Orchards

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In South Africa codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is an established key pest of apple and pear fruit. Previously control methods have been based predominantly on the use of broad spectrum insecticides, particularly organophosphates. However, concerns regarding the development of resistance to insecticides, the environmental impact and sustainability of ongoing pesticide use have resulted in the use of alternative control methods. These include the use of insecticides, including insect growth regulators, the sterile insect technique and the use of pheromone based mating disruption, all used within an integrated pest management (IPM) strategy. Here we present research currently being conducted into the use of entomopathogenic nematodes (EPNs) as inundatively applied microbial biopesticides for the biological control of codling moth in

South African apple and pear orchards. This study included a survey conducted throughout different habitats in South Africa, with the purpose of isolating endemic EPN isolates from soils. Nematodes obtained from the survey were identified to the species level using molecular techniques. The most promising isolate was selected and assessed in the laboratory for efficacy and morphological suitability for infection of codling moth larvae under different laboratory conditions such as temperature, humidity and nematode concentration. Quantitative spray-deposition assessements were carried out in the field by adding Yellow Fluorescent Pigment® to selected adjuvants and water containing nematodes which was then sprayed onto the bark of trees to determine and visualize actual spray-quantaties penetrating the cryptic habitats where codling moth larvae diapause during winter periods. Overall field-performance was also evaluated by testing the selected isolate in the field at different concentrations. The conclusive possibility of using EPNs as part of an IPM approach to codling moth control in South African orchards will be discussed.

Efficacy of *Heterorhabditis baujardi* LPP7 (Nematoda: Rhabditida) applied in *Galleria mellonella* (Lepidoptera: Pyralidae) Insect Cadavers to *Conotrachelus psidii*, (Coleoptera: Curculionidae) larvae

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The guava weevil, *Conotrachelus psidii*, is a major pest of guava in Brazil causing severe reduction in fruit quality. We assessed its susceptibility to *Heterhorhabditis baujardi* LPP7 infective juveniles (IJs) in the greenhouse and under field conditions applying the nematodes in cadavers of 7th instar *Galleria mellonella* larvae Field persistence of these nematodes in the soil was evaluated through *G. mellonella*-baiting. Insect cadaver concentrations of 2, 4, and 6 applied in pots in the greenhouse experiment caused significant mortality compared to the control. Significance differences were observed in the field between control and treatments only when 6 cadavers per 0.25 m^2 were applied. Infective juveniles from the cadavers persisted 6 weeks after application in the field, but decreased greatly thereafter. Our work demonstrates that *H. baujardi* LPP7 IJs emerging from *G. mellonella* cadavers can be efficacious against guava weevil fourth instar larvae. Also, we demonstrated the long term persistence of IJs in the soil.

Cold Tolerance Mechanisms of Entomopathogenic Nematodes

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Insect parasitic nematodes of the genera *Steinernema* and *Heterorhabditis* have considerable potential as biological control agents, since they can be cultured *in vitro* in large quantities. A major barrier to their large-scale commercial application, however, is the lack of a suitable technique for their long-term storage. Attempts to base a storage technology on desiccation and anhydrobiosis have met with limited success. However, some promising results have been obtained using freezing. We summarise this work and describe how our understanding of the cold tolerance mechanisms of Antarctic nematodes may assist in the development of a storage technology for entomopathogenic nematodes based on freezing.

An Entomoparasitic Adult Form of *Bursaphelenchus luxuriosae*

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An entomoparasitic adult form of *Bursaphelenchus luxuriosae* was found in the tracheal system and body cavity of its carrier host, *Acalolepta luxuriosa* (Cerambycidae). Because of its characteristic morphology, the nematode was not originally identified as a *Bursaphelenchus* species, but was considered to be an aphelenchoid insect parasite. However, the molecular sequence (SSU barcode) of the parasitic form was identical to a cultured population of *B. luxuriosae* and was subsequently confirmed as conspecific. Morphologically, the parasitic form shares several characters with the phoretic dauer juvenile stage of *Bursaphelenchus* nematodes and the parasitic juvenile stage of *Parasitaphelenchus* species. The parasitic form has a dome-shaped head without a clearly separated lip region and degenerate digestive organs, *i.e.*, stylet, metacorpus and pharyngo-intestinal valve. The parasitic form also has several similarities to mycophagous adults; a typical set of seven caudal papillae and clearly defined cucullus on the spicules of males and a long vulval flap, postuterine sac, and similar arrangement of the reproductive system of females. Besides these characters, the parasitic form exhibits several apomorphic attributes, *eg*., four vacuole-like dots (assumed to be a sensory organ) at its lip region, elongated and thin spicule (lamina / calomus complex) of males and conical tail of females. Molecular phylogeny was used to infer that *Bursaphelenchus luxuriosae* is basal to the ‘xylophilus’ group and adult insect parasitism is presumed to be an autapomorphy. This is the first confirmed report of an entomoparasitic adult form in the genus *Bursaphelenchus*.

The Life Cycle of *Sphaerularia vespae*, a Novel Parasite of Hornets

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Sphaerularia vespae, a newly described species in 2007, was found in gynes (potential queens) of a hornet, *Vespa simillima*. This parasitic nematode sterilizes the host gynes, as has been shown in *S. bombi*, a well-known parasite of bumblebees. To study the life cycle of *S. vespae*, *V. simillima* and other hornet species were collected through the year and dissected. The infective female and developing uterium (hypertrophied uterus with the reproductive system everted through the vulva) of *S. vespae* were found in overwintering gynes of *V. simillima*. After the gynes had overwintered, the uterium continued to develop then laid eggs in the early summer in the bodies of gynes. Hatched nematode juveniles escaped from the posterior end of gynes through the ovarioles. The infective female of *S. vespae* was found in the overwintering chambers of gynes. Prevalence of *S. vespae* in *V. simillima* gynes was approx. 55 % in 2007. The mature uteria, deposited eggs and hatched juveniles were also found in another hornet, *V. dybowskii*, but its prevalence was low, at only about 3 %. No mature uteria were found in three other species of hornets, *V. analis*, *V. crabro*, or *V. mandarinia*. These observations revealed that *S. vespae* infects the overwintering gynes of hornets and depends largely on *V. simillima* to complete its life cycle.

Selection of a South African Entomopathogenic Nematode for Control of Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) using Laboratory Bioassays

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Codling moth, *Cydia pomonella* (L.), is the primary pest of apples and pears in South Africa. Larvae use cryptic habitats such as bark and litter under trees to diapause in. Wooden fruit bins, used to transport and store fruit, are also utilize by codling moth larvae as suitable cocoon sites. Indigenous entomopathogenic nematodes (EPN) were collected and assessed as an alternative to control diapausing larvae hidden in wooden fruit bins. Nematode isolates were screened and selections made on the combined percentage of larval and pupal mortality using 24-well plates. To match a suitable EPN for the control of codling moth in cryptic habitats, corrugated paper rolls were used. To simulate more natural conditions holes were drilled into sections of old bin wood and final instar codling moth larvae allowed to spin cocoons over a period of 24 hours. These bin wood sections containing cocooned larvae were used for final selection of nematode isolates. The best candidate (*H. zealandica,* SF41) was then mass produced by a commercial organization. The formulated product of *H. zealandica* was used to determine an effective concentration to be used for the commercial treatment of fruit bins. In total, 40 EPN isolates were screened to select a suitable candidate for the control of codling moth. High percentage mortality was found for larvae, with generally lower mortality for pupae. Using the bin wood sections with cocooned codling moth larvae, concentrations of >50 IJ/ml showed no significant increase in mortality. *Heterorhabditis zealandica* has thus been shown as a good candidate for the potential control of diapausing codling moth larvae in fruit bins.

Identification of *Heteroprhabditis* Species by SEM and Molecular Techniques

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It is very difficult to use morphological characters or morphometrics to identify species in the genus *Heterorhabditis*. Body length can only be used to divide all species into 2 groups. In the first group, the infective juvenile (IJ) average body length is longer than 600 µm: *H. megidis*, *H. downes*i, *H. marelatus*, *H. zealandica*. In the second group, IJ is shorter than 600 µm: *H. amazonensis*, *H. baujardi*, *H. bacteriophora*, *H. floridensis*, *H. georgiana*, *H. indica*, *H. mexicana*, *H. safricana*, *H. taysearae.* Vulval pattern can be used to identify for a number of species and gubernaculum structure can be used for species identification but it is difficult to obtain. Molecular techniques using sequence analyses and phylogeny can be used to identify all species of *Heterorhabditis.* Sequence length of ITS regions flanked by primers 18S and 26S, length of ITS1 and ITS2, distances between species, numbers of changes on branches in a phylogenetic tree and the numbers of autapomorphies in the analysis of ITS rDNA regions are reliable for species identification. Additionally, D2D3-LSU and ND4 can be used for the same purpose. Phylogenetic trees using ITS and D2D3 group *Heterorhabditis* species into 2 groups: the *megidis* group includes *H. bacteriophora*, *H. downes*i, *H. georgiana*, *H. marelatus*, *H. megidis*, *H. safricana, H. zealandica*, while the *indica* group includes *H. amazonensis*, *H. baujardi*, *H. floridensis*, *H. indica*, *H. mexicana* and *H. taysearae.*

Distribution and Evalution of Entomophilic Nematodes (Heterorhabdus and Steinernema) in Different Agroecosystems

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A survey of soil faunal nematode (*Steinernematids* and *Heterorhabditids*) was conducted in different agro ecosystem of Tamilnadu, India. About 1000 soil samples were collected and processed for nematodes distribution. In our study the nematode bionomics and distribution was assessed with *G. mellonella*. The collective samples were positively 12% *Heterorhabdus* spp. and 20% *Steinernema* spp. and identified as *S. carpocapsae, S. glaseri* and *H.indica and H. bacteriphora*. In addition the collected strains were evaluated and screened for persistence in different temperature (15 to 35°C) and pH ranges (5-8) and the infectivity was also examined in the root grub (*Holotrica serrata)*. Persistence was determined using baiting nematode-inoculated soil and in the liquid medium in different temperature. Further infectivity was assessed at different temperature against *H. serrata* using a filter-paper bioassay and sand-well bioassay to determining nematode establishment. In our observation infectivity and production rate was more in sand well bioassay method than filter paper both *Steinernema* sp. and *Heterorhabdus* sp. and the optimum temperature for infection range 25 - 28°C. Hence, the distribution of epns acts as a biological control potential on insect pest and diverse distribution in the agroecosystem due to geographical provision. Since the epns are ubiquitous and vital for both ecofriendly and economically for sustainable management in an agroecosystem.

Potential of EPN in Management of Cotton Bollworms in Pakistan

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Cotton is the most important cash crop of Pakistan and plays a vital role in the country's economy. Thus protecting cotton from insect pests including bollworms is of prime importance and about 80% of the insecticides used in the country are meant for cotton. However, the indiscriminate use of chemical pesticides has disturbed agro-ecosystem and costs over US$195 million per year to the nation in terms of environmental and social costs. This necessitates to search and research for eco-friendly pest management options including the use of entomopathogenic nematodes (EPNs). In Pakistan, studies on EPN were initiated in 1996 and so far, six species; two new and four as new records have been identified. Pathogenicity and efficacy trials of indigenous EPN isolates have also been done with positive results. This paper presents the results of a field trial wherein four EPN isolates viz., *Steinernema pakistanense, S. asiaticum, S. feltiae* and *Heterorhabditis indica* were assessed for their infectivity against the cotton bollworm complex. EPNs cultured in *Galleria mellonella* L. and stored in distilled water at 5°C were kept at room temperature for 24 hours before use. The number of bollworms on plants before and 24 hours after EPN spray @ 1000 juveniles/100 ml water were assessed for mortality percentage of insects. All four species of insects, viz., *Helicoverpa armigera*, *Earias insulana, E. vitella* and *Pectinophora gossypiella* were found susceptible to infective juveniles of the four EPN species; *S. pakistanense* was the highly virulent EPN. The intensity of infestation varied with the species and life stage of the insects. There is a dire need to focus further research on these EPN isolates to explore and exploit their potential as an alternative to pesticides in Pakistan, especially in IPM programmes.

Survival Strategy of *Caenorhabditis japonica* Dauer Juveniles

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Caenorahbditis japonica forms phoretic association with the monophagous shield bug *Parastrachia japonensis* showing egg-guarding and provisioning behaviors. Dauer juveniles (DJs) of *C. japonica* are mainly found from the dorsum under the scutellum of adult female bugs and are quiescent on the bug throughout a year except for the provisioning period. During the provisioning, *C. japonica* leaves the bug and propagates in and around the nest of the bug in the litter of deciduous forest. Since the provisioning period of the bug is limited to early summer and they spend most of a year aggregating on the leaves of trees under the reproductive diapause, *C. japonica* DJs seems to survive on the bugs waiting for the next bug's provisioning nearly for a year. To elucidate the physiological condition of DJs on the bug, longevity and desiccation tolerance of *C. japonica* DJs were studied. When propagated *C. japonica* DJs were kept in M9 buffer or soil at 25ºC, survival rate dramatically declined within ten days and all DJs died in 20 days. When the propagated DJs were directly exposed to 97% relative humidity (RH) for one week, all of them died. However, when DJs were loaded to the shield bug in the laboratory and exposed to 97% RH, more than 60% of DJs survived. Moreover, when the bugs collected from a mountain were exposed to 97% and 85% RH, more than 80% of the naturally associating DJs on the bug survived. These results

suggest that *C. japonica* DJs appear to have short longevity and weak desiccation tolerance but they are able to extend their longevity by entering quiescent and being protected from desiccation on the bug.

Morphological and Behavioral Variation in Infective Juveniles of Mutant *Steinernema feltiae*

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Mutagenesis is a powerful tool used in genetic improvement of cultivated plants and animals', worldwide. New mutant alleles can change the organisms' phenotypes, including those of commercial significance. As with the aid of a simple cross-breeding technique point mutations can be easily transferred to existing strains, new recombinant genotypes can further widen the range of phenotypic variation available for subsequent works on strain improvement and their use in the practice.

In entomopathogenic nematodes from the genera *Steinernema* and *Heterorhabditis* the process of spontaneous and chemically-induced mutagenesis has been explored for over 15 years. However, with the exception of *Steinernema feltiae*, only a few distinctive mutations have been identified in the examined species. Our study on *S. feltiae* has revealed a marked sensitivity of this species to chemical mutagens. Over sixty point mutations altering morphology and/or behavior of infective juveniles (IJ) have been identified so far. Most of them are maintained in mutant strains in the laboratory culture. All of the mutant phenotypes (except for segmented - Seg)] fall into categories earlier described for *C. elegans*, such as dumpy - Dpy, roller - Rol, twitcher and other uncoordinated -Unc, variable abnormal - Vab, and long - Lon. The main advantage of the use of entomopathogenic nematodes in this research is that all the new phenotypes presented by IJs can be related with their practical significance, expressed as the nematode ability to disperse in the soil, find the host insect, and penetrate into its hemocoel.

IJs of most of the identified mutants are less effective than wild-type *S. feltiae* in some or all of these functions. However, mutations in two genes, i.e. *Sflon-1* and *Sfrol-1*, showed to have positive effects on the nematode activity: Increased body dimensions of IJs in the long mutant (*pn49*) are associated with the improved ability to penetrate the soil and infect insects at greater soil depths than the wild type. Also, the changes in the muscle arrangement of roller mutants (*pn16, pn36*) positively influence the nematodes' penetration into the host. Cross-breeding and transfer of these mutations to other strains helps to improve the nematode activity in the soil. Reported findings suggest that these alleles present real value for genetic improvement of IJs' efficacy in commercial strains of *S. feltiae*.

Discovery of *Beddingia siricidicola* Associated with *Sirex noctilio* in Ontario, Canada

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Nematodes of the European woodwasp, *Sirex noctilio*, a recent discovered invasive alien insect species in Ontario, were discovered and identified as *Beddingia siricidicola* Bedding (Neotylenchidae) by morphological and molecular methods. Sphaerulariid form of female reproducing ovoviviparously was found within the haemocoel of the siricid; the juveniles were successfully reared on several isolates the symbiotic fungus *Amylostereum areolatum*; the resulting mycophagous adults were compared with paratypes: the distance between hemizonid and excretory pore is between 20-50 µm. Attempts were made trying to induce the infective form from the mycophagous stage. The ribosomal DNA ITS, 18S regions, and mitochondrion CO1 were PCR amplified and sequenced. The sequences were almost identical to those of B. siricidicola from Australia.

TOPIC SEVEN – ANIMAL-PARASITIC NEMATODES

Parasitic Infections of Himalayan Yak *Bos (poephagus) grunniens*: Current Scenario

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For centuries, the yak and its hybrids with domestic cows (dzomo/dzo) have been contributing to the socio-economic status of their owners in desolate regions of the Greater Himalayas. Studies on the prevalence of parasitic diseases in these animals were undertaken in Ladakh (Jammu and Kashmir), Sikkim and villages near the India and Nepal Himalayan regions. Visceral organs of necropsied animals were observed for the presence of adult metazoan parasites, fresh or preserved faecal samples were examined for the eggs of helminth parasites and protozoan cysts, and blood smears were examined for haemo-protozoa and microfilariae. In all, examination of 225 faecal samples, 180 blood smears and the visceral organs of thirteen yaks and dzomo/dzo was undertaken. On necropsy, visceral organs revealed various adult liver and stomach flukes, gastrointestinal nematodes, tapeworms, cysts of *Coenurus* spp. and hydatid cysts, as well as *Setaria cervi* worms and large and/or small sized Sarcocystis cysts. On coprological examination, egg prevalences of 10% for *Fasciola* spp., 6.6% for various amphistomes, 10% for *Moniezia* spp., 76.4% for *Strongylate* spp., 24% for *Neoascaris* spp. and 13.7% for *Nematodirus* spp. were recorded. Identification of infective larvae from the faecal cultures showed that a majority of eggs (86.3%) in the host faeces were contributed by nematodes belonging to *Trichostrongyle* spp., *Ostertagia* spp. and *Cooperia* spp. This was followed by *Chabertia* spp. (6.5%). *Haemonchus* spp., *Bunostomum* spp. and *Nematodirus* spp. together contributed only 7.2% of the eggs found. Among protozoan infections, *Eimeria brasiliensis* and *E. zurnii* were common. None of the blood smears evidenced any haemoprotozoa or microfilariae. Likewise, none of the animals were positive for *Trichuris* spp.

A Survey of Morphologic Characters and Distribution of Intestinal Helminthes in Stray Dogs in the West of Iran

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Human infection, especially with helminthes parasites, is an emerging health issue, as the human environment is increasingly shared with infected animals, either pets or wild life. In this survey, the intestinal contents of 83 stray dogs were collected from the West Azarbaijan, Kordestan and Kermanshah provinces in the west of Iran. Following autopsy of the animals, their small intestines were removed, slit open and the epithelium of the intestine scraped into a jar. Recovered helminthes were fixed in alcohol and the cestodes were stained with carmine. The parasites were identified according to the keys and guidelines given by Yamaguti (1961), Anderson (1992) and Khalil et al. (1994). The percentage of different species recovered from these animals is listed as follows:

Toxocara canis (6.02%), Toxascaris leonina (32.53%), Ancylostoma caninum (3.61%), Oxynema sp. (1.35%), Rictularia affinis (12.05%), Taenia hydatigena (53.01%), Taenia ovis (7.23%), Taenia multiceps (4.82%), Echinococcus granulosus (13.25%), Dipylidium caninum (38.55%), Mesocestoides lineatus (26.50%) and Macracanthorhynchus hirudinaceus (4.82%).

Findings of Drilonematoidea in Vietnam

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Search of nematodes parasitic in Vietnamese earthworms began 20 years ago. From nearly 200 nominal pheretimoid species (Blakemore, 2006), a quarter was examined including formalin-preserved specimens from the collection of Dr Thai Tran Bai, Hanoi Pedagogic Institute, and animals collected alive during our field trips in Vietnam. Fourteen earthworm species were registered as hosts for Drilonematoidea. Four oligochaete species served as hosts for more than single nematode species. *Amynthas leucocircus* (Chen, 1933) collected in Ba Vi National Park in the Northern Vietnam, were particularly prone to nematode infection: 7 drilonematid and 4 oxyurid species were described from this earthworm species. Discoveries from Vietnamese pheretimoid earthworms include representatives of Homungellidae (*Homungella* and *Perodira*), Ungellidae (*Synoecnema*, *Siconema*, *Thainema*, *Unicorninema*) and Drilonematidae (*Adieronema*, *Iponema*, *Mesonema*). The first family, Homungellidae, until now was reported exclusively from South East Asia. Ungellid nematodes are represented in Vietnam mainly by Synoecneminae subfamily which includes highly specialized parasites lacking male spicular apparatus and found in a state of permanent copulation. However, recently a new species and genus of the other subfamily, Ungellinae, was firstly discovered. It is differentiated from the rest of Ungellidae by the presence of a single strongly developed cephalic hook. In this respect it reminds the genus Homungella but clearly differs by presence *vs* lack of spicules and gubernaculum and different shape of sperm. High diversity of drilonematid nematodes provided by pheretimoids native to Vietnam allows resolve relationships within their higher taxon, Drilonematoidea. Recently, phylogenetic analysis based on 18S rDNA partial sequences has revealed an affinity of species of Ungellidae (*Siconema*), together with Homungellidae (*Perodira*) and Drilonematidae (*Dicelis*), to the free-living cephalobid genera (Spiridonov *et al*., 2007). RFBR support 07-04-9005 Viet_a.

Intestinal Parasitic Nematodes of Vietnamese Invertebrates: New Findings

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The hind gut of invertebrates is a characteristic habitat for several taxa of parasitic nematodes. These latter are especially diverse in tropical habitats. Some higher taxa of intestinal nematodes of invertebrates can be found only in tropical hosts (e.g. nematodes of the superfamily Ransomnematoidea). Several surveys in Vietnam revealed impressive diversity of intestinal nematodes of invertebrates. The dissections of Passalidae beetles both in the North and South of Vietnam demonstrated heavy infestation of these coleopterans with the nematodes of the family Hystrignathidae, which were reported from Australia, Americas and Madagascar, but not from mainland Asia. SEM study of Vietnamese hystrignathids revealed the presence of unusual structure in Vietnamese Hystrignathidae - anchor-like structure on male tail terminus. Such structure was described before by Leidy for *Hystrignathus rigidus* from North American passalids. Extremely rich fauna of intestinal nematodes was discovered in the hind gut of large wood-burrowing cockroaches of the subfamily Panesthiinae. The nematodes of at least 6 genera are sharing this habitat. Together with the nematodes of known genera the representatives of two putatively new ones were reported. Several species of angiostomatids – parasitic nematodes of the alimentary tract of terrestrial mollusks – were discovered in Vietnamese snails. The presence of three species of *Heth* nematodes with their unusual and characteristic anterior end morphology is reported and illustrated in SEM. Several new species of Vietnamese *Rhigonema* were studied by morphological and molecular methods. At least three species of *Travassosinema* nematodes were found in the hind guts of different Vietnamese diplopods. Relationships of some taxa of intestinal nematodes from Vietnamese invertebrates were elucidated with phylogenetic analysis of 18S and D2D3 28S rDNA sequences. Ultrastructure of cuticle and oesophagus was examined for some rhigonematid and ransomnematid nematodes. Organization of *Travassosinema* umbraculum (cephalic alae) was studied in TEM. RFBR

Evaluations of Different Methods of Mass Production of Entomopathogenic Nematodes

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Entomopathogenic nematodes are very promising biopesticides capable of controlling a variety of economically important insect pests. But application of entomopathogenic nematodes was limited because of faulty mass production. In order to find suitable mass production technology for entomopathogenic nematodes to meet different markets' needs, several methods of mass production were adopted in this experiment.

Heterorhabditis bacteriophora（Hb-1）and *Steinernema carpocapsae*（Sc-2）were mass-produced by White trap, Lowtek, liquid and solid methods, respectively. The last instar of the greater wax moth, *Galleria mellonella* (L.) was used as host insect for entomopathogenic nematode culture by traditional White trap method. The mealworm, *Tenebrio molitor* (L.) was used as host by Lowtek. The medium of liquid and solid culture was composed of tryptic soybroth, yeast extract, oil of soybean, and sponge was used as substrate. The quality and yield of the nematodes were investigated and the costs were calculated.

The results showed that the infecting ability and living rate of the two species (Hb-1 and Sc-2) were slightly different, and White trap was the highest and Lowtek liquid culture was the lowest. The yield of solid cultured Hb-1 and Sc-2 was highest, 5.69×10^5IJs (infective juveniles)/g and 6.33×10^5 IJs/g medium, liquid culture was higher than White trap and Lowtek was the lowest. The cost of Lowtek was lower than other three methods, 0.79RMB/million IJs and 0.83 RMB/million IJs, secondly solid culture, and liquid culture was the highest. Therefore, Lowtek method can be used as low volume in local market which the equipment was simple, and solid culture can adopted as requiring large numbers of nematodes for extensive field which developed technology and large capital were needed.

TOPIC EIGHT – MARINE AND FRESHWATER AQUATIC NEMATODES

Abundance and Biomass of Benthic Meiofauna in the Summer in the Beibu Gulf, China

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The benthic meiofauna was quantitatively investigated in the Beibu Gulf in summer 2006. The mean abundance of the meiofauna amounted to 514±380 ind/10cm^2 and the mean biomass of it amounted to 580±449 μg (dwt) /10cm^2. Free living marine nematodes were the dominant group in all stations, which representing about 85% of the total meiofauna. Benthic copepod and polychaete were the second and the third in abundance. Different with the abundance, polychaete constituted 39% biomass, then, is nematodes (32%) and copepods (9%). 62% meiofauna distributed in 0～2 cm layer of the sediment. The abundance and biomass of meiofauna increasing along with the distance decreasing from the shore. Correlation analysis showed there were significant negative correlation between abundance of meiofauna and salinity, water depth, pH. There were not significant positive correlation between abundance of meiofauna and temperature, median grainsize.

Three New Species of the Genus Synonchium Cobb, 1920 (Chromadorida: Selachinematidae) from Mangrove Areas of Pakistan

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The mangroves areas of Pakistan are unique being representative of the largest arid zone mangroves in the world. Mangroves swamps support a high biological diversity of microorganism. Pakistan has a rich marine fauna in the coastal and inshore water of the Arabian Sea. During a survey of the nematofauna of mangrove areas of Arabian Sea of Pakistan, several samples were collected. Amongst the marine nematodes recovered two new species viz., *Synonchium oblongii* n. sp., and *S. pakistanense* n. sp., of the family Selachinematidae were encountered, which are described with measurement, description, drawing, light microscopic photographs and a key to the species. The Selachinematid nematodes are characterized by having annulated cuticle with transverse rows of punctuations, multi-spiral amphids, buccal cavity ornament, in the form of mandibles, precloacal supplements, cup-shaped or setose but never tubular, testes usually paired, ovaries always paired opposed and reflexed. *Synonchium oblongii* n. sp., closely related to *S. depressum* Gerlach, 1954 in having conoid tail, amphid three time as broad as high and mandible with three teeth, but it differs from *S. depressum* in combination of characters: in slender body, smaller tail and smaller spicules. Specimens of *S. pakistanensis* n. sp., are characterized by three similar sized teeth on the mandibles, presence of distinct punctuation on the cuticle, bluntly rounded tail and presence of ventromedian supplement in male. *S. pakistanensis* n. sp., comes close to *S. capense* Heyns & Swart, 1998; *S. mediterranum* Vinciguerra & Orselli, 1997 and *S. siculum* Vinciguera & Oreslli, 1997 but differs from them in a number of characteristics. A list of free-living marine nematodes encountered during the surveys is also incorporated.

Meiofaunal Community with Special Reference to Nematodes in Trawled and Non-trawled Areas of Subtropical Hong Kong

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Bottom trawling is a common fishing activity in coastal waters of SE Asia, including Hong Kong. Many studies showed that bottom trawling can modify sediment characteristics and benthic communities. This study attempted to investigate the impacts of bottom trawling on benthic environment and meiofaunal communities, particularly marine nematodes. Field sampling was conducted from April 2006 to January 2007 at one trawled and one non-trawled sites in subtropical waters, Hong Kong. Sediment parameters including total organic carbon (TOC), total Kjeldahl nitrogen (TKN), total phosphorus (TP), water content and silt/clay fraction were also analyzed. Results showed that levels of TOC and TKN were significantly higher ($p<0.05$) at the trawled than non-trawled sites. A total of 15 meiofaunal groups were identified; free-living marine nematodes were the most dominant group, accounting for 94.6% of total abundance. Two-way ANOVA results showed that nematode abundance at the trawled site was significantly higher than that at the non-trawled site ($p<0.05$). Pearson correlation analysis also showed that the abundance of meiofauna and nematodes had significantly positive correlations with the contents of TOC and TKN. A total of 144 species or taxa of free-living marine nematodes, belonging to 79 genera, 28 families and 5 orders, were identified. Multivariate analysis showed that there were distinct nematode communities at the trawled and non-trawled sites, with different dominant species. However, there were no temporal or spatial differences for nematode species number, Shannon diversity index, Margalef's species richness, Pielou's species evenness as well as trophic structure over the study period of 9 months. Results of correlation analysis using method of matching biotic to environmental patterns revealed that water content and TKN were the most important factors influencing nematode communities at the study areas.

Preliminary Observations on Nematodes from Coastal Patagonian Sediments (Chubut, Argentina)

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This paper focuses on two new species belonging to *Neochromadora* (CHROMADORIDAE) and *Cobbia* (MONHYSTERIDAE), two very cosmopolitan genera inhabiting coastal sediments systems, providing their distribution on East coastal Patagonia.

Seven littoral areas have been sampled at three latitudes. The samples have been fixed in 5% formalin with rose Bengal in the field. The geographical position (GPS), salinity, temperature, granulometry, organic matter, DPR layer and dissolved Oxygen have been taken. Nematodes were extracted using the elutriation/decantation/LUDOX TM method and specimens mounted on slides.

The Nematodes found were identified up to the lowest taxonomy level possible, and photographs were taken using a Photomicroscope. For nematode identifications the keys of Platt & Warwick and Warwick et al. were used.

Two probably new species belonging to this two genera are described from Patagonian coastal areas, Chubut province of Argentina: *Neochromadora* sp. is characterized by gubernaculums and spicules shape, the presence of a thick cuticle plate on subventral tail area; esophageal bulb shape and length of cephalic and somatic setae. It has been found in upper to low littoral sandy areas. *Cobbia* sp. is characterized by the amphid position and size and length of cephalic setae. It has been found in low littoral sandy beaches.

These species have been described in the project titled: 'Biodiversity of meiofauna communities in sand beaches of Río Negro and Chubut' supported by -GEF- Secretaría de Medio Ambiente de Nación- CONICET, Argentina.

Effect of *Meloidogyne incognita* Resistance Genes in Cotton on the Reproduction of *M. mayaguensis*

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Meloidogyne mayaguensis was first reported in the continental United States in 2002, and some isolates can reproduce on cotton. *Meloidogyne incognita* is the single most important pathogen of cotton in the United States, and efforts to incorporate resistance to *M. incognita* into cotton cultivars are ongoing. Our objective was to determine whether resistance to *M. incognita* in cotton will also confer resistance to *M. mayaguensis*. Two greenhouse experiments showed that gall and egg mass indices, eggs per gram of root, and reproductive factor (Rf) differed significantly among cotton genotypes resistant or susceptible to *M. incognita* when inoculated with *M. incognita*, but not when inoculated with *M. mayaguensis*. Results indicated that resistance to *M. incognita* in cotton does not confer resistance to *M. mayaguensis*; however, on cotton susceptible to *M. incognita*, inoculation with *M. mayaguensis* resulted in gall and egg mass indices, eggs per gram of root, and Rf less than on plants inoculated with the same number of *M. incognita*. On cotton resistant to *M. incognita*, the two nematode species reproduced similarly.

Variability in Infection and Reproduction of *Meloidogyne javanica* on Tomato Rootstocks with the Mi Resistance Gene

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Grafting became widely used in the late 1990s with the appearance of a disease known as 'vascular collapse'. Most tomato rootstocks are interspecific hybrids of *Solanum lycopersicum* × *S. habrochiates* or *Solanum lycopersicum* × *Solanum* spp. The response of 10 tomato rootstocks with the Mi resistance gene to an initial inoculum of a Mi avirulent population of *Meloidogyne javanica* was determined in pot tests conducted in spring and summer. In a field test, the rootstocks were subjected to continuous exposure to high population densities of the nematode. The presence of the Mi resistance gene in the rootstocks was determined using the PCR- based co-dominant markers REX-1, developed to detect the introgressed Mi region in hybrids of *S. lycopersicum* × *S. peruvianum*, and the Mi23, designed for *Solanum* hybrids. Nematode infectivity (egg masses), and reproduction (eggs g -1 root) was highly variable in the spring tests. Rootstocks cvs. PG76, Gladiator, and MKT-410 consistently responded as highly resistant (Pf/Pi < 1 and reproduction index [RI] < 10%), and were as efficient as the resistant tomato cultivars. The relative resistance levels of rootstocks cvs. Brigeor, 42851, 43965, Big Power and Heman varied depending on the susceptible cultivar used for reference or the duration of the test. Rootstocks cvs. PG76 and

Heman, and the resistant tomato standard cv. Caramba showed high levels of resistance in the summer test. In the field, seven rootstocks showed high levels of resistance and one (cv. Heman) showed intermediate level. In all experimental conditions Beaufort and Maxifort responded as susceptible to *M. javanica* (Pf/Pi > 50; RI > 50%). The detection of false positives for root-knot nematode resistance in cvs. Beaufort and Maxifort was attributed to their genetic background. The root-knot resistance phenotypic data indicates that Mi23 like REX-1 is not able to distinguish between resistant and susceptible genotypes in the tomato hybrid rootstocks.

Changes in Resistance of PI88.788 to Field Populations of Soybean Cyst Nematode (SCN)

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PI88.788 has been the major source of resistance to SCN for about three decades, and is estimated to be present in about 97% of varieties resistant to SCN. Recently, researchers in the Midwest have observed that varieties with PI88.788 do not seem to be as resistant as they were formerly. Either the resistant soybean varieties have changed or the field populations of SCN have themselves changed in response to long exposure to PI88.788. Goals of this NCSRP funded research were to 1) Determine the current effectiveness of PI88.788 as a source of resistance to SCN in TN, IL, IN, and Ontario, Canada; and 2) Determine the reaction to other sources of SCN resistance in areas where PI88.788 may no longer be as effective. The areas were chosen on the basis of length of time that soybean varieties with PI88.788 resistance have been widely used. In TN, the area where PI88.788 has been used the longest, every field population recovered with a high SCN population density was able to reproduce on PI88.788, and no Hg-Type 0 populations were found. In Ontario, where varieties with PI88.788 have been used for a shorter period of time, PI88.788 proved to be resistant to many field populations; but a larger than expected number of Ontario populations were found to develop on PI548.402 (Peking) and also on PI90.763, two sources of resistance not generally present in varieties grown in Ontario. While many field populations in IL and IN were unable to develop on PI88.788, other field populations were capable of reproducing on PI88.788. PI548.402, PI90.763, and PI437.654 continued to be effective sources of resistance to such populations. One SCN population from Ontario had a low level of reproduction on PI437.654, while none of the populations from IN or IL were able to reproduce on PI437.654.

Evaluation of Tobacco Germplasm for Resistance to *Meloidogyne* Species in Diverse Regions of the World

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Root-knot nematodes (*Meloidogyne* species) are a diverse group of plant parasites and are found across temperate and tropical regions of the world. Use of nematicides has reduced the losses to root-knot nematodes on tobacco (*Nicotiana tabacum* L.) in some countries but nematicides are not available or rarely used in many developing countries. Worldwide losses are estimated to reach 15% of production. Use of fumigant nematicides, reduce root-knot nematode losses, but are an increasing environmental concern. The most reliable and least costly method of nematode control is host resistance. Ninety-five percent of all nematode damage on tobacco is causes by one of four major species of root-knot nematodes: namely, *M. incognita* races 1-4, *M. arenaria* races 1 and 2, *M. javanica* and *M. hapla*. Tobacco germplasm is available with reported resistance to some populations of root-knot nematodes however, comprehensive testing of tobacco germplasm for host reaction to a diversity of *Meloidogyne* spp., races and populations has not be conducted. Tobacco germplasm (11 entries), reported to be resistant to one or more *Meloidogyne* spp. was obtained from commercial breeders in southern Africa, USA and Brazil and evaluated for resistance to *M. incognita* races 1,3,4, *M. arenaria* races 1 and 2, *M. javanica* and *M. hapla* in a controlled greenhouse trial. The tested entries included: NC 95, CC13, CC33, RJR 35, PVEH 270, PVEH 272, NC 119, Okinawa, STNCB 2-28, K RK 26, K 30R and a susceptible control. The germplasm was evaluated for nematode reproduction under field conditions in replicated field trials in Malawi, Zambia, Tanzania, Brazil (3 locations) USA (2 locations) and France. At least one tobacco cultivar significantly lowered root galling or egg mass production within each nematode species or across each field location ($P = 0.05$). The status of breeding for resistance in *N. tabacum* will be discussed.

Introgressing Root-knot Nematode Resistance into Local Maize Genotypes

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Local maize production is hampered by parasitism of economically important root-knot nematodes (*Meloidogyne* spp.). The demand for the identification of resistant maize germplasm is growing, since chemical control is seldom cost-effective, especially under dry land production conditions and most crop rotation practices favour the build-up of high root-knot nematode populations. Two acquired maize lines have been identified with resistance to *M. javanica* (RF-values < 1) in a greenhouse trial. The resistance of these two lines, together with that of the F_1 progeny resulting from crosses between the resistant and susceptible parents was subsequently verified in a field trial, where a mixed population of *M. javanica* and *M. incognita* was established artificially. Consequently six F_2 populations resulting from these crosses were evaluated for resistance to this mixed root-knot nematode population. Root-knot nematode numbers were generally low in the roots of the majority of F_2 plants from population 1 (0 to 113 17 per 50 g roots). Fifty-one percent of these F_2 plants maintained between 0 and 100 root-knot nematodes individuals per 50 g roots. Although root-knot nematode numbers in plants from the other five F_2 populations were also generally low, the majority of these plants maintained more than 100 root-knot nematodes per 50 g roots. Development of root-knot nematode-resistant maize germplasm will benefit local producers by minimising yield losses as well as by keeping root-knot numbers below damage threshold levels in cropping systems.

Survey of Plant Parasitic Nematodes and Evaluation of Germplasm Sources Resistance to Root-knot Nematode on Banana in China

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During the survey of plant parasitic nematode and the germplasm resource of banana, 172 soil and root samples were collected from forty countries of Guangxi, Hainan, Guangdong and Yunnan, and the nematode species, distribution and population density were investigated. Nematode identification resulted showed that *Meloidogyne* spp., *Rotylenchulus* spp., *Helicotylenchus spp.*, *Tylenchus* spp.., *Hoplolaimus* spp., *Aphelenchoides* spp., *Rotylenchus* spp. and *Aphelenchus* spp. were often occurred and isolated from those sample, meanwhile, *Meloidogyne* and *Rotylenchulus* are dominate species and their distribution widely, average detection rates were 71%, the second was *Helicotylenchus*, average detection rates were 57%. The maxism population density in the banana rizhosphere was *Rotylenchulus*, average population density were 249 nematodes per 100 grams soil, the second is *Helicotylenchus* and *Meloidogyne*. The evaluation of forty-three banana germplasm resources resistance to root knot nematode- *Meloidogyne incognita* were conducted. Some middle resistance and tolerance material were found. We thanks National Nature Science Foundation of China (30660102) and Science Research and Development Funding of Guanxi Academy of Agriculture Sciences (2005005 (Z)) for financial support, and thank Prof. Houbing Chen, South China Agricultural University and Prof. Yeyuan Chen, Resources Institute of Chinese Academy of Tropical Agricultural Sciences for their technical support.

Selection of Virulent Populations of *Meloidogyne incognita* in Pepper

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In the Southeast of Spain, *Meloidogyne incognita* is widely distributed in greenhouses in the Region of Murcia where pepper has been a monocrop for more than 20 years. The prohibition of methyl bromide as a soil disinfectant has lead to the use of resistant root stocks in the control of nematodes. In a greenhouse contaminated with *M.incognita*, resistant root stocks were used in soil which had not been disinfected during a 5 year period, comparing the nodulation index and percentage of plants infested in soil disinfected with methyl bromide and non disinfected soil were susceptible plants without grafting were grown. In the second year there was a selection of populations which parasitized the resistant root stock cv Atlante (5.6 and 100%) and there was no difference in the susceptible plants (7.3 and 100%). After two more years of using the same rootstock another three resistant rootstocks were tested in the 4th year with these virulent populations. The three new rootstocks were not as affected (C19: 0.3 and 13.3%; DRO 8801: 0.3 and 13.3%; Snooker: 10 and 53.3%). Following a further year using these roost stocks a new selection of the populations was observed (C19: 2.4 and 76.7%; Snooker: 3.7 and 80.0%). It is suspected that there exist different sources of resistance for this nematode in commercial root stocks.

Host Plant Resistance for Management of Root-knot Nematodes in Maize

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Identification and validation of root-knot nematode resistance in maize germplasm became a crucial element for cost-effective control of these parasites over the long term. Thirty local commercial cultivars and open-pollinated varieties (OPV) of maize were screened for resistance to *M. javanica* and *M. incognita* race 2 in two separate greenhouse trials. Each seedling was inoculated with ±10 000 root-knot nematode eggs and second-stage juveniles (J2). Nematode evaluations were done after 56 days. Twenty-one of the genotypes maintained RF-values $\leq$ 1 for *M. javanica* and 18 did not support reproduction of *M. incognita* race 2. These genotypes could therefore be classified as poor hosts to the respective root-knot nematode populations. Verification of such resistance was done in two separate microplot trials to determine the effect of initial inoculation densities (Pi ± 0 to 40 000) of *M. incognita* race 2 and *M. javanica*, respectively. The *M. incognita* race-2 trial included PAN6549 (susceptible standard OPV), QS-Ob (resistant standard OPV) and MP712W (acquired resistant standard inbred line). The former two as well as AFG4410 (susceptible standard OPV) were used for the *M. javanica* trial. QS-Ob and MP712 supported significantly lower *M. javanica* and *M. incognita* race 2 numbers in both trials than the respective susceptible standard genotypes. Strong non-linear relationships were shown for both resistant and susceptible genotypes with regard to the RF values and number of eggs and J2 per root system. Results from this study demonstrate the benefits that poor-host status of maize varieties could have in controlling root-knot nematode numbers in fields, particularly of small-scale farmers. This root-knot nematode resistance could also be used in breeding programmes to transfer resistance to varieties or cultivars that have preferred agronomic characteristics.

Virulence of Field Populations of *Heterodera glycines* in Illinois, USA

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The soybean cyst nematode, *Heterodera glycines*, is responsible for major economic losses to soybean producers. In Illinois, over 80% of its 4 million hectares of soybean are infested with yield-reducing population densities of the nematode. The primary means of managing *H. glycines* is through the use of resistant soybean cultivars; unfortunately, over 90% of the resistant cultivars are derived from the same source, known as plant introduction (PI) 88788. The nematode has responded to the wide deployment of this source of resistance by adapting to it. Rotation with cultivars derived from alternative sources of resistance (PI 548402 and PI 437654) is recommended for fields in which the *H. glycines* population has adapted to PI 88788. Field survey results suggested that these sources of resistance are not mutually exclusive; *H. glycines* populations adapted to one source of resistance do not revert (lose their adaptation) when challenged with a second source. Analysis of virulence profiles from 1,053 *H. glycines* field populations confirmed a highly significant positive correlation between virulence on PI 88788 and 548402, but no relationship between virulence on PI 88788 and 437654. Virulence is measured as a female index: development of females on a soybean line relative to development of the same isolate on a standard susceptible soybean line under the same conditions. However, analysis of virulence profiles from 229 tests on inbred lines of *H. glycines* showed the converse: a significant positive relationship between virulence on PI 88788 and PI 437654, but no relationship between virulence on PI 88788 and PI 548402. These relationships must be clarified in light of current management recommendations.

Pyramiding Cereal Cyst Nematode Resistance genes *Cre5* and *Cre6* to Improve Resistance in Bread Wheat

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The cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) is an important disease in many wheat-growing regions of the world. Until recently variability for resistance to CCN was limited within bread wheat. Consequently wide crosses between wheat and wheat relatives were made to increase genetic diversity for resistance genes against CCN within wheat. Amongst the wild relatives, *Aegilops ventricosa* has been found to be resistant to CCN. Bread wheat lines introgressed with *Aegilops ventricosa* chromosomes confer varying levels of resistance to CCN pathotypes. The *Cre6* on chromosome $5N^v$ confers a high level of resistance to pathotype *Ha13* and partial resistance to pathotypes *Ha12* and *Ha41*, while *Cre5,* on chromosome $6N^v$ displays partial resistance to the CCN pathotypes *Ha12*, *Ha13* and *Ha41*. The aims of this study were to develop wheat germplasm containing both *Cre5* and *Cre6* and to evaluate its resistance to CCN pathotypes *Ha41*, *Ha12* and *Ha13*. The results suggest that bread wheat *Ae. ventricosa* introgression lines with a combination of both genes (*Cre5* and *Cre6*) expressed significantly better resistance to the three pathotypes – *Ha12*, *Ha13* and *Ha41* than bread wheat *Ae.* v*entricosa* lines with either *Cre5* and *Cre6* alone.

Nematode Susceptibility in New Synthetic Banana Hybrids of *Musa acuminata* Resistant against *Mycosphaerella* Leaf Spot Diseases

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Host status of Yellow Sigatoka and Black Leaf Streak disease resistant new hybrids from Cirad, (cvs 916, 918, 919, 920, 921 and 924) to the burrowing nematode *Radopholus similis* and to the lesion nematode *Pratylenchus coffeae* were assessed under controlled conditions in a growth chamber on andosol, at 24-28°C and 80% RH. Banana plants produced by tissue culture were allowed to acclimate and grow for 6 weeks prior to inoculation. Susceptibility to nematodes was evaluated by inoculating the hybrid plants with 400 nematodes per plant. Forty-five days after inoculation, the nematodes were extracted from the entire root system carefully collected and weighted separately. Two cvs of Grande Naine (*Musa* AAA, Cavendish subgroup, ITC1256 and cv902) and one cv of Yangambi Km5 (*Musa* AAA, Ibota subgroup, ITC1123) were used as susceptible and resistant controls, respectively. Results on reproductive factors (Rfs) and root infestations showed that three of these hybrids (cvs 918, 919, 924) were not different from the resistant control Yangambi Km5 with a lower reproduction of *R. similis*. Similarly, four of these hybrids (cvs 918, 919, 920, 924) showed a lower multiplication of *P. coffeae*, not different from the resistant control. These preliminary results are indicatives of a partial resistance of cvs 918, 919 and 924 to both nematodes, adding an important extra value to these hybrids, formerly breed to resist to *Mycosphaerella* leaf spot diseases.

Red Alert on the Plant-parasitic Nematodes of Banana

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A mutant of 'Grande Naine', the cultivar MA13 (*Musa* AAA, Cavendish subgroup) has been selected in the field in Martinique for its good horticultural characteristics (hardiness, bunch conformation, productivity…). This peculiar cultivar always demonstrated significant lower susceptibilities to the burrowing nematode *Radopholus similis* and to the lesion nematode *Pratylenchus coffeae.* In addition, the cv MA13 exhibited singularly redder pseudostem as compared to others Cavendish clones. On the basis of these observations, we selected in field conditions some others 'coloured' mutants among different banana subgroups: cv MA13_Green (*Musa* AAA, Cavendish subgroup), cv dwarf Mossi_Green (*Musa* AAA, Red subgroup) and cvs 920_Red, 920_Green and 921_Red (*Musa* AAA, CIRAD hybrids). The susceptibility to nematodes of classic cultivars and associated mutants was evaluated under controlled conditions in a growth chamber on Andosol (volcanic ash soils representative of the French West Indies banana production area), at 24-28°C and 80% RH. Forty-five days after inoculation of each banana clone with 400 *R. similis* or *P. coffeae* per plant, the entire root system was carefully collected and weighted, and nematodes were extracted. *In vitro* micropropagation has no effect on the colour stability of the pseudostem. Reproductive factors and root infestations showed consistent trends, with an increase in *R. similis* root infestations for green mutants (up to 54 %) and a decrease for red mutants (up to 61%) compared to classic cultivars. Surprisingly, the opposite consistent trend was observed for *P. coffeae* root infestations, with a decrease for green mutants (up to 43 %) and an increase for red mutants (up to 76%) compared to classic cultivars. These results provide first evidence that colour criterion, representative of the anthocyanins content, could be a good and fairly simple indicator of nematode susceptibility in selection process.

Nematode Control and Other Benefits of Resistant Trap Crops

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Regular, proper cultivation of resistant intercropping is not only an indispensable measure for controlling nematodes and other plant diseases but guarantees also soil quality with lasting effect and significantly contributes to the guarantee and the increase of yield and thus to the profitability and sustainability of crop cultivation.

Nematode control: P. H. Petersen is a breeding company which is highly specified in designing nematode controlling trap crops. Starting with the first fodder radish *(Raphanus sativus)* cultivar PEGLETTA which is able to reduce the sugar beet cyst nematodes *(Heterodera schachtii)* population about 80 % and a special developed mustard *(Sinapis alba*) cultivar MAXI the reduction capacity of the newest varieties is meanwhile more than 90% with the use of resistant varieties like COLONEL, CORPORAL or ACCENT. Also against *Meloidogyne sp*. P. H. Petersen selected fodder radish varieties (DEFENDER, COMET) that suppress multiplication of these nematodes. In potato crop rotations special varieties like

SILETTA NOVA reduce stubby root nematodes *(Trichodorus ssp)*, which are the transmitter of the *Tobacco Rattle Virus* in order to reduce the corky ring spot syndrome in potatoes.

Additional benefits: Growing trap crops changes soil quality into into soil with higher water infiltration and better respiration. It prevents wind erosion and protects water and nutrients. Fodder radish and mustard are characterized by a very good early vigour, which helps to suppress weeds and voluntary crops.

Biofumigation: In addition to these present benefits of growing catch crops which are more or less related to rooting system and resistance, P. H. Petersen has selected plants with an additional benefit by using the green parts of the plants for biofumigation.

Absence of Resistance Association between Cereal Cyst (*Heterodera filipjevi*) and Root Lesion (*Pratylenchus thornei*) Nematode in Spring Wheat Sister Lines

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Both the sedentary Cereal Cyst Nematode (CCN) and migratory Root Lesion Nematode (RLN) are known to be economically important nematodes of wheat production systems in several parts of the world. In Turkey they commonly occur together in cereal production systems and the main method of control is through the identification and production of resistant germplasm. An advanced spring bread wheat (F9) breeding population was developed in CIMMYT Mexico using Middle-Eastern landrace (AUS4930 7.2) which has been identified as resistant to both CCN (*H. avenae* Australian pathotype Ha13 and *H. filipjevi* HF1 in Turkey) and in addition has partial resistance to RLN - *P. thornei*. This source was crossed with the widely adapted CIMMYT line Pastor. In Turkey 40 of these advanced F9 sister lines were screened for their reaction to the Turkish population of *P. thornei* and *H. filipjevi*. The results clearly demonstrated that 75% of the population (30 lines) have partial resistance to *P. thornei*, however only 3 of these also had effective resistance to *H. filipjevi*. As the resistance to CCN is well documented to be controlled by a single gene and the AUS4930 7.2 source is suggested to be allelic or closely related to the well published *Cre1* on chromosome 2BL. Recent mapping work (Toktay *et al.*) has confirmed as with other sources of *P. thornei* resistance to be quantitative and the AUS4930 7.2 is associated with the regions 1B, 2B and 6D. Although they may share one chromosomal region in common from this work there is no suggestion to consider this relates to the genetic control of both nematodes. The inference of this work is that lines with multiple nematode resistance are required to be screened against both nematodes, which will enable the identification of effective sources of dual resistance to identified and deployed in wheat breeding programs.

TOPIC TEN – HOST PLANT RESISTANCE AND GENETIC MARKERS DEVELOPMENT

Identifying Members of *Mi-DS4* Mediated Hypersensitive Response in *Nicotiana benthamiana* using VIGS

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Although resistance conferred by the *Mi-1* gene has proven to be highly effective for root-knot nematode (*Meloidogyne* spp.) control in tomato, the signalling and defensive pathways required for resistance are largely unknown. Recent development of virus-induced gene silencing (VIGS) technology allows assessment of the functional role of genes by targeting their transcripts for degradation. To identify new candidate genes involved in *Mi-1*-mediated resistance, we have undertaken a large-scale screen of a cDNA library related to plant pathogen defense using tobacco rattle virus-based VIGS in *Nicotiana benthamiana*. The *Mi-1*-mediated resistance in tomato is partly characterized by a hypersensitive response (HR), consisting of localized cell death. Transient expression of *Mi-DS4* construct, a constitutive active form of *Mi-1*, in *N. benthamiana* leaves induces HR as well, which was used to phenotype the silenced plants. To decipher whether the absence of HR was specifically related to *Mi-DS4*-mediated HR and not caused by the silencing of a gene involved in the cell death process itself, we also transiently expressed a constitutive active form of another resistance gene (*Pto*) that mediates HR in *N. benthamiana*. This system has allowed us to examine so far 2,000 clones identifying about 2% new candidates involved specifically in *Mi-DS4*-mediated HR. Further analysis of homologues of these candidates in tomato will provide insights into the *Mi-1*-mediated signaling leading to nematode resistance.

GM-crop Impact Assessment on Soil Ecosystems by DNA Barcode-based Monitoring of Nematode Communities (ERGONema)

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Based on the recommendation of the Dutch Advisory commission on Genetic Modification (Cogem), the Dutch government initiated a research programme (Ecology Regarding Gene-modified Organisms; ERGO) on the better ecological understanding of non-target effects of genetically-modified (GM) crops on soil ecosystem functioning. We will study such effects for the interactions between GM-crops, nematodes and soil fertility in our ERGONema project, starting 2008. The closest interactions between any crop and its environment take place in the rhizosphere and during litter decomposition. A potentially relevant hazard scenario is that GM-crops affect organisms, with consequences for soil fertility. Nematodes constitute a diverse group within the soil fauna, feeding on bacteria, fungi, other nematodes and plant-roots. This intimate feeding relationship with all major players in direct contact with plant residues implies that GM-crop effects in the rhizosphere and on the decomposer community will be reflected in the nematode community. However, for large scale ecological analysis of communities non-morphological characteristics should be used. DNA barcodes are being developed for individual nematode families, and quantitative PCR can be used to monitor changes in the nematode community with unprecedented resolution. ERGONema aims to deliver a validated Ecological Risk Assessment (ERA) to evaluate potential risks of GM-crops on biodiversity and fertility, using an accurate and affordable DNA-based tool.

Activities:

- Establishment of the response window of a high impact, non-GM crop (control) on nematode communities, using newly developed DNA-barcodes;
- Establishment of the response window of a high impact, non-GM crop on soil fertility;
- Determine relationships between changes in nematode communities and soil fertility;
- Integration of the scientific findings of activities 1 and 2 into a draft ERA-guidance.
- Validate the newly developed procedure in GM-crops under field conditions.

Backgrounds:
http://www.soq.wur.nl/UK/ and http://www.nem.wur.nl/UK/
http://www.rivm.nl/en/aboutrivm/organization/mev/ler/index.jsp
http://www.nwo.nl/nwohome.nsf/pages/NWOA_6MBGNF_Eng

From Virology to Nematology: A New Approach to Plant Resistance to Nematodes

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Root-knot nematodes are economically important plant parasites with a wide host range. They develop a unique interaction with cells of host plant roots that involves the induction of highly specialised giant cells from which they feed. Giant cells are the sole nutrient source for these nematodes that enable them to complete their life cycle and to reproduce. Strategies to develop synthetic resistance include inhibiting feeding cell function, but the requirement for this approach is strict control of gene expression to nematode feeding sites using a giant cell-specific nematode-responsive promoter. However, this approach has achieved limited success because a completely specific nematode-responsive promoter, which shows no other expression in any other cell type in the plant, has not been identified, although some promoters that drive high expression in giant cells have been reported. An alternative approach is to use two nematode-responsive promoters rather than one to develop the required specificity of gene expression. We are developing such an approach, based on a Tobacco Yellow Dwarf virus (TYDY) replicase gene and a target gene with modified structure which is not expressed in the absence of the virus replicase. Expression of the target gene requires over-lapping activities in giant cells of two different promoters, and this approach may provide the tight control of target gene expression that is needed to restrict it only to giant cells. The current status of this work will be presented that may lead synthetic plant resistance to nematodes by inhibiting development of host feeding cells.

Mapping Nematode Resistance in Rice

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The root knot nematode *Meloidogyne graminicola* is an obligate biotrophic parasite and a major pest of rice for which resistant varieties are not available in *Oryza sativa* L.. Six *O. sativa* varieties displayed differing degrees of nematode susceptibility, while an *O. glaberrima* variety was resistant. Nematode infection in two *O. sativa* varieties (Bala and Azucena) was investigated in a time course experiment. *M. graminicola* consistently caused more galling and had higher reproductive success in Azucena compared with Bala. Quantitative trait loci for partial resistance to *M. graminicola* were identified using the Bala x Azucena mapping population of recombinant inbred lines. Gall numbers were estimated for 144-156 RILs two and four weeks after inoculation. A total of five and two significant or putative QTLs (LOD score $\geq$ 3.2 or $\geq$2.4 respectively) for nematode resistance were detected in the two experiments. The five QTLs in the two-week experiment were on chromosomes 1, 2, 6, 9 and 11 and explained 5.2 – 9.0 % of the total phenotypic variation. In the four-week experiment, two QTLs were detected on chromosomes 6 and 7, explaining 9.6 and 10.3% of variation. The QTL on chromosome 6 was detected in both experiments. For two of the QTLs detected, Azucena was the donor of the resistant alleles, suggesting it will be possible to breed plants with greater resistance than the more resistant parent. Partial resistance genes are thought to be non-specific and effective against all races of pathogens and therefore are valuable for development of durable resistant varieties.

TOPIC ELEVEN – PARASITISM, HOST REACTIONS AND GENE EXPRESSION

Molecular Characterization of *Coffea arabica* Resistance to *Meloidogyne incognita*

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Coffee (*Coffea spp.*) is an important world trade *commodity*. The *C. arabica* species is representative of 76% from the total annual production in Brazil, the largest world coffee producer. Among the pests and diseases affecting the *C. arabica* crop, root-knot nematodes cause major losses, notably *Meloidogyne incognita, M. exigua*, *M. coffeicola* and *M. Paranaensis*. Development of nematode-resistant *C. arabica* varieties by genetic engineering constitutes a promising alternative for the nematode control, since conventional breeding is quite difficult. Sequencing analysis efforts are in progress to enrich the coffee genomic data, to date, have no representative data available for coffee roots. We proposed the use of functional genomics to isolate sequences involved in *C. arabica* resistance to the root-knot-nematode *M. incognita.* The first step was the identification of a resistant genotype (UFV 408-28, Epamig), which enabled the study of the cellular aspects of its interaction with *M. incognita.* Around 10,000 J2 were inoculated per plant and root tips from 2 to 50 days post inoculation (dpi) were analyzed. More than 10,000 cutting samples (4 µm) were evaluated by optical microscopy under white and UV lights. We observed the normal development of nematode in the susceptible genotype, whereas in the resistant genotype, after penetration, nematode was impaired to accomplish the feeding state. Resistant infected roots showed high accumulation of phenolic compounds and necrotic cells next to the nematodes. All our results indicated a specific gene-for-gene resistance of the *C. arabica* UFV to *M. incognita* as responsible for the hypersensitive-like response phenotype found. The resistance level found in the genotype UFV 408-28 allows the construction of ESTs libraries for further molecular investigation. Generation of specific ESTs derived from compatible and incompatible interactions are currently in progress. Functional and expression patterns of genes potentially involved in the molecular resistance will be addressed to help understanding this mechanism in coffee plants.

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Characterization of RKN-regulated WRKY-like Genes from Tomato

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In tomato, *Mi-1* is a resistant gene originally identified to confer resistance to root-knot nematode (RKN; *Meloidogyne* spp.). Later, it was shown that *Mi-1* also confers resistance to potato aphids (*Macrosiphum euphorbiae*), whiteflies (*Bemisia tabaci*) and tomato psyllids (*Bactericerca cockerelli*). We used TOM1 cDNA microarrays to identify the transcriptome changes during RKN/tomato compatible (susceptible, Moneymaker; *mi*) and incompatible (resistant, Motelle; *Mi-1*) interactions 24 h post inoculation. Ten percent of the up-regulated genes (P <0.05 and ≥2-fold change) were transcription factors. About 30% of the up-regulated transcription factors were specific to the incompatible interaction. Within this group were representative of gene encoding bZIP, MADS box, bHLH, MYB and WRKY transcription factors. Two novel WRKY like genes, specifically up-regulated during the incompatible interaction, were further characterized to understand their role in RKN/tomato and potato aphid/tomato interactions. Time-course expression studies with RKN infected roots and potato aphids infested leaves showed that these genes are up-regulated very early in the incompatible interactions in both roots and leaves. Further characterization of these WRKY like genes and their role in *Mi-1*-mediated and basal defense to RKN and potato aphids in tomato will be presented.

Functional Characterisation of Pathogenicity Genes Identified in Expressed Sequence Tags of the Potato Cyst Nematode *Globodera pallida*

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The potato cyst nematode *Globodera pallida* causes extensive damage to crops throughout the world and is extremely difficult to control with existing natural resistance. Like other biotrophic plant parasitic nematodes *G. pallida* induces complex changes in its host and proteins secreted from the pharyngeal gland cells are thought to be important in this process. Identification and functional characterisation of such proteins is a major goal of our work. We have recently undertaken an EST project on cDNA libraries made from invasive stage juveniles and early parasitic stage nematodes of *G. pallida.* Several thousand ESTs have now been obtained from these libraries and subjected to bioinformatic analysis. We have also downloaded a further 4400 *G. pallida* ESTs from dbEST and included these in our analysis. Homologues of candidate parasitism genes including cell wall degrading enzymes, chorismate mutases, RanBPM-like proteins and proteins involved in ubiquitination were identified in the EST dataset. Novel candidate parasitism genes were identified in a screen of over 18,000 cDNA clones for genes upregulated in parasitic stage nematodes. Several of these novel genes were shown to be expressed in the dorsal gland cell of *G. pallida* by *in situ* hybridisation studies. Functional studies of several candidate parasitism genes are currently in progress.

Manganese Superoxide Dismutase in *Meloidogyne incognita* Isolates Selected for Virulence on Tomato

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Root knot nematodes (*Meloidogyne* spp.) are important pests of a wide range of crops, including tomato. Resistance of tomato to root-knot nematodes is conferred by the single dominant gene *Mi-1*, which is present in all commercially available resistant tomato cultivars, at present. However, several resistance-breaking populations are being collected world-wide. Two isolates coming from the same standard population of *Meloidogyne incognita*, one selected for virulence against *Mi-1* and the other left avirulent, have been used in this study. Manganese superoxide dismutase activity (Mn-SOD) was higher in virulent infective juveniles compared with avirulent counterparts. m-RNA was extracted from those two isolates and converted into cDNA. Species-specific primers of Mn-SOD were used for PCR and one single PCR product was generated from each isolate (EMBL accessions nos. AM285679 and AM285680). Such products were sequenced and the deduced amino-acid sequence recognized as a mitochondrial dimeric manganese SOD. The amount of transcripts was quantified by realtime-PCR. The virulent isolate showed an amount of transcripts 4-fold higher than that present in the avirulent isolate. The up-regulation of manganese SOD gene, which was associated with a high activity of the enzyme, has been found to be a characteristic of pre-parasitic juveniles of *M. incognita* isolates selected for virulence on tomato.

Expression Analysis of Plant Defensin Genes in Nematode Induced Feeding Sites

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Arabidopsis thaliana is a host for the sugar beet cyst nematode *Heterodera schachtii*. Juvenile nematodes invade the roots and induce the development of a syncytium which functions as a feeding site for the nematode. Transcriptome analysis of syncytia induced in the roots of Arabidopsis by *Heterodera schachtii* was performed with Affymetrix GeneChips using microaspiration to isolate pure syncytium material. Initial data analysis has shown that defense gene expression is repressed similar to the results obtained for galls induced by the root knot nematode *Meloidogyne incognita* in Arabidopsis roots (Jammes et al., 2005). However, an exception is the expression of a group of plant defensin genes. *Pdf2.2* and *Pdf2.3* were strongly expressed both in control root sections and in syncytia, and *Pdf2.1* was among the most strongly upregulated genes in syncytia. The expression pattern of these genes in Arabidopsis plants and their response to infection by *H. schachtii*, *H. glycines* and *M. incognita* has been studied using GUS-lines, *in situ* RT-PCR and RT-PCR.

Functional Characterisation of Transcripts Expressed in Early Stage *Meloidogyne javanica*-induced Giant Cells Isolated by Laser Microdissection

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Meloidogyne javanica induces giant cells and feeds from them during its development and reproduction. To study the cellular processes underlying the formation of giant cells, we used laser capture and microdissection to isolate the contents of early stage giant cells 4 and 7 days post infection (dpi), and generated cDNA libraries from mRNA from batches of 100 giant cell samples. Eighty seven (250 EST clones) and 54 (309 EST clones) individual transcripts were identified from the 4 and 7dpi libraries respectively. These have roles in metabolism, stress response, protein synthesis, cell division and morphogenesis, transport, signal transduction, protein modification and fate, and regulation of cellular processes. Expression of 11 of 25 transcripts was upregulated in giant cells between the 4^{th} and 7^{th} day after infection. For 4 genes expression was higher than controls at 4dpi and at 7dpi. Levels of expression for another 4 genes increased only at 7dpi. Genes not affected by nematode infection include phenylalanine ammonia lyase and MTD1, the product of the latter gene accumulates in nodules in *Medicago truncatula*. Phi protein, a cell-cycle related homologue in tobacco, expressed 8.5 times higher in giant cells than surrounding cells at 4dpi and reduced to 6.7 times higher at 7dpi: it may be important in early stages of giant cell formation. Together with a pectinesterase gene and a proteasome subunit beta type 2-A involved in cell division and morphogenesis, Phi protein preferentially localises in cytoplasm of giant cells at about 5dpi. The identification of highly expressed transcripts in developing giant cells adds to knowledge of genes responsive to nematodes. Characterisation of transcripts matching unknown proteins, and cDNAs with no significant matches in gene databases, will increase understanding of nematode-host interactions, and may provide candidate genes and/or promoters which could be used in nematode control strategies.

TOPIC TWELVE – PATHOGENICITY AND NEW HOST RECORDS

Host Range of *Scutellonema bradys* on Tuber Crops

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Scutellonema bradys is recognized only as a pest of yam (*Dioscorea* spp.), upon which it causes serious damage, while its affects on other root crops is unknown. This study was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria in 2004 and 2005. Four of the major tuber crops grown in West Africa, cassava, cocoyam, sweet potato (two cultivars) and potato, compared against yam were inoculated with 2000 *S. bradys* four weeks after planting in sterile soil in pots and harvested six months after planting. Un-inoculated tubers were similar in number and weight than *S. bradys*-inoculated tubers. Damage to potato tubers by *S. bradys* was comparable to yams and was significantly greater than for other plants. However both cultivars of sweet potato showed moderate symptoms of damage. The total nematode population in potato roots and tubers was similar to yam, but more than the populations for both sweet potato and cassava, while cocoyam supported the lowest density of nematodes. *S. bradys* was found to be parasitic on both roots and tubers of potatoes and sweet potatoes, roots only of cassava, while cocoyam did not support *S. bradys*.

Scutellonema brachyurus (Steiner, 1938) Andrássy, 1958 a Quarantine Endoparasitic Nematode for Medic and Ornamental Plants in the North of Iran

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The spread of plant pest and diseases by human activities, including nematodes has sometimes caused economic and environmental damage. All through a survey conducted for identification of medic and ornamental plants` parasitic nematodes in the north of Iran, some root and soil samples of *Aloe vera*, *Agave Americana*, *Calendula officinalis*, *Dracaena marginata*, *Ficus benjamina*, *Lycopersicum esculentum*, *Opuntia microdasys*, *Pandanus veitchii* , *Sterlitzia reginae*, and *Syngonium vellozianum* were collected during 2006. Nematodes were extracted by centrifugation and quarantine parasitic nematode, *Scutellonema brachyurus* (Steiner, 1938) Andrássy was identified.

The population density was 50-430/200 g soil. In 45% of cases the soils were sandy. Extraction of a very high population of the nematode from infected roots of favorite hosts- *Aloe vera* and *Lycopersicum esculentum* indicated on endoparasitic behavior of the nematode. The cultured population characteristics are as below:
n = 25, L = 765 (695-823) µm, L' = 754 (683-812) a = 23.5 (21.1 – 24.9), b = 7.1 (6-7.8), b' = 5. 4 (4.6 -5.9), c = 71.3 (57.9-84), c' = 0.56(0.5 -0.6), V = 60.5 (57.4–63.1), Stylet = 29.2 (27 – 32) µm, Tail = 10.8 (9-12) µm. *Male*: Not detected.

This nematode has been probably entered to the country through imported seedlings, tubers or bulbs of ornamentals. Efforts to stop further spread of this nematode- because of its vast host range and damage capability, must be taken.

Efficacy of Fumigant and Non-fumigant Nematicides for the Management of *Meloidogyne chitwoodi* in Idaho Potatoes

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Two field experiments were conducted at the University of Idaho, Idaho, USA to study the efficacy of different combinations of Vapam HL, Temik 15G, Mocap 6EC, Vydate C-LV and different application methods of fosthiazate for the control of *Meloidogyne chitwoodi* in potato. The experiments were laid out in a randomized complete block design with seven and six treatments in a field each with five replications for the first and second experiment respectively. Mocap treatments were surface broadcast using a tractor-mounted plot sprayer and Vapam was applied as broadcast by fumigation bar. Temik and Admire were applied in furrow at planting. Vydate was applied at planting and chemigated subsequently after planting. Preplant treatments (PPI) of fosthiazate were broadcast sprayed or shanked at 6 inch deep or shanked followed by rototilling or broadcast surface spray with the nozzles attached to the front of the rototiller. Potato cv. Russet Burbank seed pieces were planted on 25th April in rows three feet apart. Weeding and other normal cultural practices were followed. Five months after planting, the tubers were hand-harvested on 2nd October from 15 feet of the middle two rows of each plot and weighed. The tubers were graded and evaluated for nematode infection. Data from both experiments indicated that there was an increase in total yield in different combinations of treatments compared to control plot. Percent of nematode infected tubers in treated plots ranged from 6 to 27.4 and 5.6 to 35.5 for the first and second experiment respectively. Lowest level of nematode infection was observed in the Mocap 2gal + Temik 20 lb + Vydate 2.2 pt (6.0 %) applied plots and; Fosthiazate 4.5 ai/A + Temik 20lbs at planting (5.6 %) applied plots in the first and second experiment respectively.

Yield Loss Potential of *Pratylenchus penetrans* and *Meloidogyne* spp. on Onion

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Experiments were conducted under green house and microplot conditions to determine the yield loss caused by different initial population densities of *Pratylenchus penetrans* and *Meloidogyne hapla*, on onion. For the green house study, seeds of onion cultivar Tioga planted in pots filled with sterile soil mixture was inoculated with respective nematodes at the rate of 0, 2, 4, 8 or 16 nematodes/cc soil. In the microplot experiment Tioga seedlings were inoculated at the rate of 0, 2, 4, 6, 8 *M. hapla*/cc or 4 *M. incognita*/ cm^3 soil. At harvest, the data on fresh and dry weight of the plant and root were recorded along with the nematode population in the soil and root. Data indicated that *P. penetrans* significantly reduced all onion growth parameters except the leaf fresh and dry weight with the increasing inoculum levels. Significant reduction in plant parameters began at two *P. penetrans*/ cm^3 soil. Bulb weight reduction ranged from 31.5% at 2 *P. penetrans*/ cm^3 soil to 64.2% at 16 *P. penetrans*/ cm^3 soil in microplots. Maximum plant total dry weight reduction was 84.2% at 8 *P. penetrans*/ cm^3 soil under greenhouse conditions. Inoculation of *M.hapla* and *M. incognita* on onion under green house conditions showed that total plant dry weight reduction ranged from 40.6% to 59.6%. Results from the microplot experiment indicated that plant parameters were significantly reduced at all inoculum levels, and the maximum reduction in bulb weight was 41.3% at 8 *M. hapla*/ cm^3 soil. The microplot experiment also showed that significantly more damage was caused by *M. hapla* than *M. incognita* when the two species were inoculated at the same density.

Effect of *Meloidogyne ethiopica* on the Growth of Grape (*Vitis vinifera* L.) in Pots

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The effect of initial population densities of a Chilean population of *Meloidogyne ethiopica* on the growth of the grape cv Merlot was investigated in 600 cm^3 clay pots. Each pot was artificially inoculated with 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 or 512 eggs and second stage juveniles/cm^3 soil and transplanted with one 60-day-old seedling of the grape cv. Merlot. The plants were maintained on benches with 6 replications for each initial population density in a glasshouse at 26 ± 3 °C for 45 days. Data of height and fresh top and root weight of the plants fitted to the curves of the Seinhorst model, $y = m + (1 - m)z^{P-T}$. Tolerance limits (T) to the nematode for plant height, fresh top and root weight of grape were 1.3, 0.6 and 0.45 eggs and second stage juveniles/cm^3 soil, respectively. The minimum relative yields (m) were 0.06, 0 and 0.2 at $Pi \geq 64$ eggs and second stage juveniles/cm^3 soil for fresh top and root weight and height of grape, respectively. Maximum nematode reproduction rate was 307.2-fold at the lowest initial population density. These results provide evidence of the serious damaging effect caused by the Italian population of *M. javanica* to common bean.

Topic Thirteen – Interactions of Nematodes with Microorganisms

Diversity of Endophytic Bacteria Isolated from Pine Trees with Pine Wilt Disease

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The characteristic rapid death of maritime pine, *Pinus pinaster*, after infection by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, suggests the involvement of other organisms in pine wilt disease (PWD). Microorganisms are common companions of plant roots, but few reports describe their presence in the aerial parts of plants. Endophytic bacteria are ubiquitous in nature and colonize a broad spectrum of plant species. They colonize plants internally without harming them and have been reported to promote their growth and stimulate plant defence mechanisms. They may be involved in improving plant health but they have also been seen as one potential cause of the low regeneration capacity of mature trees. The bacteria carried out by the PWN through pine trees may also contribute to the rapid wilting of the maritime pine trees. Fifty-one bacteria strains were isolated from infected pine wood and PWN trails, these were RAPD typed and identified by sequence of the 16S rDNA gene. The endophytic microbial community isolated included 17 RAPD types. *Betaproteobacteria* were isolated from pine trees but the majority of the strains identified belonged to the *Gammaproteobacteria* group. Six different bacteria strains were isolated from the nematode trails. Although they belonged to the *Gammaproteobacteria* group, they were distinct from the endophytic bacteria. These nematode-related bacteria were phylogenetically closely related to bacteria strains previously isolated from wood-boring organisms.

Interactions between Population Densities of the Nematode *Globodera pallida* and *Rhizoctonia solani* Diseases of Potatoes under Controlled Environment Conditions

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The potato cyst nematode *Globodera pallida* and the soil borne fungus *Rhizoctonia solani* AG3 are problematic and widespread on potatoes in the UK, limiting the efficient potato production. Previously the combined presence of *G. pallida* and *R. solani* has been shown to cause a damaging disease complex of potato but until now no study has investigated the effect of nematode densities on *R. solani* disease of potato. This study explores interactions between *G. pallida* and *R. solani* by comparing disease development in potato plants inoculated with a range of *G. pallida* juvenile densities with *R. solani*. The treatments included inoculating a range of *G. pallida* juvenile densities with *R. solani,* and organism alone at 2, 4 and 6 weeks after planting in the controlled environment conditions.

A sequence of potato plants samples were taken to assess any effects of nematode infestation on the incident of *R. solani* disease severity. The results showed that the severity of stolon infection and stolon pruning caused by *R. solani* significantly increased with a high density of *G. pallida* juveniles. A regression analysis revealed a positive relationship between the number of nematodes juveniles present in the roots and incidence of stolon infection, stolon pruning and stem canker by *R. solani.* The various *R. solani* disease severities were more likely to be greater when potato plants are infested by *G. pallida* at an early stage of their development. For the first time an interaction between *G. pallida* and *R. solani* disease has been demonstrated.

Endophytic *Beauveria bassiana* Controls *Radopholus similis*in Tissue Culture Banana

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Beauveria bassiana is an entomopathogenic fungus and one of the most widely used and studied as an insecticidal biopesticide. Among the pests that it has been targeted against is the banana weevil (*Cosmopolites sordidus*). However, banana and plantain production is also heavily affected by plant parasitic nematodes, especially *Radopholus similis*. Recent work at the International Institute of Tropical Agriculture in Uganda discovered that the fungus can infect east African highland banana plants endophytically, without adverse effect to plant growth. Assessment of banana plants harboring endophytic *B. bassiana* was subsequently undertaken with the objective of determining the potential of *B. bassiana* to control *R. similis* in addition to banana weevils. Experiments were conducted using five month old tissue culture bananas (cv. Nabusa) with and without *B. bassiana,* inoculated with and without 1000 *R. similis* juveniles at 1, 2 and 3 months after *B. bassiana* inoculation. Plant height, pseudostem girth, percentage dead roots, feeder root score, fresh shoot weight and fresh root weight were not affected by the presence of *B. bassiana* at 12 weeks after nematode inoculation. Necrotic root index was significantly affected by both *B. bassiana* inoculation and *R. similis,* populations of which were significantly higher in treatments without *B. bassiana* than *B. bassiana* inoculated treatments. Results indicate for the first time the antagonistic affect of *B. bassiana* on *R. similis* in banana, offering promise for its potential as a biocontrol agent with multiple target effects.

Interaction between *Meloidogyne incognita*(race 2) and *Verticillium dahliae* on Olive Seedlings (*Olea eurapaea* L.)

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As olive (*Olea europaea* L.) is native to north of Iran, at the south end of the Caspian Sea and also because of its cultivation development, study on its important pathogens in the area such as *Meloidogyne incognita* and *Verticillium dahliae* is necessary. In a two year program on olive in Gorgan during 2003-2005, interaction between *M. incognita*(Race 2) and *V. dahliae* on 2 years olive seedlings c.v. zard roghani were studied in a randomized complete block design with four replications and five treatments in greenhouse conditions. Treatments included control, nematode alone, fungus alone, nematode 15 days prior fungus and fungus 15 days prior nematode. The results indicated that there is a positive interaction between *Meloidogyne incognita*(Race 2) and *Verticillium dahliae* that the severity and speed of wilting by *V. dahliae* increase in the presence of nematode; although, no symptoms of wilting were observed in treatments without fungus. This result confirmed the role of nematode in increasing of wilting in treatments that contained nematode and fungus. The amount of growth parameters in the presence of both pathogens together, showed a higher decrease than those treatments which nematode or fungus were lonely. The height of shoots decreased significantly in the treatments of nematode 15 days prior fungus. The minimum dry matter weight of root and shoots were measured in the treatment of nematode 15 days prior fungus, 4.85 g and 17.2 g, respectively. This project was financed by Gorgan University of Agricultural Sciences and Natural Resources.

Mycorrhizal Fungi against Potato Cyst Nematodes: Understanding the Interaction and Potential for Integrated Pest Management

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The inadequate control of the potato cyst nematode (PCN) *Globodera pallida* (relative to the other PCN species, *G. rostochiensis*) by short persistence granular nematicides has been associated with the later hatching of *G. pallida* juveniles, compared to that of *G. rostochiensis*. Our project aimed to investigate the interaction between arbuscular mycorrhizal fungi (AMF) and PCN, with the goal of improving management of *G. pallida*.

In laboratory bioassays and pot trials, inoculation of potato roots with Vaminoc, a commercial AMF multi-species mixture, or with the single AMF species *G. intraradices* or *G. mosseae*, had a significant stimulatory effect on the early hatch of *G. pallida* but not *G. rostochiensis*, eliminating the difference in hatching date between the two species. This effect, which was cultivar non-specific, was demonstrated by molecular exclusion liquid chromatography to be the consequence of increases in the amounts of *G. pallida*-selective hatching factors (host-specific semiochemicals present in potato root leachate, PRL) in the mycorrhizal PRL profile. Mycorrhization also increased crop tolerance to *G. rostochiensis*

and compensated for 75% of yield loss sustained by the non-AMF *G. pallida*-infested plants. Acceleration of the early *G. pallida* hatch in the field in response to AMF inoculation of potato tubers enhanced the efficacy of the nematicide aldicarb by significantly reducing nematode invasion and multiplication in roots by almost 40% as compared to non-AMF plants. Despite the increased amount of phosphate in the agricultural soils, high levels of AMF colonisation were detected, suggesting that PCN control by AMF inoculation can be feasible for field-grown potatoes.

This research has undoubtedly contributed to a better understanding of the complex interrelationships between potato plants, PCN and AMF. More importantly, it has opened up novel avenues in PCN management by applying AMF as part of an integrated PCN management strategy.

Effects of Selected Rhizosphere Microorganisms and Carbon on Soybean Cyst Nematode Population Density and Reproduction in Different Tillage Regimes

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Soybean cyst nematode (SCN) population density can be influenced by tillage practices. Data were collected over two growing seasons on total and active bacteria, total and active fungi, and protozoans (amobae, flagellates, ciliates) in the soybean rhizosphere to determine whether the levels of these organisms were related to differences in SCN population density and reproductive rate under different tillage regimes. Data were also collected on the levels of soil carbon in the tillage regimes. In 2006 and 2007 the population density of SCN was highest in treatments which had been tilled since 1979 (long-term tilled) and also in treatments which had been tilled for the last four years but previously were no-tilled. The highest levels of total bacteria were found in the long term tilled and no-tilled for the last four years (previously tilled) treatments. The long term no-till treatments had the lowest levels of total bacteria but the highest levels of flagellates. The SCN reproductive rate during this study was highest in the treatments which had been tilled since 1979. Levels of active bacteria, total fungi, active fungi, cilliates and amobae were similar in all treatments. SCN reproduction (Pf/Pi) was less than 1 in all treatments except the long term tilled plots in 2007 due to drought conditions.

AMF-induced Bioprotection against Migratory Plant-parasitic Nematodes: Which Mechanisms are Responsible?

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About 80% of all vascular plants on earth are colonized by symbiotic arbuscular mycorrhizal fungi (AMF). Plants with a mycorrhized root system benefit from improved water and nutrient uptake, which results in enhanced growth and survival. In addition AMF can reduce the occurrence and impact of several soil pathogens and plant-parasitic nematodes. The AMF-induced bioprotection has been reported for many nematode species in a wide range of agricultural crops, but until now the mechanisms involved have seldomly been investigated for AMF-nematode interactions. In the presented study the interaction was considered between the migratory nematode *Radopholus similis* and the banana plant *Musa* cv. Grande Naine, colonized by the AMF *Glomus intraradices* or *G. mosseae*. By using a split-root experimental set-up in the first part of this study it was shown that not only a local mechanism is responsible for the bioprotective effect, but also a systemic plant response. In the second part, a step-by-step analysis based on the different nematode infection phases was used to reveal the mechanisms responsible for the AMF-bioprotective effect. First, attraction of the nematodes toward plant roots was studied in both *in vitro* and *in vivo* root exudate experiments, for which exudates were collected from mycorrhized and non-mycorrhized plants. In both experiments the non-mycorrhized exudates resulted in a neutral to attracted nematode movement while nematodes were repelled by the mycorrhized exudates. Secondly, in the penetration experiment a significantly lower *R. similis* penetration was observed in mycorrhized banana roots. Considering the results of the root exudate experiments, a changed root exudation in mycorrhized plants seems to be at least partially responsible. Finally, the nematode reproduction was studied. After 8 weeks, the final population was significantly higher in control plants, while the reproduction ratio was significantly higher in mycorrhized plants.

Analysis of Expressed Sequence Tags (ESTs) of a Fungivorous Nematode *Aphelenchus avenae*

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The fungivorous ability and anhydrobiotic survival capability of *A. avenae* have potentially increased its importance over the other free living soil nematodes. Despite the availability of the genome sequence of *Caenorhabditis elegans*, the amount of genetic information available for other free living nematodes remains very limited. To address this, we present an analysis of over 3000 expressed sequence tangs (ESTs) from the *A. avenae*. A cDNA library was constructed from mixed-stage *A. avenae* and used to generate ESTs. Clustering analysis showed that 3075 ESTs could be grouped into 2174 clusters ranging in size from a single EST (1835 cases) to 45 ESTs (1 cases). A great majority of the clusters showed similarity to the genes of *C. elegans* and parasitic nematodes with known or predicted function and one third of the expressed transcripts did not have significant similarity to any of the sequences in current databases thus possibly representing novel genes. To the best of our knowledge, the fungivorous nematode, *A. avenae* has no association or has only very rarely been found in

association with plants. However, with *A. avenae* ESTs, we identified set of genes with significant homology to the genes encoding plant cell wall degrading enzymes including cellulase, pectate lyase, expansin and polygalacturonase. These were previously characterized as the plant parasitic nematode genes and were thought to be involved in the host-parasite interaction. The presence of genes encoding plant cell-wall degrading enzymes in *A. avenae* demands an elaborate investigation to know functional roles of these genes in its life cycle.

The Relationship between Endoparasitic Nematodes and Arbuscular Mycorrhizal Fungi in Soybean Cultivars

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Abstract: Endoparasitic nematodes (EPN) have detrimental effect on plant growth but also substantialy affect nutrient dynamics in ecosystem. Association of plants with arbuscular mycorrhizal fungi (AMF) increases plant access to immobile soil minerals, and therby increase plant growth rates. Mycorrhizae influence the colonization of roots by other microorganisms and may reduce the susceptibility of roots to nematodes. Since, there is direct competition for space, the mutual antagonism between EPN and AMF has been observed. The objectives were to determine the effect of soybean cultivars on EPN density in roots and root length colonization by AMF and their correlation. The cultivar effects and relationship between EPN and AMF colonization in soybean roots has been observed in roots of seven soybean cultivars, in randomized complete block design experiment. Significant differences were found between cultivars for the root infection by EPN and AMF colonization depending on the month of investigation. Relationship between the cultivars concerning interaction between EPN and AMF was found to be positive or negative depending on the cultivar and the month. Three cultivars (OS-1-0, OS-2-I, and OS-3-I) had strong negative correlation while the strength of correlations of other cultivars were weak. Our results suggest that mutual inhibition between EPN and AMF in soybean roots existed depending on the cultivar and the plant genotype as such affects their relationship.

Suppression of Root-knot Nematode Reproduction on Solanaceae Plants using Synergistic Effect of Non-pathogenic *Fusarium* and Attenuated Tobamovirus

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The degree of reproduction of *Meloidogyne incognita* (Mi) on tomato plants varied according to the inoculation timing of non-pathogenic *Fusarium oxysporum* strain F13 (F13) and Mi J_2 to plants: i.e. the numbers of Mi egg masses and galls on tomato plants in pots were more or less greater than the control treatment when the tomato plants were inoculated with F13 ($5x10^4$ cfu/ml soil) prior to or simultaneously to Mi inoculation (1 J_2 /ml soil), but were less than the control when the F13 was inoculated two days after Mi J_2 inoculation. Numbers of Mi galls on tomato plants in pots were significantly less than the control ($p<0.05$) when the plants were previously infected with attenuated Tomato Mosaic Virus (ToMV: Tobamovirus group) strain $L_{11}A$ and inoculated with F13 ($7.5x10^4$ cfu/ml soil) one day before nematode inoculation (10 J_2 /ml soil) to plants. Similarly, numbers of Mi galls on pepper plants were less than the control by ca. 70% when the pepper was previously infected with attenuated Pepper Mild Mottle Virus (Tobamovirus group) strain No. 16 and inoculated with F13 ($1x10^5$ cfu/ml soil) two days after nematode inoculation (3.1 J_2/ml soil). In a vinyl house microplot experiment, average root-knot index (Zeck, 1971) was as low as 1 in plots where tomato seedlings previously infected with the attenuated ToMV strain $L_{11}A$ were transplanted then two days later given a F13 soil treatment ($1.2x10^{10}$ cfu/plant), while the root-knot indices in plots of only attenuated ToMV or only F13 were 6.5 and 4.5, respectively. The fruit yield of tomato (ca. 12 kg/plant) from the plot of attenuated virus and F13 was as high as from the control plot treated with D-D fumigant, while the yields were ca. 8.5 kg/plant at the inoculated plots receiving only attenuated virus or only F13.

New Approach to Plant Parasitic Nematodes and Fusarium Wilt Association on Some Field and Vegetable Crops

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Plant parasitic nematodes often play a major role in disease interactions. This is not surprising when the habits of these pathogens are considered, including relationships established with host plants, mode of parasitism, and other facets of their life cycles. Also, fungi of various types constitute a significant portion of the soil microflora. Some of them are recognized plant pathogens; others are not normally pathogenic under prevailing conditions. At any rate, important disease complexes involving certain fungi and nematodes have been investigated.

Our studies focus on the association between plant parasitic nematodes and soil-borne fungi, particularly that between root-knot nematodes and *Fusarium*, wilt .We have come to the conclusion, that the interaction between root-knot nematode increased the incidence of *Fusarium* wilt and the severity of wilt on the hosts investigated. We found that *M. incognita* increases the incidence of *Fusarium* wilt on both wilt resistant cotton cultivars and wilt susceptible cultivars and invasion by juveniles of root-knot nematodes enabled races of *Fusarium oxysporum* f, sp. *Lycopersici* to attack tomato varieties normally resistant.

Fusarium wilt-root-knot nematode complexes were subjected histological analyses to help in understanding the relative contributions of each complex component, and may give insight into basic aspects of the interaction.

On soybean, cotton and tomato giant cells and xylem elements of both *Fusarium* – resistant and susceptible plants were haveily invaded by *F. oxysporum* . Giant cells, although extensively colonized, were highly sensitive to fungal attack, and became apparently devoid of contents soon after early invasion by the fungus. Abundant fungal growth in tissues was observed. This clearing of giant cells was accompanied by gradual debilitation of fungus hyphae occupying the tissues. Occasional invasion of the female nematode and the egg mass by the fungus were clearly observed. These investigation stressed that fungal colonization was not resticed to the galled area of a nematode-infected plant . Vigorous hyphae extended up into the xylem above the soil line, considerably removed from the site of nematode activity, even in wilt-resistant plants.

Another genus of sedentary endoparasitic nematodes *Heterodera*, has been incriminated in complexes with the *Fusarium* wilt fungus. Reported such a complex on soybean involving *H.glycines*, and stated that the cyst nematodes were more effective in predisposing plants to *Fusarium* wilt than was *M. incognita*. Soybean, however, are somewhat more tolerant to root-knot nematodes than to cyst nematodes, and this could explain the observation. Complete expression of this disease syndrome appears to be dependent upon the presence of both *Hoplolaiums unifformis* and *F. oxysporum* f. pisi. *H. uniformis* is a migratory ectoparasitic nematode, and is thus quite different from either *Meloidogyne* or *Heterodera* spp. The sting nematode, *Belonolaimus longicandatus* is a highly aggressive pathogen of cotton, as well as other crops. This migratory ectoparasite is at least as important as root-knot nematodes in promoting *Fusarium* wilt in cotton, and is, therefore, an extreme hazard to this crop.

Effects of *Mesocriconema xenoplax* on Growth, Nutrition and Colonization by Arbuscular Mycorrhizal Fungi of Grapevine

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Ring nematodes (*Mesocriconema xenoplax*) reduced vine biomass 33%, fine root mass 75%, and arbuscule development in roots of grapevines (*Vitis vinifera*) in a 3-year field microplot study. Based on these data, two greenhouse studies were conducted to test the hypothesis that ring nematodes suppress arbuscules by competing for root carbohydrates. In the first experiment, a factorial experiment with 'Pinot noir' grapevines was conducted with 3 levels of initial nematode population densities (0, 0.1, 1.0 g^{-1} soil), 2 levels of light to affect photosynthesis (full sun, 50% sun), and 2 levels of arbuscular mycorrhizal fungi (AMF) (+/-). Growth of –AMF vines was extremely poor and data are not presented. Nematodes had little influence on plant growth after a single growing season. Root biomass, and sugar and starch concentrations in fine roots were reduced by low light, but the final nematode population densities and arbuscule frequencies in roots were unaffected by light. Nematodes reduced arbuscules and starch concentrations in fine roots, but did not affect root colonization by AMF hyphae. Nematodes reduced plant P and K uptake at the highest density. In experiment 2, 'Pinot noir' vines were infested with 1.0 nematodes g^{-1} soil and subjected to 3 regimes; full sun, 15% sun, and partial defoliation. Plant biomass was only reduced by nematodes in the shaded treatment in the first year, but was reduced by nematodes in all regimes in years 2 and 3. Nematodes reduced root starch and arbuscular colonization of roots at the end of year 1. These findings support the hypothesis that ring nematodes suppress arbuscules in roots via competition for carbohydrates. However, since arbuscules were not more severely altered by nematodes under low light, other factors appear to play a role in ring nematode-AMF interactions in grape roots.

Reducing Variability in Field Trials: Principal Component Analysis a Possible Solution?

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Anyone who has done field research trials knows about the frustration of, after completing the trials, having non-significant results due to excessive variability in the trial site. This variability is often related to something else other than nematodes. Pre-selection of trial sites is usually based on visual observation of the soil and crop growth. A number of samples, or a composite sample, may be taken to assess the number and types of nematode taxa present at the site. At-plant soil samples are usually taken to establish the baseline composition of the nematode community for each plot. However this is usually after the plots have been laid out and the trial planted. Plots for field trials are usually laid in a randomised pattern with different treatments blocked together into replicates. The number of replicates depends on the number of treatments and available space. All of this 'trial design' is often done at the desk-top using random number generators to assign treatments to plots within replicates. However we propose a more scientific approach where all plots of trials are pre-sampled before designing the trial maps and before assigning treatments to plots.

To investigate this technique, three cultivar x nematicide trials were planted in late 2007. For all three trials, the trials were measured, pegged and soil samples taken from each plot. These samples were then sent for: soil physical (%sand, %silt, %clay), chemical (pH, P, K, Ca, Mg) and plant parasitic nematode (*Helicotylenchus, Meloidogyne, Paratrichodorus, Pratylenchus, Xiphinema*) analyses. Data for all of the variables were then put into matrices and Principal Component Analyses (ADE-4 software package) used to categorise the individual plots into clusters. Using this data, replicates could then be grouped, irrespective of their position on the trial, such that treatments with similar abiotic and biotic factors were placed within the same replicate. Thus the inherent variability within a trial site was 'transferred' from being random to being evenly spread out between replicates, ensuring less variability between treatments, and, a better chance of the results obtained being due to nematode differences and not due to other factors.

Occurrence of Soil-transmitted Helminths in Developing Country Women

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Objective: To find the occurrence pattern and prevalence of the soil transmitted helminths in women of child-bearing age group in developing country Nepal.

Methods and Materials: The study was conducted in 7 districts at an altitude of 2100 metres above sea level. Faecal samples of 2478 women of child-bearing age (15 to 45 years) were taken randomly and examined for the ova of soil transmitted helianthus during year 2007. The data were analysed and edited by EPI info program.

Results: The occurrence pattern was 53.0%, 20.0% and 2.7% for Hookworms, *Ascaries lumbricoids* and *Trichuris Trichuria* respectively. Both *Ascaries* and *Hookworm* prevalence rates noticeably increased with increasing age, with the highest infection rate between the ages of 36 - 45 years while *trichuris* infection was highest in women of 15-25 years of age.

Conclusion: Due to the lack of medicine and healthcare facilities in remote areas, there is a high prevalence of hookworm and *Ascaries* in women of child-bearing age and intervention is needed according to WHO guidelines. The government should make special policies and programs for health care access in these areas.

Role of Soil Nematodes as a Bioindicator of Soil Health and the Influence of Human Interventions on their Composition

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A survey to characterize nematode communities in different land management systems in Zimbabwe and to explore the potential of using nematode communities indices as bioindicators to infer soil process and quality was carried out during 2007 in Zimbabwe. Soil samples were collected at random at two depths, i.e. 5 – 15cm and 15 – 30cm from land under continuous maize cropping and at the Botanical Reserve Gardens (uncropped). The results from continuous maize fields showed reduction in diversity of plant parasitic nematodes. Plant parasitic nematodes were in higher proportion over free-living nematodes. *Pratylenchus* spp., *Scutellonema* spp. and *Helicotylenchus spp*. were the dominant plant-feeding species. At the Botanical Reserve Gardens populations of free living nematodes mainly fungivorous Dorylaimids were abundant followed by predatory Mononchids and Dorylaimids. Maturity index, an indicator of the ecological successional status of soil community based on colonizers-persisters (c-p) scale were employed and results showed high index in the Botanical Reserve Gardens due to presence of many nematodes with high c-p values i.e Mononchids and Dorylaimids. Their assemblage can be related to reduced soil disturbance in the ecosystem. Most nematodes with high c-p value are most susceptible to soil disturbances and their abundance in particular habitat, that habitat is considered more stable and suitable for agricultural production. This study found that soil dwelling nematodes may be able to give insight on the effect of particular disturbances on the soil ecology.

Population Densities of *Tylenchulus semipenetrans* Related to Yield of Clementine Mandarin in Spain

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Mandarin Clementine represents 75% of the hectarage in Catalonia where 77% of the orchards are infested with *T. semipenetrans*. To determine the relationship between nematode populations and yield of mandarin, trials were conducted in three drip irrigated orchards of Clementine mandarin cv. Clemenules (El Pla and Mariclaire) or cv. Hernandina (Martorella) for two consecutive years. The orchards were sampled in March and October (El Pla and Martorella), or only in March (Mariclaire) to determine soil and root population densities. Fruits were collected in a single picking. The economic threshold (nematode population at which the value of the damage caused is equal to the cost of control) was calculated for each mandarin cultivar and year considering the gain threshold (value of harvested mandarin that equals the cost of control), and the relationship between population densities and yield. Yield of cv. Clemenules was related to densities of females/g root in spring (R^2 = 0.469; P = 0.0001) but unrelated to juveniles in soil. Yield of cv. Hernandina, however, did not relate either to females/g root or juveniles in soil probably due to the alternate behaviour of this cultivar. The relationship between relative yield of cv. Clemenules and densities of females/g root in spring was described by the Seinhorst damage function model ($0.035 + 0.965\ (0.9995)^{(Pi - 287)}$, R^2 = 0.4782, P < 0.0001). The tolerance limit of cv. Clemenules on citranges Carrizo and Troyer was 287 females/g root. The estimated economic threshold considering the price of mandarins (0.25 and 0.17 €/kg year one and two, respectively) ranged from 330 to 710 females/g root if nematicides were used to control the nematode (lower and upper nematicide treatment cost considered). These tolerance limit and economic threshold values can be taken as a benchmark and will be helpful to provide guidance to the growers.

Effect of Soil Factors on Nematode Faunal Profile

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Soil ecosystem has high biodiversity interacting in complex relationships between food web organisms and abiotic factors. Soil nematodes is one of the most abundant and diverse group with several trophic levels that play an important role in soil functioning. They control the cycling of nutrients, influence microorganisms dispersion, are food source for other organisms, with some species acting as parasites or pathogens. Moreover, their high diversity and ecological importance make them interesting bioindicators for soil health, quality and ecosystem resilence. The aim of this work is evaluate the effect of different physicochemical soil variables on nematode community and food web status and select those variables which are most likely to influence variation in the basal, enrichment and structure indicator guilds. The response was studied in 89 soil samples from 3 natural areas and 15 agricultural fields, where 18 physicochemical soil variables and 12 chemical pollutants (heavy metals and organochlorine pesticide residues) were also evaluated. Thus, soils factors are conjunctly interacting in ecosystem, hearchical partitioning model (HP), as statistical approach, was used to avoid spurious results due to multicollinearity between soils factors. After HP was performed, spearman correlation analysis was used to determine significant association between soil factor and nematode faunal profile. The disturbance level assessed for experimental plots was low-moderate (56%), high (17%), stressed (5%) and undisturbed (22%), corresponding with a food web condition of maturing, disturbed, degraded and structured, respectively. The enrichment index (EI) in all disturbed plots was ≥50, indicating that food web status is due to anthropogenic disturbances rather than effect of limiting natural resources. However, a clearly association between food web condition status and agricultural management could not be established because some conventional horticultural and organic orchard crops showed structured condition as natural areas ones, and the most crop fields showed maturing condition. We consider that this fact is indicating high stability and resilience of nematode community despite of the agricultural practices impact.

Does Long-term Organic Fertilization Simultaneously Enhance the Structural and Functional Stability of Soil Ecosystems?

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The stability of soil ecosystem function depends on soil community, of which nematodes can be used as model organisms to represent the soil food web. To test whether organic fertilization enhances the stability of soil ecosystem structure and function simultaneously, we compared the resilience and resistance of nematode community and overall soil function (decomposition of plant residues) of two soils (degraded soil and its counterpart with long-term organic fertilization) after applying an experimental stress of copper, heat, chloroform or drying as well as a control treatment, respectively. In soils before applying stress, organic fertilization sharply promoted soil physi-chemical, microbiological properties, nematode abundance, diversity and structural complexity and soil function. After different stress applied, however, neither the stability of nematode community nor soil function was consistently higher in organically fertilized soil, but depended on the type of experimental stress. Soil function with organic fertilization showed significantly lower resistance (P<0.05) after heat or drying but higher (P<0.05) resilience irrespective of stress type. Further, we found different respects of nematode community showed distinctive responses even imposed by a similar stress. Nematode abundance, diversity, trophic structure exhibited significantly higher resilience and/or resistance following the stress of copper, chloroform or drying in organically fertilized compared to degraded soil, but both maturity index (MI) and structure index (SI) showed reverse trends after applying copper, heat or drying stress. Thus, the higher abundance and complexity of nematode community initially established in soil do not mean higher stability of community and function after stress. Our results indicated that, besides organic fertilization, both the historical legacy of stresses soil originally undergone and the type of stress soil subsequently received determined the structural and functional stability of soil ecosystem. The pre-cautionary roles of nematodes' response for indicating soil functional performance under currently increasing natural and anthropogenic stress require further study.

Impact of Temperature on Population Dynamics of *Bursaphelenchus xylophilus* in European Conifer Saplings

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Temperature has a significant effect on the development of Pine Wilt Disease (PWD) in trees infested with *B. xylophilus*. It is known that increasing temperature enhances the development of all life stages of the nematode *in vitro*. The effect of temperature on the population dynamics of *B. xylophilus* and on PWD development in *Pinus sylvestris*, *Larix decidua* and *Picea abies* was studied by inoculating four to five year old saplings with 4800 nematodes. Nematodes were initially isolated from naturally infested *Pinus pinaster* and reared on *Botrytis cinerea*. Experiments were carried out in climate chambers at 15, 20 and 25°C. Nematodes were extracted from shoots and roots at seven sampling dates over a 61 day period to cover the period of infection, nematode establishment and development and pathogenesis of the plants. Temperature had a mayor affect on the population dynamics of *B. xylophilus* in the susceptible *P. sylvestris* and *L. decidua* tree species. Temperature did not significantly influence the pathogenicity of *B. xylophilus*, with maximum mortality in *P. sylvestris* and *L. decidua* reached at temperatures exceeding 20°C. Wilt symptoms were not detected at 15°C in any conifer species. At 20°C the maximum population density in shoots of susceptible tree species was approximately 2500 nematodes per gram dry matter. Nematode populations increased to approximately 4000 nematodes in *P. sylvestris* and in *L. decidua* to 2700 nematodes per gram dry matter in shoots at 25°C. It was shown that wilt was induced in *P. sylvestris* and *L. decidua* before nematodes reached a density of 1000 nematodes per gram dry matter.

Pyrosequencing for Analysis of Nematode Diversity

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It is well established that nematodes play an important role in ecosystem processes, yet the relevance of nematode species diversity to ecosystem ecology is still an enigma. Because nematode identification of all individuals at the species level using standard techniques is difficult, laborious, and extremely time consuming, the characterization of nematode communities continues to be resolved at higher than the species level leaving ecological analysis partially ambiguous or superficial. Novel cloning-independent pyrosequencing may offer a potentially rapid tool to inventory nematode fauna at previously unparalleled levels of resolution at faster speeds and lower cost. The main objective of our study was to assess the suitability of 454 GS FLX technology for nematode species identification from metagenomic samples.

Two ~ 400 bp fragments of rRNA loci flanked by "universal" primer pairs (NF1 - 1573R 18S and D3A - D3B 28S) were used to identify nematode taxa. We hand-picked 44 known nematode species in known frequencies to set up 4 artificial metagenomic samples. Two metagenomic samples consisted of DNA of all nematode species extracted and amplified together (18S mPCR and 28S mPCR). Two additional samples came from pooling PCR products from single nematode species PCR reactions (18S sPCR and 28S sPCR). All metagenomic samples were A-Amplicon sequenced on GS FLX and run separately on a 16-chambered plate. The total number of reads ranged from 4159 to 14771 per sample. Out of all reads, ~82% comprised reads of at least 200 bp and within those reads ~91% were identified as matching our referenced 44 species. While ~52% of reads gave 100% identity match, about 30% of reads varied by 1-2 bp, and the rest of the reads varied by >3 bp. Although neither diagnostic region recovered all nematode species, 18S resulted in higher species recovery (~90%) than 28S barcode (~80%) and the use of both barcodes improved the detection level of nematode species. Of the >199 bp reads that were unmatched to our nematode database (~8%), the majority were identified as fungal and ~0.4% were identified as chimeras. The frequency distribution of reads did not mirror the frequency distribution of nematode species.

Overall, results strongly support the suitability of 454 technology for identification of all nematode individuals from environmental samples. At this point, however, the use of the distribution reads for inferring the relative abundances of species within a nematode community is premature.

Effects of Cadmium, Lead and Zinc on the Entomopathogenic Nematode *Steinernema feltiae*

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Nematodes have been well documented as useful bioindicator organisms for environmental monitoring. This project investigates the suitability of the entomopathogenic nematode *Steinernema feltiae* as a sentinel species of heavy metal pollution in areas of mining activity in Ireland, where in recent years, public health concerns were raised. The nematode is widely occurring in Ireland and can be easily extracted from soil and cultured in the laboratory. In addition, the third stage infective juveniles provide suitably age synchronised individuals for toxicity testing. Experiments were carried out in which the effects of cadmium, lead and zinc were investigated on several traits of the nematode. Preliminary results revealed that short term heavy metal exposures of the nematodes did not affect their insecticidal ability against *Galleria mellonella* larvae, with the exception the case of lead at the higher dose tested. On the other hand there were clear negative effects on nematode progeny production, indicating that fecundity in *S. feltiae* could be developed as suitable end point for heavy metal toxicity. The nematodes used in this study belong to a native Irish strain isolated in Bull Island Co. Dublin. Efforts are on going to isolate *S. feltiae* from the mining areas of interest so that long term effects could also be determined. This project endeavours to combine physiological and behavioural nematode responses with data on genotoxic effects to be used as environmental risk assessment tools for cadmium, lead and zinc pollution.

Long-term Changes in Soil Nematode Communities under the Impact of Fertilizers

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Changes taking place in the communities of soil nematodes of an artificially sown meadow under the impact of annually applied mineral fertilizers have been studied in a field experiment for nine years. Changes in the species composition, eco-trophic community structure, and the number of nematodes from different genera depend on the fertilizer applied. The application of mineral fertilizers had a more pronounced effect on the nematode community in the plots without background manuring; the soil manuring weakened the mineral fertilizer effect. The most significant changes in the soil nematode numbers were observed for the trophic groups of bacterial feeders and plant feeders during the first 5-6 years of the experiment. The population of bacterial feeders increased in response to the increasing rates (from 60 to 180 kg/ha annually) of the complete mineral fertilizer *NPK* and nitrogen-containing fertilizers. The population of plant feeders increased in response to the potassium fertilizers. The spectra of nematode genera sensitive to *NPK* and to the particular nutrients have been identified with the use of parameters, including the maturity index of nematode communities, the biotope preferences of the particular nematode genera, and the general pattern of nematode habitats. It was found that the nematode community structure stabilized by the seventh--ninth year of the succession; after this, the effect of annual application of mineral fertilizers on the nematode community structure was not very significant. The results obtained can be used to assess the effect of mineral fertilizers on the soil fauna and to suggest optimum application rates of mineral fertilizers ensuring the sustainable development of meadow herbs.

Effect of Cadmium and Lead Salts on Soil Nematodes

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Sensitivity of soil nematodes from 15 genera extracted from spruce forest at different concentrations $CdSO_4$ (1.5, 3.0, 6.0 mg/l) and $PbSO_4$ (16, 32, 64 mg/l) was studied under laboratory conditions. Toxicity of *Cd* and *Pb* was estimated on mortality of individuals. Distilled water was used as control. In water 50% of nematodes died in 11 days, the majority of taxa passed away in 16 days. Representatives of *Plectus* and *Eudorylaimus* were the most sensitive to such environmental conditions; their death was registered in 7 and 11 days respectively.

Nematodes from genera *Plectus, Eudorylaimus* and *Tylenholaimus* were the most sensible to *Cd* (mortality were observed in 2 days). Representatives of *Aphelenchoides, Rhabditis* and *Acrobeloides* showed a high resistance. They survived up to 15 days (dose 1.5 mg/l). With increasing cadmium dose up to 3.0 and 6.0 mg/l death of nematodes was registered in 9 and 8 days respectively.

The first ones who responded earlier to *Pb* (in 3 days) were nematodes from genera *Metateratocephalus, Teratocephalus*, *Eudorylaimus*. Representatives of genus *Plectus* lost

the vitality on 4-8 days. Nematodes from genera *Eucephalobus, Cervidellus, Malenchus, Ditylenchus, Paratylenchus* exhibited higher resistance to lead. The representatives of *Rhabditis, Aphelenchoides, Cephalobus* and *Acrobeloides* were the most resistant. Mass nematode mortality was fixed in 12-13 days for low *Pb* concentration and 11 days –for high concentrations (32, 64 mg/l).

On the whole, cadmium was more toxic to nematode populations. It was exhibited in early terms of total nematode mortality (in 8-9 days for *Cd*, 14 - for *Pb*). Research was supported by the Programme of Fundamental Research of Biology Department, RAS, № 01.2.006 08823.

T-RFLP Analysis of Nematode Assemblages

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Soil degradation is a global problem, threatening many ecosystems. Loss of carbon from degraded soils to the atmosphere offsets reductions in CO_2 emissions made elsewhere. Furthermore, degraded soils place sustainable agricultural production under severe pressure in terms of monetary costs by decreased crop yields and need for increased fertiliser application.
Monitoring soil health is therefore essential for their protection. A number of indicators have been proposed one such method is the use of biological indicators, for example profiling of soil nematode assemblages. Nematodes are considered a model indicator group being abundant and ubiquitous in all ecosystems, with a short generation time making them responsive to environmental change. However, traditional characterisation of nematode assemblages is based on classical morphology which is both time consuming and problematical resulting in a low-throughput of samples.

Alternatively molecular approaches to classical characterisation of soil nematode assemblages can be applied. Here, we describe an alternative approach of Terminal Restriction Fragment Length Polymorphism (T-RFLP) of small subunit ribosomal DNA. Two approaches are described, the first a *non-directed T-RFLP* entailing digestion of fluorescently labelled PCR product with a single enzyme, combined with multivariate analysis of the resulting fragment profile. Application of this method on agricultural plots under differing management regimes has revealed significant differences in nematode assemblage composition with addition of compost to barley plots which equates well to classical analysis of the same plots. The second approach utilises a *directed T-RFLP* method where, from collected sequence information, a restriction digest has been designed to separate nematode taxa present at the study sites into terminal restriction profiles with fragments of known size. We envisage that these resulting semi-quantitative profiles may be combined with existing biological diversity indices to provide a high-throughput robust ecological monitoring tool.

Comparison of Nematode Fauna of Plantings of *Eucalyptus* Species in the Fleurieu Peninsula of South Australia.

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Soil was sampled randomly from five small plantings of *Eucalyptus globulus* (Tasmanian Blue gum) present on a property located in Back Valley, Fleurieu Peninsula South Australia. These plantations were 7, 5 and 3 years old and planted either close (2m) or wide (4m) apart. The original vegetation consisted of mainly small stands of remnant pink gum (*Eucalyptus fasciculosa*) with braken as the understory. The braken was sprayed before cultivation in preparation for planting. Nematodes were extracted from the soil and the nematode fauna observed and counted. The nematodes were separated into 5 main trophic groups (free-living dorylaims, predators, bacterial feeders, fungal feeder and plant parasites). Nematodes within each trophic groups was further separated into general morphological groups depending on genera or sub-family. The nematode fauna was then compared between plantings and significant differences were found. A diversity index was also calculated. The highest numbers and diversity of nematodes was found in the 7 year old stand of wide spaced trees. The lowest number and diversity was found in one of the 3 year old closely spaced plantings. Comparisons were also made between the plantings of *Eucalyptus globulus* and 5 year old stands of wide spaced trees of *Eucalyptus saligna* (Sydney blue gum), *Corymbia maculata* (Spotted gum), *Eucalyptus occidentalis* (Flat top yate), *Eucalyptus cladocalyx* (Sugar gum) and *Eucalyptus fasciculosa* (Pink gum). There were significant differences between all stands of gums and the numbers and composition of the different nematode groups. There was a gradual increase in nematodes the older the plantings of trees although there was also an effect seen of topography and soil within the sample area.

Development of PCR-DGGE for Nematode Community Analysis: Selection of a PCR Primer Set

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To develop PCR-DGGE for nematode community analysis, we compared four primer sets (nem1-nem2 (gnem1 h): Foucher and Wilson 2002, NEMF1-NEM896r (gNEM h): Waite et al. 2003, MN18F-22R (gMN h): Bhadury et al. 2006 and SSU18A-SSU9R/GC (gSSU h): Okada and Oba, in press). A database survey showed that SSU and nem1 had no remarkable mismatches between any nematode sequences, while not less than three bases mismatches were found in NEM and MN. In addition, no amplification from several species of order Rahabditida was observed with NEM. Next, we tested bands resolution on DGGE gel. SSU and NEM produced clear bands. However, nem1 and MN produced faint band. Profiles of cloning library of four nematode samples constructed with SSU and NEM were compared to conventional method (hCONV h). Three families (Nordiidae, Nygolaimidae, Leptolaimidae) observed by CONV were not detected with both primer sets, but these taxon were minor. Four families of the order Chromadorida and Rahabditida were not detected with NEM. NEM had another disadvantage: it produced multiple bands even from cloned single sequence, probably because the degenerate sequence in the primer design. To examine quantitative performance of SSU, we prepared artificial communities by mixing nine species

of cultured nematodes at different ratios. The intensities of specific DGGE bands of each species were then measured. A correlation between the biomass and the band intensity was found in each nematode species. We concluded that SSU was the most suitable for PCR-DGGE of nematode communities, because it could amplify wide range of nematode taxa to produce clear band patterns, and could obtain some quantitative information.

Comparison of Nematode Community Similarities Assessed by Polymerase Chain Reaction–denaturing Gradient Gel Electrophoresis (PCR–DGGE) and by Morphological Identification

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Molecular biology techniques for nematode community analysis that are high throughput and operable for nonexperts are in high demand for soil biological assessments. In the development of such techniques, the closeness of their analytical results to those obtained by the conventional method is of key importance. In this context, we compared similarity relationships of nematode community structures between polymerase chain reaction–denaturing gradient gel electrophoresis (DGGE) and individual-based morphological identification (CONV) using 12 nematode samples recovered from crop field soils in four localities in Japan. First, we determined whether the primer set chosen to amplify the 18s rRNA genes of nematode taxa was suitable for DGGE by comparing community structures of four selected samples between clone libraries (CLs) and those revealed by CONV. We considered the primer set to be suitable because we found significant correlations in the structures between CLs and CONV in three of the four samples examined. Then we determined the community structure of 12 samples by both DGGE and CONV, and calculated distance matrices to examine if analytical results were similar between the two methods. The correlations in matrices were 0.400–0.603, depending on the types of distance measures, and were always significant between the two methods. In comparing dendrograms drawn based on the matrices, DGGE and CONV were actually similar to some extent in that samples from a single locality tended to group together, although some localities were split in DGGE. Given these results, we expect DGGE to become a useful and efficient tool for nematode community analysis, especially for nonexperts.

Nematode Succession during Controlled Microbial Composting

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Composting is the heat-producing, aerobe disintegration of organic materials by several organisms which form a complex and fast changing community. Until now only the dynamics of the bacterial community has been thoroughly investigated in relation to decomposing processes. This research focuses on the taxonomical composition and the succession of the nematode community during the composting process. Nematodes show several characteristics which make them ideal bio-indicators to analyze the ecosystem quality. Furthermore, the nematode diversity and density in mature compost are considered crucial to construct a solid soil food web. To characterize the nematode community, a compost heap prepared according to the CMC-method (Controlled Microbial Composting) was surveyed at different time intervals and at the same time several variables (temperature, pH, conductivity and moisture) were determined. The quantitative and taxonomical analyses of the compost samples clearly showed an abundant presence of nematodes in compost (except during the heat peak at more than 71 °C). The most important genera were *Rhabditis, Cephalobus, Aphelenchoides, Diploscapter, Panagrolaimus* and *Mononchoides*. Some major shifts in the nematode community occurred during the process. The first nematodes appearing after the heat peak were bacterial feeding enrichment opportunists followed by the bacterial feeding general opportunists. Later in the process fungal feeding nematodes became gradually more important. These apparent temporal changes in nematode community and associated indices illustrate the potential of nematodes to assess the maturity of compost.

Unraveling the Nematode Community Structure of Different Plant Species and Habitats in the Sub-arctic Coastal Environment at Churchill, Manitoba, Canada: a Preliminary Analysis

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Nematodes, being the most abundant soil metazoans and important recyclers of soil nutrients, are useful indicators of ecological processes in the soil. As they are heterotrophic, they are ultimately dependent upon plants. We hypothesize that different plant communities and habitats will have distinct nematode communities as they are determinants of soil food web structure. The objective of our study was to characterize nematode communities in the sub-arctic coastal environment in Churchill, Manitoba, Canada, in relation to different plant communities and habitats. We sampled soil from the Hudson Bay coast to inland habitats, in the following order; coastal *Elymus* sand dunes, tundra *Dryas* heath, polygonized-peat plateau, hummocky eutrophic fen, and *Spruce*-lichen forest. These sites have various non-vascular (mosses and lichens) and vascular (grasses, sedges and other higher plants) plant communities. We found 50 genera of nematodes with *Eumonhystera*, a bacterivore, as the dominant taxon comprising 21% of the total nematofauna followed by *Filenchus* (12%), *Rhabdolaimus* (8%), *Plectus* (5%) and *Prodesmodora* (5%). Bacterial-feeding nematodes had the highest relative abundance in all sites except in the plant-feeder dominated *Spruce*-lichen forest. Habitats containing moss had high moisture levels and favored bacterial

feeders. *Pratylenchus* dominanted in the coastal dune site vegetated with the grass, *Elymus*. This research is continuing in 2008 with multivariate analysis to be done from a more extensive dataset of nematode communities from plant communities and habitats, and soil properties. These findings, in addition to providing lacking information of soil nematode communities in subarctic environments, are also expected to provide a basis for long-term monitoring of climate change impacts on soil food webs in an environment sensitive to climate warming.

Community Structure and Vertical Distribution of Soil Nematodes in Soybean Fields under Different Rotation Systems

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Soil nematodes are numerous, species-rich and widely distributed, and play essential roles in soil organic matter decomposition, plant nutrient mineralization and nutrient circulation. More and more scholars pay attention to the important function of soil nematodes in agroecosystems. The study was conducted in a soybean field under continuous cropping for 15 years, alternate-year of wheat-soybean and wheat-maize-soybean rotation systems in Hailun Agricultural Ecology Experimental Station ,CAS so as to elucidate the character of nematode distribution, dynamic change and vertical distribution of nematode community.

In total, 8 order, 19 family and 43 genera nematodes were identified and 29 genera were shared by the 3 soybean rotation systems; another 14 genera were different among soybean rotation systems in that their distribution was affected by crop rotations and soil environment. The total number of soil nematodes represents an increase-and-then-decrease trend in continuous cropping for 15 years, while increasing continuously in alternate-year of wheat-soybean and wheat-maize-soybean rotation at the soybean growth stages. The number of *Ditylenchus*,*Acrobeloides*, *Mesorhabditis*, *Protorhabditis*, *Panagrolaimus*,*Filenchus* and *Coslenchus* was different among soybean rotation systems.

The total number of soil nematodes displayed a gradual decrease trend with depth under each rotation systems .The majority of nematodes were presented in the 0-30 cm soil layers. The numbers of trophic groups in soybean field were higher than that in bare field. Predators-Omnivores were less presented.

Skimming the Surface: The Effects of Organic Amendments and Tillage on Soil Nematode and Protozoan Communities

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Soil microfauna (nematodes and protozoa) have been shown to be responsive to soil manipulations, but few experiments have investigated whether these responses vary across the soil profile. We investigated the response of the soil food web to organic amendments and tillage at two depths in a field experiment. Organic amendments were incorporated into a tomato:bean:corn rotation through application to soil in the spring and fall over a three-year period. We hypothesized that the addition of amendments would positively influence protozoa and nematodes primarily in the surface layers of the soil, but that this effect could be extended into deeper layers via tillage. Soils were sampled (0-5 cm and 5-25 cm) at the initiation and at the end of the growing season. Organic amendments had positive effects on most measured variables on both sampling dates, but these results mainly were confined to the top 0-5 cm of the soil. For example, organic amendments enhanced soil organic matter content in the top 0-5 cm of the soil profile but not in the deeper layer. Amended soils also had higher respiration rates in the top layer than control soils, and this effect was correlated with higher densities of both protozoans (amoebae and flagellates) and nematodes (specifically Rhabditidae and Panagrolaimidae). Nematode density was further enhanced by tillage in amended soils, but tillage had no effect on the other measured variables. Nematode and protozoan densities were lower in the 5-25 cm layer but did not vary between treatments. The results of this study suggest that soil microfaunal responses to organic amendments tend to be confined to the surface layer (0-5 cm) of soil regardless of tillage. Future research evaluating the influence of agricultural management practices on soil microfauna should employ sampling schemes to account for stratification of resources.

TOPIC SIXTEEN – ORGANIC AMENDMENTS AND MANAGEMENT

Efficacy of Neem Seed Granules, *Trichoderma viride* and *Pseudomonas fluorescens* Alone or in Combination against *Meloidogyne incognita* infecting Cucumber and Tomato

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The neem, *Azadirachta indica* tree is known to possess nematicidal properties in its different parts as well as in seed but large quantities needed to give desired reduction in nematode population densities. Biological control of nematodes using various antagonistic fungi, bacteria etc. offers an attractive alternative but effects are slow. Integration of two or more methods provides better results. However, it is necessary that these methods are compatible with each other or are made compatible by suitable modifications such as by formulation or application technologies. Neem is known for its abilities to suppress certain kinds of fungi and bacteria besides nematodes. Similarly, a number of fungi and bacteria are known to possess antibiotic properties against other fungi and/or bacteria. A neem seed granular formulation (NSG) was developed and tested for its compatibility and integration with the fungus, *Trichoderma viride* and the bacteria, *Pseudomonas fluorescens.* Application of these three alone and in combinations significantly reduced the root galling of cucumber and tomato due to *Meloidogyne incognita,* except in case of NSG alone and combination of all the three, i.e., NSG + *T. viride* + *P. fluorescens*. *P. fluorescens,* NSG + *P. fluorescens* and NSG + *T. viride* improved plant growth at par with carbofuran. The least effective combination was NSG + *T. viride* + *P. fluoroscens.* It was at par with *T. viride* and NSG alone, giving no additional advantage due to the combinations. *In vitro* investigations indicated certain degree of incompatibility among the three components. NSG suppressed growth of *P. fluorescens* and *T. viride*, however, inhibition was temporary. But *P. fluorescens* caused considerable reduction in growth of *T. viride*. These results show that NSG and the bioagents could significantly, but not completely reduce the infection and reproduction of *M. incognita* on cucumber and tomato.

Plant Parasitic Nematodes Associated with Sugarcane in Kenya and their Management using Host Resistance and Crop Mixtures

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A study to identify plant parasitic nematodes associated with sugarcane in western Kenya and to determine the factors influencing their abundance and distribution was conducted in Nzoia, Mumias, West Kenya and Busia zones. Soil samples were collected from randomly selected farms in each zone. 15 genera of plant parasitic nematodes were recovered from sugarcane rhizosphere. The most predominant were *Pratylenchus*, *Scutellonema* and *Meloidogyne* species with densities of 21%, 18% and 13% respectively. Other nematodes found in association with sugarcane were in the genera *Rotylenchus*, *Aphelenchoides, Paratylenchus, Tylenchus, Helicotylenchus*, *Tylencorhyncus, Xiphinema, Ditylenchus, Hoplolaimus,*

Belonolaimus, Trichodorus and *Longidorus*. Soils in Nzoia were the most heavily infested having 55% of total nematodes recovered from all the zones. Sandy soils haboured 40% more nematodes compared to clay soils. Crop cycle, altitude, AEZ and organic products were also found to influence the parasites. Greenhouse tests were conducted to determine relative host resistance status of sugarcane varieties grown in Kenya. Seven varieties namely CO421, CO617, CO945, EAK70-97, KEN83-737, KEN82-808 and KEN82-216 were selected for evaluation. All the varieties tested showed a higher level of resistance to nematodes compared to the standard, N14. A study to determine the influence of different intercrops of sugarcane on the population dynamics of plant parasitic nematodes was undertaken. Five food crops were selected namely bean, soybean, pigeon pea, maize, and cowpea. Nematode numbers were 81% lower in a CO421/bean mixture compared to N14/bean. Significant differences ($P \leq 0.05$) were also observed in different sugarcane/soybean mixtures. *Aphelenchoides* and *Pratylenchus* species were significantly ($P \leq 0.05$) influenced by different types of intercrop with their numbers highest in CO617 and least in KEN83-737. Overall intercropping resulted in reduction of numbers of nematodes except *Scutellonema* species whose numbers increased in sugarcane/bean mixtures.

The Effect of Cover Crops on the Biology of the Yam Nematode, *Scutellonema bradys*

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Management of *Scutellonema bradys* is important in the sustainable production of yams (*Dioscorea* spp.). Cover crops that can be selected for inclusion in yam cropping systems were screened for their antagonism of *S. bradys*. Two week old seedlings of *Aeschynomene histrix*, *Cajanus cajan, Stylosanthes guianensis, Tagetes erecta* and *Dioscorea rotundata* (control) were inoculated with 1000 eggs of *S. bradys.* The experiment was laid out in a randomized complete block design with three replicates and 50 plants per treatment to accommodate daily destructive sampling. Roots of plants were harvested from between 1- 42 days after inoculation and assessed for nematode penetration and development within the roots. Exudates from roots were also collected and the *in vitro* effect of 1 mm stock solution assessed against 50 eggs of *S. bradys* in glass blocks. Hatching and juvenile mortality were recorded daily over a 10 day period. *S. bradys* juveniles were observed in yam roots and roots of *C. cajan* at three days after inoculation, but not in roots of *S. guianensis* until the 12th day or in *T. erecta* roots until the 17th day. Adult females produced eggs 26 days after inoculation in yam roots, whereas no females or eggs occurred in the roots of *C. cajan* by the end of the study. Juveniles observed in roots of *S. guianensis* and *T. erecta* did not emerge into adults by the end of the study. Egg inhibition and juvenile mortality in exudates of *T. erecta* was 71% and 80% respectively compared to the control, while *A. histrix* and *S. guianensis* inhibited egg hatch by 59 and 46% respectively. *S. guianensis* and *T. erecta* appear to produce exudates that affect the penetration of juveniles into their roots and at the same time disrupt the biology of those that penetrate. *C. cajan* however, permits rapid penetration of juveniles after which their development is arrested.

The Nematicidal Properties of Cysteine Proteinases and their Potential to Control Plant Parasitic Nematodes

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Natural plant cysteine proteinases produced progressive damage to the cuticle of the plant parasitic nematodes Meloidogyne sp. and Globodera sp. (Stepeck et al, 2007). Enzymes from a number of different plant fruit and latex extracts (e.g. actnidain from kiwi, bromelain from pineapple and papain from papaya) are active against some migratory and sedentary plant parasitic nematodes. Significant nematicidal effects up to (90% mortality) were observed in preliminary in vitro laboratory assays against a range of nematode species using different cysteine proteinases applied against M. incognita.

The mechanism of action of these plant enzymes is not understood; however an attack on the protective cuticle of the nematode is evident involving blistering and eventually, total digestion of the cuticle. Surprisingly, these enzymes were not effective against the free living nematode *Caenorhabditis elegans* and some beneficial nematodes. Kiwi fruit extracts showed no effects on the mobility and cuticle damage of animal parasitic nematodes, but were able to digest the cuticle of plant parasitic nematodes. These results indicate that there are distinct differences in the composition of the cuticles of these nematodes.

Previous research at Rothamsted has shown that by affecting nematode mobility we can interfere with nematode infection of plants (Fioretti, *et al.*, 2002; Sharon, *et al*., 2002; GB Patent Filing 46082). The nematode cuticle and/or chemosensory organs represent key targets for nematode control as these structures are exposed to the environment and are easily accessible to attack. We hypothesise that lower concentrations of these enzymes in soil will affect nematode mobility and therefore affect host finding. The cysteine proteinases are potential candidates for developing nematode-resistant crop varieties and transgenic plants, which secrete these enzymes into the rhizosphere to interfere with nematode infection.

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Effect of Biofumigation on Population Dynamics of *Pratylenchus* spp.

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The lesion nematode (*Pratylenchus* spp.) is a major constraint to agricultural and horticultural crops, causing severe yield losses in conventional as well as organic farming systems. Due to the wide host range of *Pratylenchus* crop rotation for nematode control is difficult. A relatively new and promising control measure is biofumigation. The method makes use of crucifer species and varieties with high concentration of glucosinolates. The crop is mulched into the soil at flowering when glucosinolate concentration reaches its peak. Due to enzymatic hydrolysis of the glucosinolates nematicidal isothiocynates are released into the soil. The efficacy of biofumigation to control *Pratylenchus* spp. in temperate regions was tested in 2006 and 2007 at two field sites on a commercial farm in Northern Germany. The two field sites differed in *Pratylenchus* species compostion; field site A was primarily infested with *P. crenatus*, field site B with *P. penetrans* and *P. neglectus*. The biofumigation crops were planted after harvest of the previous crop in late July and incorporated mid September. On field site A the biofumigation treatment Terraprotect (*Brassica juncea* cv Energy x *Raphanus sativus* cv Defender) reduced *P. crenatus* population density by 50%, while *R. sativus* cv Defender caused 30% decrease. On field site B all 10 biofumiagtion treatments reduced the population density of *Pratylenchus* spp.: reproduction rates ranged between 0.2 (*Raphanus sativus* cv Colonel) and 0.85 (*Brassica juncea* cv Terrafit).

Bionematicide Effects of Canola Extracts on *Heterodera schachtii in vitro*

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Tissues of several species of *Brassica* family produce compounds such as glucosinolates which have nematicidal effects. A study was carried out *in vitro* conditions to assess the potential of canola extracts on percentage hatch of eggs and mobility of juveniles of sugar beet cyst nematode. Two commercial Canola cultivars were grown until flowering onset, tops were cut, frizzed at −20°C; leaves were chopped in a blender, passed through filter papers and 3 concentrations of stock, 50 and 10% were made. One ml of each concentration was placed in each well of tissue culture plates to which 50 cysts and/or J2 of *Heterodera schachtii* were added and treatments with 10 replicates were kept at 20 and 15°C respectively. After 72 h, about 100, 80% and less than 1% of juveniles were immobile in concentrations of stock, 50 and 10% of both cultivars respectively; all juveniles in water control were alive by the end of the experiment. After 21 days, average 8% of eggs within cysts were hatched in stock solution of both cvs compared with 50% of those in water control.

Effects of Salicylic Acid and Amino Butyric Acid on *Meloidogyne javanica in vitro*

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Root knot nematodes are limiting factors on many agricultural products and farmers' income in Iran; their existence is either not recognized or are being treated extensively with nematicides specially in the greenhouses or high value crops. Resistance of plants to nematodes can be induced by applying chemicals like salicylic acid (SA) and DL-*β*-Amino-*n*-butyric acid (BABA). In two experiments, 1 ml of each three SA and two BABA concentrations or distilled water were placed into each well of tissue culture plates to which 50 eggs and/or second stage larvae of *Meloidogyne javanica* were added. Treatments in 5 replicates were kept for 2 weeks at 25°C for hatching test and 4 days at 15°C for mobility experiment. Fewer eggs hatched in higher concentrations of both chemicals than in control, mean control being 23% for SA and 37% for BABA treatments. Under these conditions neither of both chemicals had any significant effects on larvae mobility.

Effects of Cover Crop Systems and Organic Manures in the Management of *Mesocriconema Xenoplax* and other Pests in an Organic Peach Orchard

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The Peach Tree Short Life syndrome (PTSL) associated to ring nematode (*Mesocriconema xenoplax*) has been a problem in peach and plum orchards in South Region of Brazil since about 1980. In order to investigate alternatives to the *M. xenoplax* control, the impact of three organic amendments (vermicompost, cattle manure and castor cake) and three ground cover crop systems (*Vicia sativa-Sorghum vulgare*, *Raphanus sativus-Arachis hypogea* and *Avena strigosa-Pennisetum americanum*) was investigated in an organic orchard of the peach Ametista cv. naturally infested with this nematode. During the fall and spring seasons, for 4,5 years, 20kg of each organic waste was incorporated into the soil and the cover crops were sowed in plots ($10m^2$) containing two adult peach plants. Peach plots whithout weeds or maintained with native vegetation were used as controls. Four months after the manure incorporation and seeds planting (9^{th} crop season ending), soil samples were collected to determine the *M. xenoplax* reproduction factor (FR). At the last peach harvest (2007), the incidence of *Anastrepha fraterculus* was checked in the in the peach fruits. There was a reduction in the *M. xenoplax* FR by using all the cover crop systems tested as well as by incorporating castor cake and vermicompost amendments into the soil. In the treatments using *V. sativa-S. vulgare*, *R. sativus-A. hypogeae* and cattle manure, the incidence of *A. fraterculus* on fruits was reduced. When the soil was supplemented with the castor cake a tonic effect in the plants was observed, as well as an increase in the number of branches, nitrogen levels in the leaves and some delay in the fruit ripening. Based on these results, the continued use of some cover crops and organic amendments may result in a considerable *M. xenoplax* suppression in the soil, what should contribute to increase the peach tolerance to PTSL.

Evaluation of Millet and Rapeseed as Rotation or Green Manure Crops to Control Nematodes in Orchard Replant Sites

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Four annual crops including Canadian forage pearl millet (*Pennisetum glaucum*) hybrid 101, velvetbean (*Mucuna* spp.), rapeseed (*Brassica napus*) cv. Dwarf Essex, and buckwheat (*Fagopyrum* spp.) were evaluated as rotation or green manure crops for suppression of dagger (*Xiphinema americanum*) and lesion (*Pratylenchus* spp) nematodes. Two sets of field plots (3 m × 3 m) were established in Pennsylvania and one in Connecticut, four replications per treatment were arranged in a randomized complete block design. All plots were planted with seed from the same seed lot to eliminate variability. Nematode population levels were determined three times for each treatment in each set of plots; pre-plant, end of season and three weeks after the crops were incorporated as green manure. Canadian forage pearl millet suppressed lesion nematodes but was a good host for dagger nematodes. Dwarf Essex rapeseed reduced populations of dagger nematodes only after incorporation as green manure but did not suppress lesion nematode populations. All sites had similar results although the dagger nematode data was not statistically different at one location in Pennsylvania. Buckwheat was included as a susceptible control and the data showed that it was a good host for both lesion and dagger nematodes. Velvetbean has been reported to have nematicidal activity but our results showed that the variety used in these experiments appeared to be a good host for both lesion and dagger nematodes and was not nematicidal as a green manure. Many old orchard sites are infested with lesion and dagger nematodes and both nematodes have the potential to cause problems on young trees in replant sites. Rotation crops offer an environmentally friendly alternative to chemicals for nematode control but as this research demonstrates, it is important to know which nematodes are present and what effect a rotation crop may have on the population.

In vitro Screening of Nematicidal Activity of Volatile Fatty Acids in Liquid Hog Manure to *Pratylenchus penetrans* and *Caenorhabditis elegans*

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Application of acidified liquid hog manure (LHM) in microplots and field studies reduced the total numbers of plant parasitic nematodes and lesion nematodes below threshold levels. Previous reports indicated that short-chain volatile fatty acids (VFAs), products of the bacterial anaerobic fermentation of the LHM, were responsible for the suppression of the microsclerotia of the wilt fungus *Verticillium dahliae* in potato fields. Those reports prompted us to examine whether these VFAs might have the same effect on lesion nematodes, *Pratylenchus penetrans,* which interacts synergistically with *V. dahliae* leading to Early Dying Syndrome of potatoes. We have also examined their effects on the model nematode *Caenorhabditis elegans*. Acute toxicity tests (bioassays) were conducted to: (1) Compare the sensitivity of *P. penetrans* and *C. elegans* to the VFAs, (2) Screen the VFAs (acetic, propionic, butyric, isobutyric valeric, isovaleric and caproic acids) in the LHM based on their nematicidal effect on *P. penetrans*, (3) Determine whether individual VFAs in the LHM interact with each other in their toxicity to *P. penetrans*. In the bioassay, 300 nematodes were exposed to increasing concentrations of the VFAs in a buffered solution of citric acid-NaOH at pH 4.5. The concentration of interest is LC_{95} (the concentration that kills 95% of the nematodes in 24 hr). *P. penetrans* was more sensitive to acetic acid than *C. elegans* whereas to the larger chain VFA, caproic acid, sensitivity was similar. Individual VFAs vary in their lethality to *P. penetrans*. Valeric acid was the most lethal (LC_{95}= 6.8 mM) while Isobutyric acid was least lethal (LC_{95}= 45.7 mM). Individual VFAs didn't interact in their toxicity to *P. penetrans* and their effect was additive. Our results indicate *C. elegans* cannot be used as a surrogate to *P. penetrans* in toxicity studies using VFAs. The efficacy of LHM to control lesion nematodes can be evaluated by assessing the VFA content in the manure prior to application. This evaluation is facilitated by the fact that the interaction of individual VFAs is additive.

Acidified Liquid Hog Manure Kills Plant Parasitic Nematodes but Alters Nematode Communities and the Soil Food Web

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Volatile fatty acids (VFAs) in liquid hog manure (LHM) suppressed plant parasitic and lesion nematodes in field, microplots and laboratory experiments. However, their effects on nematode communities are unknown though informative in indicating if VFAs kill free-living nematodes and if soil food webs are disturbed. Nematodes have proved to be a good tool for determining the condition of the soil food web and thus examined in this study. A microcosm experiment was conducted using nematodes faunal analysis as a tool to determine: (i) whether the VFAs in the LHM have specific or general toxicity to various groups of nematodes, (ii) if they could cause 'biological vacuum' in the soil where soil organisms are killed allowing colonization by opportunistic organisms, and (iii) the degree of disturbance they could cause to the soil food web. Acidified LHM (pH= 5.5) was added to pasture soil harboring a wide range of nematodes, representing different trophic levels and to which *Pratylenchus penetrans* was added. Nematodes analysis was conducted at intervals over a period of 28 days to examine the changes in the nematodes community. Nematodes were identified to the genus level. Within days of application of LHM, total numbers of nematodes in soil were reduced with all nematode taxa being affected including *P. penetrans*. Fungal and bacterial feeders' nematodes re-colonized the soil 7 days after LHM application indicating the presence of a 'biological vacuum' caused by application. After 4 weeks, the structure index in the manure treatments had decreased while the enrichment index increased compared to the control and acid-only treatments. LHM application caused moderate disturbance to the soil food web with the creation of 'biological vacuum' and subsequent colonization by opportunistic organisms. LHM can be used as an effective method to control plant parasitic nematodes including *P. penetrans*. However, it should be used in situations to mediate intense disease pressure and to not replace cultural methods and promotion of soil health in controlling plant diseases and pests.

Volatile Fatty Acids in Liquid Hog Manure are Responsible for Suppression of *Pratylenchus penetrans* under Acidic Conditions

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Acidified liquid hog manure (LHM) reduced the total numbers of plant parasitic nematodes and lesion nematodes, *Pratylenchus penetrans,* in previous microplots and field studies. Acidic conditions generating non-ionized forms of short-chain volatile fatty acids (VFAs) in LHM were also previously shown responsible for the suppression of microsclerotia of the wilt fungus *Verticillium dahliae*. In this study we examine if VFAs are similarly responsible for the killing of lesion nematodes exposed to LHM under acidic conditions. LHM was collected from a lagoon at a commercial hog farm in Manitoba and the manure centrifuged to remove particulates prior to use in this study. The concentration of VFAs in the LHM from highest to lowest concentration was (acetic = 190.3 mM, propionic = 50.7 mM, isobutyric = 22.8 mM, butyric = 53.9, isovaleric = 12.5 mM, valeric = 8.5mM and caproic = 8.7mM). A mixture of VFAs equivalent to those in the LHM was then prepared using commercially available VFAs and the test mixture and LHM compared to their ability to kill *P. penetrans* juveniles in a solution bioassay. The bioassay consisted of 300 *P. penetran*s juveniles exposed to 0, 5, 10 and 15 % (vol/vol) of the LHM and the VFAs mixture in a buffered solution of citric acid-NaOH at pH 4.5. LHM was slightly more lethal to *P. penetrans* than the VFA mixture, killing 60% and 40% of the nematodes population respectively with the 5% test concentration. With the 10% test concentration, LHM killed 99% of the nematode population while the VFA mixture killed 88 %. Complete killing of the nematode population was achieved at 15% for both LHM and VFA mixture. These results indicate that the VFAs can account for the majority of the lethal effect LHM to juveniles of *P. penetrans* under acidic conditions. Our findings indicate that other factors in LHM could be responsible for additional slight killing of *P.penetrans*.

Modern Technologies for Potato Growing and Protection from Potato Cyst-forming Nematode in North-West Russia

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Some wastes of the wood processing industries and temperature pre-treatments of seed materials (DROP-technology) are suggested as ecologically friendly technologies for crop growing and pest control in the North. Conifer bark, sawdust and sodium lignosulphonates (co-products of pulp and paper industry) were found to possess nematicidal properties, i.e. decrease potato cyst-forming nematode *Globodera rostochiensis* Woll. (PCN) populations 3-5 fold. These methods of pest control have the following features and advantages: the simplicity of application into the soil (mulching and solution), low toxicity for soil-inhabiting organisms and plants, enhancement of potato productivity. We took out two patents: ‘A method for fighting the potato nematode’ (1996) and ‘A nematicide against the potato cyst nematode’ (2006).

DROP-technology is based on temperature pre-treatment of potato tubers for a short period of time, which includes daily temperature drop from optimal temperatures to temperatures of 4-6°C. The technology solves a problem on reduction of chemical growth retardant applying, provides an increase in crop productivity, increment in plant resistance to unfavourable environmental factors and PCN. It was established that DROP-technology led to increment in plant cold tolerance, which remained at high level for a long time during de-acclimation period. Experiments conducted on the base of this technology on potato seedlings infected by PCN showed a considerable rise of potato resistance to the infection. In the field experiment 3-fold decrease in nematode population were obtained.

The technologies are available for agricultural purposes in Russian Federation, especially in the North, and can be used for raising the efficiency of agriculture. The research was supported by RFBR (№ 08-04-98833).

Potential Nematicidal Effect of Anaerobic Fermentation Residue (Biogas and Bioethanol) on *Pratylenchus penetrans*

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The nematicidal action of anaerobically digested slurry and fermentation residues was evaluated *against Pratylenchus penetrans* in laboratory experiments using a soil naturally infested with nematode. When the soil was added with three different types of anaerobically digested slurry [(1) cow manure fermented at high temperature (CMH), (2) pig slurry fermented at medium temperature (PSM), and (3) municipal solid slurry fermented at medium temperature (MSM)] at a rate of 100 mg NH_4-N/kg soil and incubated for 7 days, the number of *Pratylenchus penetrans* significantly decreased although that of total nematodes did not change in most cases. In order to know the suppressive mechanism, ADS was aerated using a pump (1.5 L min^{-1}) to examine the effects of volatile fraction such as ammonia on *Pratylenchus penetrans* and then applied to the soil as the same rate as the original ADS. The similar result was obtained. When the suppressive effect of NH_3, acetic acid, propionic acid and butyric acid on *Pratylenchus penetrans* was compared, NH_3 showed the strongest effect at the same concentration. When the NH_3 solution at concentrations of 10 mg/L to 100 mg/L was adjusted to ca. pH 6, corresponding to pH of the soil used, the suppressive effect decreased compared with that of the pH non-adjusted NH_3 solution, but was still observed. These results suggested that ammonia toxicity was not the sole factor to decrease the population of *Pratylenchus penetrans* but also other suppressive mechanisms could be involved in ADS. When the soil was applied with fermentation residues such as ethanol and acetic acid productions at rates of 100 mg-N/kg and 200 mg-N/kg soil, significant suppressive effect was observed on *Pratylenchus penetrans*, after two weeks incubation. Thus, residues from anaerobically fermentation such as biogas and bio-ethanol could be considered as a potential nematicidal organic waste.

Crop Rotation as a Management Tool for Root-knot Nematode (*Meloidogyne incognita*) in Carrot

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A greenhouse experiment was conducted at Benguet State University, La Trinidad, Benguet, Philippines from 2006-2007 to assess the effect of crop rotation on root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood 1949 in carrot. Carrot (*Daucus carota*) cultivar New Kuroda was planted as the main crop and was rotated with 6 semi-temperate vegetable crops namely, broccoli (*Brassica oleraceae* var. botrytis), cabbage (*B. oleraceae* var. capitata), garden pea (*Pisum sativum*), mustard (*Brassica juncea*), onion leeks (*Allium cepa*), snap beans (*Phaseolus vulgaris*) including potato (*Solanum tuberosum*), strawberry (*Fragaria* ^{x}*ananassa*) and sweet corn (*Zea mays* var. rugosa). These rotation crops were planted subsequently during the 2nd and the 3rd cropping period while carrot was planted during the 1st and the 4th cropping period. The cropping sequence was as follows: (i) carrot during the dry season of 2006; (ii) rotation crops during the dry season of early 2007; (iii) rotation crops during the wet season of 2007; (iv) carrot during the dry season of late 2007. Carrot in rotation with strawberry, cabbage and onion were the most effective in reducing the population densities of *M. incognita* in the soil. These were significantly different ($P \leq 0.05$) from the other rotation crops. Planting of carrot after strawberry exhibited the lowest number of galls and egg masses consistent in both primary and secondary root systems of carrot in the last cropping period. Highest marketable yield was recorded in carrot following strawberry, broccoli and sweet corn. This information is very important in devising a suitable nematode management strategy, specifically in the cropping systems used by marginal farmers in the highlands.

Effect of Application Sequences of Brassica Green Manures, Mustard Seed Meal and Nematicide on Root-knot Nematode Suppression, Starch Reserves, Yield and Juice Characteristics in Grapevine

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Inter-row cultivation of brassicas may substantially reduce *Meloidogyne* spp in vineyards but the optimal number of years of cultivation to reduce the population levels below damage threshold is less known. We investigated effects of two brassicas (Indian mustard cv Nemfix, BQMulch™) as green manure, mustard seed meal and Nemacur® on the suppression of *M. javanica* in soil and roots when applied in one to three consecutive years in potted Semillon vines. Each vine was inoculated with 500 *M. javanica* J_2 three months after planting. Two control treatments, infected and uninfected, were also maintained. Brassica seeds @ 20 kg/ha were sown in early May; plants grew until early September, and then slashed and incorporated into top soil. Mustard seed meal @ 2t/ha and Nemacur® @ 30 L/ha were also applied on the same day. Data on nematode populations in soil and roots, starch contents in shoots and roots, yield and juice characteristics were recorded from each treatment. Results

indicated that *M. javanica* J_2 populations were reduced in all treated soils compared to the inoculated control treatment. Populations were increasingly reduced from one to three years of repeated treatment. Root populations were also consistently reduced in the BQMulch™ and Nemacur® treated vines. Yields from treated vines were similar to that of inoculated vines in one and two consecutive years of treatment but increased significantly following three consecutive years of treatment. Similarly vines applied with brassica green manures for three consecutive years produced similar yields to that of uninoculated vines. Starch contents in roots (% dry weight) of brassica and seed meal treated vines were considerably higher (21-24%) than that in roots of untreated and inoculated vines (17%) after three consecutive years of application. The number of treatment years did not affect juice characteristics.

Effect of Enhanced UV-B Radiation on Reniform Nematode (*rotylenchus reniformis linford* and *oliveira*) Populations in Cotton (*gossypium hirsutum l.)*

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High density radiation (UV-B) reaching the earth's surface has increased by 6-14% since 1980 and is projected to rise further in the near future. Over the past two decades, several studies were conducted to evaluate the effects of high density radiation on growth and development of crop plants. However, few studies have addressed UV-B induced changes in plant chemistry and their influence on pests such as insects, snails, and nematodes. To quantify increased UV-B radiation effects on nematode populations, cotton plants were exposed to several levels of radiation. Plant growth, development, drymatter accumulation, pigments, and phenolics, along with the number of eggs and nematode numbers in the root zone, were recorded. We observed increased levels of leaf and root phenolic concentration with increased levels of UV-B radiation and decreased number of eggs and nematodes in the root zone. The results of this study can be used to screen cultivars for nematode tolerance.

Possible Mechanisms Decreasing the Damage to Radish by the Root-lesion Nematode in a Soil Amended with Okara and Coffee Compost

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Damage by the root-lesion nematode (*Pratylenchus penetrans*) to radish is a serious problem in Japan. The Kanagawa Agricultural Technology Center has been conducting a field study to test the effect of a compost consisting of okara (the residue of Tofu production) and coffee extraction residue since 1996 and found that the damage was consistently lower in the okara and coffee compost (3 t ha^{-1}) amended soil (OC-soil) than a soil (CM-soil) with cow manure compost (8 t ha^{-1}). In this study, mechanisms of the OC-soil were estimated by comparing the number of *P. penetrans* and total nematodes, community structures of nematodes, fungi and bacteria among the OC-soil, CM-soil and a soil amended with chemical fertilizers in 2006 to 2007. The number of total nematodes was consistently high in the OC-soil during the monitoring period. The number of *P. penetrans* was almost the same between the OC- and CM-soils before radish cultivation both in 2006 and 2007. Nematode community structure analyzed by PCR-DGGE was different among the three soils both in 2006 and 2007. Specific bands were detected in the OC-soil and nearest to be *Prismatolaimus intermedius* and *Distolabrellus veechi*. While community structures of bacteria and fungi were not different in the non-rhizosphere soil, bacterial community structure in the radish root was different between the OC- and CM-soils both in 2006 and 2007. From these results, the suppression mechanisms of damage on radish by the root-lesion nematode in the OC-soil might be related to enhanced numbers of free-living nematodes and bacteria community colonizing the radish roots.

Protective and Curative Activity of Leaf Extracts from *Lantana camara* L. against *Meloidogyne incognita* (Kofoid & White) Chitwood and its Use to Protect Eggplant Roots

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An aqueous extract from leaves of *Lantana camara* L. was assayed for its nematostatic effects on second stage juveniles of *Meloidogyne incognita*. The biological activity was nematostatic; nematodes were not killed but were completely paralyzed in S/10 concentration of leaf extract after 12 hrs. Degree of effectiveness observed, was directly related to the concentration of extract. The effect was reversible; juveniles previously paralyzed in S/10 concentration, recovered complete mobility in tap water and were able to infest susceptible eggplant cv. 'Navkiran'. The standard solution 'S' was more effective against juveniles and killed by 96% while S/2 concentration of extract killed juveniles by 75% after 48 hrs. Freeze-dried aqueous extract from *Lantana camara* L. leaf added to a sterile sandy substrate at S/10 protected susceptible eggplants from *Meloidogyne incognita* infestation. Regardless of concentration of extract added to the sand, second stage juveniles that penetrated roots of eggplant were able to complete development. This suggests a promising use of *Lantana camara* L. as both a green manure and natural alternative to synthetic chemicals in nematode management option.

Effect of Neem Based Biopesticides on *Meloidogyne incognita* and *Rotylenchulus reniformis* Attacking Tomato

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Pot experiments were conducted in two successive years to evaluate the potential of some neem, *Azadirachta indica* A. Juss, based biopesticides viz., Achook 0.15%; Neem Raj 0.15%, Fortune aza 0.15% and Neem drop on the root-knot development caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and population of reniform nematode *Rotylenchulus reniformis* (Linford and Oliveira), attacking tomato (*Lycopersicon esculentum* Mill. cv 'Pusa Ruby'). These neem based biopesticides were applied @3 ml/pot and all the biopesticides significantly reduced the nematode multiplication. The highest reduction in nematode infection was observed in pots treated with neem drop followed by Achook, Neem Raj and Fortune aza respectively. The reduction in the nematode infection simultaneously enhanced the plant growth characters, the highest being in neem drop treated pots.

Biocontrol of *Meloidogyne incognita* using Antagonistic Fungi, Plant Growth Promoting Rhizobacteria and Composted Cow Manure on Tomato

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The effects of antagonistic fungi (*Aspergillus niger, Paecilomyces lilacinus* and *Penicillium chrysogenum*) and plant growth promoting rhizobacteria (PGPR) (*Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas putida)* were assessed alone and in combination with composted cow manure (CCM) on the growth of tomato and on the reproduction of *Meloidogyne incognita* in glass house experiments. Applications of antagonistic fungi and PGPR alone and in combination with CCM caused a significant increase in the growth of both nematode inoculated and un-inoculated plants. Application of *P. lilacinus* caused a 38 % increase in the growth of nematode inoculated plants statistically similar to that caused by *P. putida.* Similarly, *A. niger* caused a 33% increase in the growth of nematode inoculated plants almost similar to that caused by *B. subtilis* or *A. chroococcum*. However, use of *P. chrysogenum* was least effective (29%) in increasing growth of nematode inoculated plants. Moreover, addition of CCM caused a 42 % increase in the growth of nematode inoculated plants. Highest increase (79%) in the growth of nematode inoculated plants was observed when *P. putida* was used with CCM followed by use of *P. lilacinus* plus CCM (73%). Inoculation of *P. lilacinus* caused a high reduction in galling and nematode multiplication followed by *P. putida, B. subtilis, A. niger,* CCM, *A. chroococcum* and *P. chrysogenum*. Combined use of *P. lilacinus* with *CCM* caused a maximum reduction in galling and nematode multiplication while use of *P. chrysogenum* with CCM was the least. Root colonization by PGPR was increased in the presence of CCM while nematodes had an adverse effect on root colonization of PGPR. *P. lilacinus* or *P. putida* may be used with CCM for the biocontrol of *M. incognita* on tomato.

Nematode Communities and Phosphorus Availability in an Andosol under Compost Application and Winter Cover Cropping

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Soil nematodes participate in the decomposition of organic amendments and mediate microbial P turnover. Nematode community analysis was conducted to develop an understanding of how P becomes available to crops under the application of organic amendments. Two types of amendments were investigated: composted cattle manure (0, 61, and 183 kg P ha^{-1}) and cover crop residues (no crop, rapeseed, and cereal rye). Cover crops were grown over winter and incorporated into the soil two weeks before compost application. In the 3 x 3 factorial experiment, soybean was planted as a test crop in 2006 and 2007 on a P-deficient Andosol in Fukushima, Japan. Compost application increased free-living nematodes in the soil. Although the bacterial-feeder Rhabditidae was dominant in the nematode community of the compost, compost application stimulated fungal-feeding nematodes to a greater degree than bacterial-feeding nematodes in the soil. The increase of free-living nematodes was less pronounced in the cover-crop treatments compared to the compost treatments; only bacterial-feeding nematodes consistently increased after the rye treatment. Bray-2 soil P (i.e., readily soluble and desorbable P) increased only in the compost treatment. Soil phosphatase activity and microbial P, both representing the potential of P mineralization, were enhanced in the treatments of compost and rye. Rapeseed had minor effects on the soil P parameters. Soybean P uptake at flowering was improved under the application of compost and/or rye residue. The different changes in the community composition of soil nematodes and the P parameters suggest that the use of a rye cover crop improved P availability to soybean differently than compost application. The density of Pratylenchidae, the prevailing plant-feeder in the soil investigated, declined in the soils applied with compost and/or rye residue while increasing in the rapeseed-applied soil. This may also have affected soybean P uptake.

Onion Stunting in the Murray Mallee, South-Eastern Australia

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Stunted patches, with reduced emergence and yields, are increasingly reported in onions grown in sandy Murray Mallee soils, under a two-cropping system that uses a herbicide-killed, cereal 'nurse' crop.

Surveys showed Lesion Nematodes (*Pratylenchus* spp.) were commonly present in both soil and onion roots, but populations did not tend to be higher in affected patches. Rarely, Stubby-root Nematode (*Paratrichodorus* sp.) was shown to be the likely cause of stunting. Other pathogens, including *Rhizoctonia solani*, *Fusarium* spp. and *Pythium* spp., are likely to be involved.

Greenhouse experiments, using naturally-infested, field soils ± fertilizers to investigate factors influencing onion disease severity, demonstrated:

- Onion plant stand negatively correlated with length of barley cover crop growth, but nullified by 14 days of fallow.
- Carry-over effects in a second season, with reduced plant stand and yields in 'stunted patch' soil previously cropped to barley.
- Lowest root rot severity in 'non-patch' soil not cropped to barley.
- Reduced emergence and growth associated with barley cover crops or addition of pea straw.
- Possible toxic effects from pea straw decomposition, with reduced yields at rates of 2 t /ha or higher.
- Growth stimulation in the following season in soil previously amended with pea straw.
- Possible allelopathic effects from a wheat nurse crop, grown for five weeks, inhibiting emergence of onions.
- Accelerated rate of damping-off in quintozene-treated soil, implicating pathogens other than *R. solani*
- Stimulated root growth in the following season in soil previously treated with quintozene.
- Reduced emergence associated with N fertilizer, deeper (20 mm) seed placement, or cooler, ambient temperatures.
- Increased emergence and growth, and reduced *Pratylenchus* population, in onions grown in soil treated with fenamiphos or lucerne pellets.
- Inhibited emergence in fenamiphos-treated soil in the following season, possibly caused by carry-over effects on beneficial organisms.

Soil Amendments and Natural Products forNematode Management in California

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In two years of field trials on carrots, natural products were tested alone, and in combination, and compared to untreated controls and a chemical standard for management of root-knot nematode (*Meloidogyne javanica*). Each trial consisted of five replicates in a randomized complete block design. Several of the treatments reduced nematode populations or improved yields.

TOPIC SEVENTEEN – NEMATODE BIOLOGICAL CONTROL AGENTS

Effects of Application of Compost and Cultivation of Antagonistic Plant on Parasitic Fungi of Eggs of Soybean Cyst Nematode (*Heterodera glycines*)

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Soybean cyst nematode (*Heterodera glycines*) is an important harmful nematode for soybean and other legumes in East Asia, North and South America. The prevention of this nematode is very difficult, therefore biological control using egg parasitic fungi is considered particularly promising. To clarify a suitable soil environment for egg parasitic fungi, multiplications of nematodes and ratio of abnormal eggs that were parasitic to the fungi was investigated in different soil conditions, namely, continuous cropping, application of compost and cultivation antagonistic plant.

Densities of nematode eggs were investigated at five fields, namely (a) continuous cropping of soybean field, (b) application of compost after continuous cropping field, (c) cultivation the crotalaria which is an antagonistic plant for soybean cyst nematode field, (d) antagonistic plant mingle into soil field and (e) application of compost after cultivation antagonistic plant field. It was observed that nematodes more decreased at (b) and (d).

Nematodes were inoculated into soils gathered from these five fields in the greenhouse and were investigated densities and ratios of abnormal eggs after cultivation of soybeans. Multiplications of eggs were higher in soils from (b) and (e). And ratios of abnormal eggs were highest in soil from (b) next in (c). The fungus mainly isolated from abnormal eggs was different in (b) and (c). The major fungus from (b) was *Paecilomyces lilacinus*. Furthermore, *P. lilacinus* was inoculated into these soils in the greenhouse and multiplication of nematode was examined. Nematode decreased most in the soil (b) and decreased least in the soil from (e).

These results suggest that application of compost accelerate multiplication of soybean cyst nematode, but compost activates egg parasite fungi such as *Paecilomyces lilacinus*, too. Therefore, the density of nematode decreases. There are possibilities that crotalaria activate other egg parasite fungi, but these effects offset each other.

Interaction between Two *Meloidogyne incognita* (Tylenchida: Heteroderidae) Biotypes and the Enthomopathogenic Nematodes *Steinernema feltiae* and *S. carpocapsae* (Rhabditida: Steinernematidae)

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The effect of entomopathogenic nematodes (EPN), *Steinernema feltiae* 'Rioja' and *S. carpocapsae* '96' strains (Rhabditida: Steinernematidae), on *Meloidogyne incognita* (Tylenchida: Heteroderidae) BXVIC (virulent) and B66 (avirulent) strains was assessed under growth chamber conditions using susceptible tomato cv. Marmande (*Solanum lycopersicum,* Solanales: Solanaceae). A complete randomized experiment design with 12 replicates/treatments was performed and repeated three repetitions over time. Treatments were: i) *M. incognita* avirulent, ii) *M. incognita* virulent, iii) *M. incognita* avirulent + *S. feltiae* or *S. carpocapsae*, iv) *M. incognita* virulent + *S. feltiae* or *S. carpocapsae*, v) *S. feltiae* or *S. carpocapsae*, and vi) control treatment without nematode inoculation. Single tomato plantlets with 2-4 leaves were inoculated with 200 *M. incognita* infective juveniles (IJ, treatments i, ii, iii and iv) and 900 IJs of *S. feltiae* or *S. carpocapsae* strains (treatments iii, iv and v). After 60 days, roots were gently rinsed prior to count galls and egg masses. Egg masses were kept in tap water, and emerging root-knot IJs were counted after 7 days. Differential effect was observed for nematode interactions. Both EPN species significantly reduced the number of egg masses and IJs/egg mass when plants were inoculated with the virulent biotype, with only *S. carpocapsae* reducing galling. However, only EPN-*M. incognita* avirulent biotype interaction with *S. carpocapsae,* significantly reduced the number of IJs/egg mass, whereas a significant increase in root galling was observed when either EPN species was applied, although the number of egg masses/plant was not statistically significant from the control treatment. Our results suggest that further studies on the EPN-*M. incognita* interaction considering different root-knot nematodes biotypes and EPN species are strongly recommended in order to clarify the possible effects of their simultaneous presence in the soil.

Influence of *Fusarium* Wilt Resistant Tomato Cultivars on Root Colonization of the Mutualistic Endophyte *Fusarium oxysporum* Strain 162 and as Biological Control of Root-knot Nematode

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The non-pathogenic endophytic fungus, *Fusarium oxysporum* Strain 162, originally isolated from the endorhiza of tomato roots, reduces damage caused by *Meloidogyne incognita*, by inhibiting juvenile penetration of and development in the root ability of the nematode antagonistic. This mutualistic endophyte colonizes the endorhiza of different Fusarium wilt resistant and susceptible cultivars of tomato (*Lycopersicon esculentum*) were investigated. The fungus was colonized the endorhiza of both wilt resistant and susceptible cultivars of tomato. The colonization percent ranged from 75% - 100% in repeated trials, three weeks after fungal inoculation. However, percent colonization declined 8 weeks after fungal

inoculation. The level of nematode biocontrol reached between 55% - 82% in repeated trials. Surprisingly, the highest level of nematode control was found in fungus wilt resistant cultivars. Although endophyte colonization decreased over time, the antagonistic fungus reduced *M. incognita* in the most of the cultivars tested.

Biodisinfection of Soil as a Means of Alternative Management of Northern Root-Knot Nematode (*Meloidogyne hapla*) Damaging Carrot

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Northern Root-Knot Nematode (*Meloidogyne hapla*) has become a troublesome pest in the Czech Republic in recent years. Crop failures were reported from root vegetables and especially from carrot cultivated in Elbe lowland. Chemical treatment is of doubtful efficacy and currently there are no registered nematocides against this species in the Czech Republic. Therefore the aim of this research was to evaluate the efficiency of biofumigation on population density of the *Meloidogyne hapla* nematode. The infested soil was recovered from a field with carrot infected with *Meloidogyne hapla*. The soil was placed in plastic containers; 5 g of cut fresh plant tissue of rapeseed (*Brassica napus*) was inserted in each container and containers were covered with transparent polyethylene sheet. After three weeks the sheet was removed and young carrot plants were replanted in containers. After another six weeks the plants were examined for the presence of root galls. The statistically significant difference between experimental variant and untreated and uncovered control variant was determined. Therefore the amount of the green mass and length of the treatment were proven sufficient to suppress the nematode population. The research was supported by the Ministry of Agriculture of the Czech Republic; project number QG 50087.

Evaluation of Biocontrol Efficacy of *Pochonia chlamydosporia* var. *chlamydosporia* Isolates on *Heterodera schachtii* on Sugar Beet

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In Iran, populations of more than 1 egg of sugar beet cyst nematode /g soil cause economic damage to sugar beet. Environmental concerns regarding nematicides usage have increased interest in the exploitation of natural biological control agents for nematodes. Strains of fungus *Pochonia chlamydosporia* var. *chlamydosporia* isolated from *Heterodera schachtii* have had variable effects on nematode control. An experiment was conducted in which seedlings of sugar beet were planted in unsterile soil infected with chlamydospores of six isolates of fungus (10^{-3}/g), after a week pots were inoculated with 11 J2 of *H. schachtii* /g soil, all pots including control without fungus and five replicates per treatment were kept in a growth chamber at 25°C with 16 h light. After two months, weight of plant parts were taken and final nematode and fungal density were determined. Number of cysts, eggs and reproduction factor were decreased in fungal treated soils; final population of eggs were reduced by 70%, 60% and 20% in soils treated with isolates 6 and 4, 5 and 3 and 1 and 2 respectively. Reproduction factors were also decreased to less than 1 in pots treated with isolates 3, 4, 5 and 6.

Antagonistic Activity of an Isolate of *Pochonia chlamydosporia* var. *chlamydosporia* on Root-knot Nematode *in vitro*

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In this study, antagonistic activity of an isolate of *Pochonia chlamydosporia* var.*chlamydosporia* was examined on *Meloidiogyne javanica*. Fungus was cultured on PDA and rate of colony growth per day were measured at different temperatures after 10 days at dark. Center of Petri-dishes containing 0.8 % water agar and antibiotics were inoculated with plugs of infected CMA with fungus, sterilized egg masses of nematodes were placed near the center and plates including control without fungus were arranged randomly in an incubator at 18°C at dark for 3 weeks. Culture filtrate was obtained by growing fungus on 1.5% malt extract broth for 2 weeks at 22°C, contents including uninfected medium were filtered; 1 ml of extracts were placed in a well of tissue culture plate, 50 J2 were added to each well and plates with 9 replicates kept at 13°C. Maximum growth rate occurred between 25-28°C; only 5% of the juveniles were immobilized in full culture filtrate after 48 h; and approximately more than 80% of the eggs were colonized by the fungus on water agar.

Gene Expression Profiles in *Pochonia chlamydosporia* in Conditions of Saprotrophic-to-parasitic Transition

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Biotypes of *Pochonia chlamydosporia* play an important role as biological control agents of root-knot and cyst nematodes in the plant rhizosphere. A better understanding of the biochemical mechanisms involved in *P. chlamydosporia* saprotroph-to-parasitic transition may enhance the practical exploitation of this fungus. To reveal the genetic and metabolic diversity of the fungus, we analysed differential gene expression under contrasting nutrient conditions. An RNA fingerprinting technique was used to generate cDNA-amplified fragment length polymorphisms (cDNA-AFLP) enabling identification of different gene expression profiles. Conidia of two *P. chlamydosporia* isolates, VC10 and VC280, preferentially parasitising *Meloidogyne incognita* (root-knot) and *Globodera pallida* (cyst nematode), respectively, were inoculated in rich medium. Germinated conidia were harvested after 5 days, washed and transferred to fresh rich and/or weak media. After 24 hours, cultures were challenged with nematode eggs or a water control. Total RNA was extracted over a pre-defined time course and used as template for cDNA-AFLP analysis. Using 12 primers combinations, the 2-b primer selective extensions yielded around 50 labelled fragments per track from the *P. chlamydosporia*-cyst nematode interaction, indicating both differentially and constitutively expressed genes. Representative bands were excised from the gel, cloned and sequenced. Most fragments ranged in size from 65 to 425 bp. The transcript derived fragment sequences (TDFs) were analysed for similarities in the Genbank database. Of the 30 differentially expressed *P. chlamydosporia* TDFs analysed in this study, 6 corresponded to sequences with known function, 15 matched to sequences coding for conserved hypothetical or unknown function proteins and 9 presented no similarities in the databases searched. In all instances the sequence similarities were of fungal origin. Based upon their similarities the

identified TDFs sequences showed identities at amino acid level to known genes and hypothetical proteins ranging from 62-87%. These included putative transcription regulators, ubiquitin modifiers, glycosyl transferases and chaperonins.

Combined Use of Three Different Microbial Agents for Management of *Meloidogyne javanica* in Tomato

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Root-knot nematodes (*Meloidogyne* spp.) are the main nematological problem for horticultural crops in Spain. A great number of microbial antagonists of nematodes have been investigated for their biological control potential but no one of then has shown enough efficiency by itself to be considered as an alternative to nematicides in intensive crops. With the aim of finding a feasible biological control tactic for root-knot nematodes, we have tried to combine the use of several nematode biocontrol agents, using different biological control strategies and thus, susceptible of working together. The bacterial hyperparasite *Pasteuria penetrans*, which can reduce the infective inoculum of nematodes in soil and its reproduction potential and a mycorrhizal fungus *Glomus mosseae* or a PGPR *Pseudomonas* spp., which can compensate partially for the damage caused by nematodes and also can induce a systemic resistance response in the host plant. Pot experiments were carried out in a protected glasshouse under controlled conditions. In all the experiments, the use of a biocontrol agent together with an additional bioprotection in the host plant increased plant growth parameters and reduced the reproduction index in nematodes after 8-10 weeks of growth. Final nematode densities were only reduced in treatments with *P. penetrans*. Treatments with *G. mosseae* increased plant growth parameters (plant height and weight) by 18-37% and reduced gall index caused by nematodes (23-45%). Treatments with *Pseudomonas* increased slightly plant growth (14-21%) but did not reduce gall index. No interactions were observed among treatments but protection conferred by the mychorrizal fungus and bacterial hyperparasite applied together was greater than that conferred by these treatments applied individually.

Pochonia chlamydosporia Reduces the Multiplication Rate of Potato Cyst Nematodes (*Globodera pallida* and *G. rostochiensis*) in Potato Crops in the UK

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Two field experiments were undertaken in 2006 and 2007 at different field sites in Shropshire to assess the effects of the nematophagous fungus *Pochonia chlamydosporia* and the nematicide fosthiazate on the multiplication rate of potato cyst nematodes *Globodera pallida* and *G. rostochiensis*. Treatments consisted of an untreated control, *P. chlamydosporia*, *P. chlamydosporia* with the nematicide fosthiazate and fosthiazate on its own. In both experiments significant reductions in the nematode multiplication rate (*Pf*/*Pi*) for *P. chlamydosporia* treated plots were observed (48 and 52% control respectively) when compared with the untreated control. The *P. chlamydosporia* treatment did not differ significantly from the fosthiazate treatments in terms of *Pf/Pi* in spite of the increased control observed, particularly in 2006 (90% and 59% respectively), demonstrating that the biological control agent was as effective as fosthiazate at reducing nematode multiplication. The combined treatment did not provide any additional reduction in *Pf/Pi* but demonstrated that *P. chlamydosporia* was compatible with fosthiazate. Over the different developmental stages of the juveniles there was evidence of parasitisation of J5 females on the plant root by *P. chlamydosporia*. Root colonization by *P. chlamydosporia* tended to be higher in the *P. chlamydosporia* treatment due to increased levels of juvenile nematodes in roots. Results from both experiments demonstrated the efficacy of *P. chlamydosporia* as a biological control agent of potato cyst nematodes and indicate its potential for use as part of an integrated pest management strategy.

In vitro Pasteuria spp. Endospores Rate of Germination and Infection on *Belonolaimus longicaudatus*

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The majority of earlier studies of rate of germination and infection of *Pasteuria spp.* on nematodes involve *Meloidogyne* species. Spore-filled females produce relatively large numbers of spores and the majority of the life stages can be easily extracted from roots. Also the existence of a unicellular cell stage in the life cycle of *Pasteuria* was unknown until recently. Ectoparasitic nematodes are much more difficult to study since they produce much lower numbers of spores and must be extracted from the soil. The development of an artificial growth media for *Pasteuria* spp. allows for detection of *Pasteuria* cells and other growth structures present in the nematode body. *Belonolaimus longicaudatus* and the *Pasteuria* spp. that parasitizes it were used in a study to determine the rate of germination and infection of *in vitro*- grown *Pasteuria* spores. *In vitro* produced spores were attached with a centrifuge technique. Approximately 200 spore-encumbered nematodes were placed in clean moist sand, in small petri plates. Nematodes were extracted from plates at 1, 2, 3 and 7 days and crushed in wells with growth medium. Plates were observed immediately and after 24 hrs and presence of cells, mycelial balls, thalli and spores were recorded. Spore germination and cell growth had begun by 24 hrs. Cells, mycelial balls and thalli were present by 48 hrs and spores were present at the 7-day sample. Spore germination is much more rapid than reported for *P. penetrans* parsitizing root-knot nematodes, but germination was determined by the presence of mycelial balls. Sporulation also occurred at a much higher rate, within 7 days, as compared to about 20 days for root-knot nematode.

Possible Mechanisms of Action of an Endophytic *Fusarium* Isolate toward the Rice Root-knot Mematode *Meloidogyne graminicola*

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Many studies have been carried out to investigate the interaction between a soilborne plant pathogen, its microbial antagonistist(s), and the host plant on non-graminaceous species, while there has been relatively litle research done on such complex interrelationships existing in cereal crops, such as rice. A biological control system for the rice root knot nematode *Meloidogyne graminicola* was recently developed at the University of Bonn, using an endophytic *Fusarium* isolate that was isolated from a rice growing region of Vietnam. This *Fusarium* isolate demonstrated good antagonistic potential against *M. graminicola* by reducing gall formation and juvenile penetration by upto 50%. *In vitro* experiment showed that secondary metabolites produced by this endophytic fungus caused nematode mortality by up to 95% after 72 hours exposure compared with nematode not exposed to fungal metabolites. Secondary metabolites also significantly inhibited egg hatching of the nematode. *In vivo* experiments demonstrated that the fungus interfered with nematode reproduction by reducing the number of females and number of eggs per female, thus reducing nematode populations in the root over time.

Effects of Increasing Inoculation Levels of *in vitro* Produced '*Candidatus'* Pasteuria usgae*'* on *Belonolaimus longicaudatus* on Turf

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Experiments were conducted to evaluate the effects of increasing inoculation rates of '*Candidatus* Pasteuria usgae*'* endospores per cubic centimeter of soil on *Belonolaimus longicaudatus* populations on turfgrass in greenhouse culture. Treatments were endospore rates of 0; 28,000; 56,000; or 140,000 endospores/cm^3 of sand with four replications at two observation dates. Endospores in liquid suspension (100 ml) were applied to 1,430 cm^3 of sand, mixed, and then potted. 'Penncross' creeping bentgrass was seeded, into pots, allowed to germinate, and then establish for 10 days prior to inoculation of 900 mixed life stages of *B. longicaudatus*/pot. Nematode samples were collected from sixteen pots at 28 days and 56 days after nematode inoculation, respectively by removing a single core (7.62-cm-diam., 10-cm-deep) from the center of each pot. Nematodes were extracted from these cores by centrifugal-flotation and counted. Effects of endospore level on number of *B. longicaudatus* were quantified using regression analysis. *Belonolaimus longicaudatus* populations were reduced with increasing levels of endospores (P = 0.007). At the 140,000 endospores/cm^3 *B. longicaudatus* populations were reduced by 33 and 45 % at 28 and 56 days, respectively compared to the 0 endospore rate.

Anastomosis in Selected Isolates of *Pochonia chlamydosporia* from Cysts and Root-knot Nematodes

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The fungus *Pochonia chlamydosporia* is an effective biological control agent against both cyst (*Globodera* spp., *Heterodera* spp.) and root-knot nematodes (*Meloidogyne* sp.). Fungal biotypes show host preference at the intraspecific level but little is known about compatibility between biotypes. If genetic exchange occurs between native biotypes and those added to soil as biological control agents, the loss of selected characteristics could reduce the efficacy of the agent. *Cordyceps* is the perfect stage of the fungus but little is known about nuclei interchange between different biotypes of the imperfect stage (*Pochonia*). Hyphal anastomosis (fusion between different branches of the same or different hyphae) is an essential step in both sexual and parasexual cycles and such compatibility is associated with hyphal fusion and nuclei interchange. A study of four *P. chlamydosporia* biotypes was conducted using monosporic cultures: two from *Meloidogyne* spp., one from *Globodera* sp. and another from *Heterodera* sp. Individual spores and agar discs of the isolates were grown on glass slides covered with an agar film in different combinations and kept in a humid chamber for five days, followed by staining with the dye HOE 33258. Nuclei, hyphal walls and septa in fungal colonies were observed using a fluorescence microscope (Axioscope) and a high pressure mercury arc lamp as the light source. It was confirmed that conidia are uninucleate and different types of anastomosis were identified among biotypes. Anastomosis occurred between paired colonies of each biotype with nuclear exchange between the compatible hyphae observed. However, hyphae of fungal biotypes from root-knot nematodes and those from cyst nematodes appeared to be incompatible and anastomosis was not observed.

Variability in Desiccation Tolerance among Different Strains of the Entomopathogenic Nematode *Heterorhabditis bacteriophora*

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The entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar is used for biological control of several soil-borne insect pests. As compared to steinernematid nematodes, the shelf life of *H. bacteriophora* is shorter and nematodes loose infectivity earlier. In order to prolong shelf life, the metabolism of nematodes during storage must be reduced by means of desiccation of dauer juveniles. Previous investigations indicate that the heritability of the desiccation tolerance is high provided they have been adapted to moderate desiccation conditions. This makes this trait an excellent target for genetic selection. Positive results in enhancement of desiccation tolerance have already been obtained. In order to start selection with a broader genetic background, this investigation evaluated the desiccation tolerance of sixty-one *H. bacteriophora* strains from different geo-climatic regions. Dehydrating conditions were produced by treating dauer juveniles with the non-ionic polymer polyethyleneglycol 600. Desiccation was measured as water activity (a_w-values). The *H. bacteriophora* strains were produced *in vivo* using the greater wax moth, *Galleria mellonella* (Lepidoptera, Pyralidae). All treatments were done with one nematode batch and repeated

three times. Significant intra-specific variations ($\alpha \leq 0{,}05$) were noted among *H. bacteriophora* strains. Mean desiccation tolerance ranged from a_w-value 0,90 to of 0,95 for non-adapted nematode populations and 0.76 to 0.99 for adapted nematode populations. Variability within one *H. bacteriophora* population increased with increasing desiccation stress. Strains from arid regions tolerated desiccation better than those from temperate regions. Results indicated three nematode strains from Israel (a_w-value of 0,845), Germany (a_w-value of 0,857) and Egypt (a_w-value of 0,86) were the most tolerant and will be crossed for production of the foundation strain, which afterwards be used for genetic improvement by selective breeding. For this purposes, the most tolerant 10% of the population will be used for each selection step.

Cultural and Physical Combinations: An Effective Control Measure against Root-knot Nematodes in Cucumber Fields

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Plant-parasitic nematodes associated with vegetable crops in growing areas in Isfahan and other parts of Iran include root-knot (*Meloidogyne javanica, & M. incognita*.) and root-lesion (*Pratylenchus* spp.) nematodes. Root-knot and root-lesion nernatodes reduce growth of vegetable crops, especially cucumber. The potential of soil-solarisation to control root-knot nematodes *Meloidgyne javanica* and *M. incognita*, and soil amendment with (cow dung 40T/ha.) alone or in integration were studied in cucumber fields. The study was conducted during the hot season at two provinces in Iran between July and August for periods of five weeks in two consecutive years. The temperature in the solarised soils was (10±1°C) higher in comparison to non solarized soils at 5 cm depth and may reaches lower temperature at deep depths. The moisture content of the soil was preserved to value more than 80% in the solarised soils. Bioassay of cucumber roots, indicates that soil solarisation alone could reduces the incidence of root-knot nematodes to 52.56%, and in amended soil to 56%, however, the integration of soil-solarisation and soil-amendment could effectively reduce the nematode incidence to 83%. Total parasitic nematode population of the genera *Aphelenchoides*, *Helicotylenchus*, *Heterodera*, *Paratylenchus*, *Tylenclus* was reduced to 71%, 69% and 79% in the respective treatments. The total free-living nematode populations of the genera *Aphelenchus*, *Cephalobus* and *Rhabditis* was increased only in amended soil and integration of both treatments of soil-amendment and soil solarisation to 30% and to 53% respectively.

Impact of *Pseudomonas*-based Biocontrol Agents and Solarization on *Mesocriconema xenoplax* Populations and Tree Survival in a Peach Tree Short Life SIte

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Soil solarization, alone or in combination with other disease management practices, has been shown to be effective in reducing inoculum density of many soilborne diseases, including nematodes. *Pseudomonas synxantha* (BG33R), isolated from a peach orchard site suppressive to peach tree short life (PTSL), was demonstrated to suppress ring nematode, *Mesocriconema xenoplax*, reproduction in field soil under greenhouse conditions and inhibit egg hatch *in vitro*. In 2005, a field study was initiated to determine the influence of combining solarization and application of BG33R plus four additional bacterial, nematode-antagonists through the irrigation system for management of *M. xenoplax* and prevention of PTSL tree death. Soil treatments include: i) solarized soil alone (S); ii) solarized soil + biocontrol cocktail (SB); iii) nonsolarized soil alone (NS); iv) nonsolarized soil + biocontrol cocktail (NSB); v) solarized soil + wheat (SW); vi) solarized soil + wheat + biocontrol cocktail (SBW); vii) nonsolarized soil + wheat (NSW); viii) nonsolarized soil + wheat + biocontrol cocktail (NSBW); and ix) methyl bromide (MBr) fumigated soil. Controls include, fumigated and nonsolarized nonfumigated soil. Four and 13 months (June 2005 & March 2006) after planting trees, ring nematode populations were greatest in three nonsolarized treatment plots (NS, NSBW, and NSW) than in all four solarized (SW, SB, SBW, S), or MBr fumigated plots. Twenty-one months after MBr application (November 2006), ring nematode population density did not differ among most of the treatment plots. In May 2006 and 2007, more trees in the nonsolarized treatment plots (NSW, NS, & NSB) developed typical PTSL symptoms and died than in the solarized (S, SW, SBW, & SB) or MBr fumigated plots. Soil solarization alone significantly reduced PTSL tree mortality.

Suppression of *R. similis* on Bananas using Root Endophytes and Organic Matter

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Radopholus similis remains an economic constraint to sustainable banana production for many countries. *R. similis* may be suppressed by enhancing endophytic organisms that are antagonistic to plant-parasitic nematodes. Endophytic *Fusarium* spp. have been identified as being able to protect banana roots from damage by *R. similis* and some isolates have been shown to be more effective at reducing *R. similis* damage than others. However, soil conditions in which bananas are cultivated vary, and this may impact on the efficacy and survival of root endophytes. This work reports on the isolation of effective *Fusarium* spp endophytes to reduce numbers of *R. similis* in the roots of bananas, and their effectiveness when grown in soil with different types of organic matter.

Endophytic *Fusarium oxysporum,* isolated from the roots of banana, were grown on sterile grain sorghum before being added to pots growing a banana plant. Banana plants were then inoculated with 1000 *R. similis* three days after re-potting bananas. The most efficacious isolate (A3) was then used for further glasshouse experiments.

Field soil was treated with carbon in the form of sucrose, cellulose, lignin or an equal mixture of all three at a rate of 45g of carbon to 1500g of soil and compared to untreated soil. The A3 *F. oxysporum* isolate was added to half of the pots. Plants were inoculated seven days later with 500 motile *R. similis* per pot and allowed to grow for 12 weeks before being harvested.

Of the *F. oxysporum* isolates tested, the isolate A3 was found to significantly reduce the number of *R. similis* in the roots of bananas, relative to untreated soil and when sterile sorghum alone was mixed with the soil. However, when the isolate A3 was added to soil with different forms of carbon it was found to be ineffective at reducing numbers of *R. similis*. Nevertheless, the addition of sucrose was able to significantly reduce *R. similis* numbers relative to untreated soil. Further work is required to develop an understanding of soil ecological conditions that favour the promotion of nematode antagonists.

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Biocontrol of Mushroom Sciarid Fly using Entomopathogenic Nematode, *Steinernema Carpocapsae* on Different Mushrooms

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The experiment for the biocontrol of sciarid fly on mushroom (Milky mushroom & Shiitake mushroom through *Steinernema carpocapsae* was conducted. Substrate of milky mushroom (*Calocybe indica*) and shiitake mushroom (*Lentinula edodes*) were prepared. To know the effect of different inoculum levels of *Steinernema carpocapsae*, 5,000, 10,000, 15,000 and 20,000 IJs / bag were inoculated. It was observed that mushroom yield in treated bags were significantly higher as compared to control in both the species of mushroom. However, the maximum yield of milky mushroom was obtained @ 15,000 IJs / bag followed by 10,000 IJs / bag. The cost benefit ration was 1:2.68 and 1:2.57 respectively. Similarly maximum yield of shiitake mushroom was obtained @ 10,000 IJs / bag followed by 5,000 IJs / bag with the cost benefit ratio of 1:2.29 and 1:2.17 respectively. The yield of mushroom further increased with an increase in the inoculum level of *Steinernema carpocapsae* up to certain level. It was therefore, concluded that the effective doses of *Steinernema carpocapsae* for the biocontrol of sciarid fly on milky and shiitake mushroom were 15,000 and 10,000 IJs / bag respectively. These doses were also found to increase the yield of mushroom and to reduce the population of sciarid fly significantly.

Effect of the Emergence and Infectivity of Entomopathogenic Nematodes under Different Host Desiccation

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Entomopathogenic nematodes have already been proved to be an effective biocontrol agent for the management of key insect pests. However, their survival capacity, storage life and transportation has been the major constraints in popularizing its to use among the growers. Successful control and long term management of insect pest through the application of dauer juveniles of *Steinernema* spp. can only be achieved when the nematodes possess good survival capacity. In view of this an experiment was conducted to improve the storage capability of EPN's under desiccation and tried to understand how the entomopathogenic nematodes (EPNs) respond to desiccation stress. This study has also explored the effects of anhydrobiosis on longevity and infectivity of infective juveniles (IJs) of entomopathogenic nematodes. We have examined the influence of desiccation conditions on entomopathogenic nematodes (EPNs) on several insect pests. Desiccation studies were conducted using the host *Galleria mellonella* (Lepidoptera : Pyralidae, wax moth) and compared three indigenous strains of *Steinernema* spp. . viz. STSLU, STUDR-1 and STUDR-2 in two desiccated form i.e. desiccated infected cadaver and desiccated infected pupae at different time intervals ranging between 1-10 month at 20º C. Maximum survival of IJs of *Steinernema* sp. STSLU was observed 18 percent after 10 month in desiccated infected pupae followed by 8 percent of *Steinernema* sp. STUDR-1 after 6th month and 13 percent of *Steinernema* sp. STUDR-2 after 5 months. However, maximum survival in desiccated infected cadaver was recorded 12 percent of *Steinernema* sp. STSLU after 4 month followed by 9 percent of *Steinernema* sp. STUDR-1 after 3 month and 6 percent of *Steinernema* sp. STUDR-2 after 3 month, respectively.

Fermentation and Application of Nematicidal Metablelites from Filamentous Fungus *Syncephalastrum racemosum* Sr18

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The metabolites from filamentous fungus, *Syncephalastrum racemosum* (Sr 18) showed very strong and wide nematicidal spectrum against some kinds of important plant-parasitic nematodes. Its characters of solubility in water and high thermal stability give it great potential for practical application in nematode control. The submerged fermentation on the scale of 30L fermenter was carried out according to the optimal culture condition in the shake flask and 5L fermenter, under the following conditions: the seeds were cultured on the stationary condition for 24h and added to 30L fermenter which contained 20L culture medium with 4% inoculation, operating at 300r/min~400r/min and 0.35~1VVM in order to control the dissolved oxygen(DO) among 20%~30%.The nematicidal mortality of one time diluted culture filtrate to the pine wood nematode(*Bursaphelenchus xylophilus*)reached above 95% after 44h~48h fermentation. The toxicity evaluation confirmed that the bio-nematicide

produced by Sr18 has low toxicity and harmless to animals eyes and skin. The bio-nematicide produced by fungus Sr.18 were used for the control of tomato and cucumber root-knot nematode (*Meloidogyne incognita*) in greenhouse and got good control effects. The result of control cucumber root-knot nematode showed that it could reduce the number of second stage juvennil(J_2) by94.68% ,and the control effect investigated at the harvest season was up to 80.27%, which is significantly higher than that of chemical and biological control nematicides. Moreover, It also could promote the growth and yield of the plant. The average height and the weight of each cucumber plant which was treated with the Sr.18 bio-nematicide were higher than that of control groups, increasing by16.32% and 20.43% respectively. The cucumber yield also increased by 28.28% which was significant higher than contrast groups with chemical and biological nematicide.

Influence of Fungicides on a Nematode-Suppressive Soil

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We identified a field in Georgia, USA that was moderately suppressive to *Meloidogyne* spp. In the greenhouse, reproduction of both *M. incognita* on cotton and *M. arenaria* on peanut was greater in microwave-heated soil than in natural soil from this field suggesting that nematode suppression was caused by a heat-sensitive organism. Because fungi antagonistic to nematodes are common in soils and are frequently associated with suppressive soils, we hypothesized that fungicides would reduce the activity of these fungi and allow greater nematode reproduction. To test this hypothesis, we collected soil from the suppressive field and placed it in 6 liter pots. Peanut was planted and 2 weeks later inoculated with 3,000 eggs of *M. arenaria*. Starting 5 weeks after planting (WAP), the peanuts were sprayed with one of four fungicide treatments: 1) five applications of chlorothalonil, 2) four applications of tebuconazole, and 3) two applications each of flutolanil, and 4) azoxystrobin. These fungicides are commonly sprayed on peanut at the rates and frequencies used. There were eight replications of each treatment and control (no fungicide). The number of eggs/g root was determined 15 WAP. In both trials of the experiment, azoxystrobin was the only fungicide that led to an increase (P = 0.0004) in nematode densities relative to the control; nematode densities in the other fungicide treatments were not different from the control. The number of eggs/g root was 20,391 in control pots and 44,315 in azoxystrobin-treated pots, a two-fold increase in nematode reproduction. Further research is underway to determine whether azoxystrobin has a similar effect on nematode densities in heat-treated soil.

Potential of Dual Purpose Intercrops for the Management of Plant-parasitic Nematodes and Beneficial Mycorrhizal Fungi in Banana-based Cropping Systems

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When damage thresholds are exceeded, *Radopholus similis* and *Meloidogyne* spp. can cause yield losses up to 70 % of bananas and plantains in poor soil conditions. Whereas chemical nematicide use is non-existent in subsistence cropping systems (87 % of the world banana production), it is currently being restricted in commercial production systems due to serious environmental and health concerns. Therefore, further research on alternative sustainable nematode strategies is becoming increasingly important. Though intercropping is common practice in subsistence banana-based cropping systems, knowledge about its effects on nematode field populations is rather scarce. Even less is known about the effects of the used intercrops on other (beneficial) soil organisms, such as arbuscular mycorrhizal fungi (AMF). AMF are known to promote plant growth and provide a bio-protective effect against plant parasitic nematodes in banana. To study the potential of banana intercrops, 7 leguminous (pigeon pea, sunn hemp, Grant's rattlebox, soybean, hairy indigo, common bean and cowpea) and 7 non-leguminous crops (wormseed, cotton, sorghum, sesame, orgho-Sudangrass, sweet potato cv. Inzovu and cv. Tapato) were screened individually for their nematode susceptibility and AMF compatibility in greenhouse conditions with inclusion of a susceptible and compatible banana cultivar (*Musa* AAA Grande Naine) as a reference crop. After identifying the promising intercrops (nematode resistance and/or AMF compatibility), 3 leguminous and 3 non-leguminous intercrops with different levels of nematode susceptibility were studied in mixed greenhouse set-ups to study their effect on nematode population build-up and AMF inoculum potential. As such, promising intercrops for reduction of nematode population and enhancement of AMF field inoculum potential were identified.

Pre-infectional Effect of AMF-induced Bioprotection against Plant-parasitic Nematodes

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Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts of more than 80% of all vascular plants. Not only do they enhance the growth of their host plant, it has also been shown that AMF are able to protect many plants against attack from a wide range of soil pathogens and pests, including several plant-parasitic nematode species. However little is known about the modes of action of this AMF-induced bioprotective effect against nematodes. Therefore the underlying mechanisms of the observed biocontrol are studied by investigating the AMF-nematode-plant interaction during the different stages of nematode infection. This study focuses on the pre-infectional attraction phase of the nematodes *Meloidogyne incognita* and *Pratylenchus coffeae* toward tomato roots (*Lycopersicon esculentum* cv. Marmande). Greenhouse experiments confirmed that plants of this cultivar, when pre-mycorrhized by the AMF *Glomus mosseae*, are infected significantly less by both nematode types. A nematode penetration experiment was conducted for which root exudates

from mycorrhized and non-mycorrhized plants were collected and applied to both mycorrhized and non-mycorrhized plants in the greenhouse. This was repeated until twelve days after nematode inoculation, when the plants were harvested for nematode counting. Further, the flavonoid content of the collected mycorrhized and non-mycorrhized root exudates was determined by HPLC analysis. The effect of the differential compounds on nematode behaviour, i.e. chemotaxis, egg hatch and viability, was then studied by using *in vitro* bioassays.

Studies on the Nematicidal Activity of Plant Extracts and their Control of Plant Disease Caused by Nematodes

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Ethanol extracts of 46 species of plants (belong to 33 families) from China were screened for nematicidal activity (NA) against *Bursaphelenchus xylophilus* and *Meloidogyne incognita* by the fungal-feeding and dipping bioassays method. *Croton tiglium* L., *Ruta.graveolens* L, *Cerbera manghas* L., *Axillary Choerospondias* Fruit, *Sapium sebiferum* (Linn.)Roxb, *Tripterygium Wilfordii* Hookf., *Dysosmavers ipelis*(Hance)M.Cheng , *Lantana camara* L. and *Artocarpus heterophyllus* Lam showed very strong NA against *Bursaphelenchus xylophilus* (80%-90%); while *Lagerstroemia speciosa*(L.)Pers, *TripterygiumWilfordii* Hookf. *Ophiopogon japonicas* (Linn.f.)Ker-Gawl., *Colocasia gigantean*, *Eucalyptus citriodora*, *Croton tiglium* L., *Sapium sebiferum* (Linn.)Roxb., *Aconitum hemsleyanum, Dysosmavers ipelis*(Hance)M.Cheng, and *Schima superba* showed strong NA against second stage juvenile of *Meloidogyne incognita* (85%-100%).Pot experiments showed that *Dysosmavers ipelis*(Hance)M.Cheng, *Celastrus angulatus*, *Eucalyptus citriodora*, *Sapium sebiferum*(Linn.)Roxb, *Croton tiglium* L, *Lantana camara* and *Cerbera manghas* L. could effectively control the root-knot disease by powder or root irrigation methods. Soil add the powder of this plants or water with the extracts of this plants could significantly reduce the nematode infection, the number of galls in root and the number of nematode in galls; and inhibit the egg hatching.

Parasites, Vibrations and the Hunt for Hosts

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Steinernema carpocapsae is thought to be an unsound choice for control of sedentary and subterranean hosts as its response to chemical stimuli is so poor. However, in repeated field trials for control of the large pine weevil (*Hylobius abietis*), the most serious pest in plantation forest establishment in boreal regions, this nematode has proven to be at least as efficacious as other commercially available nematodes that show significantly better chemical response. The target immature stages of the weevil develop in the cryptic habitat of conifer roots and stumps that grow in peat, a material often used as a biofiltration medium due to it's excellent adsorption/absorption properties for volatiles. The volatile binding efficiency of the peat may act to reduce insect borne volatile gradients and in such an environment an alternative host cue might be important. We demonstrate that entomopathogenic nematodes are attracted to vibrations through the substrate, a response to a host cue never before documented in an insect parasite, and subsequently postulate that in forest soils vibrational cues may be more important for host detection than those of a chemical nature.

Phoretic Dispersal of Entomopathogenic Nematodes by Large Pine Weevil Adults

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This study investigated whether entomopathogenic nematodes are capable of using the large pine weevil, *Hylobius abietis* as a vector for phoretic dispersal. The bioassays tested whether *H. abietis* promoted dispersal of *Steinernema carpocapsae* and *Heterorhabditis megidis* between two connected terraria filled with sand and whether transported nematodes were able to infect a host, *G. mellonella*. The two terraria were connected by an 18 cm polystyrene tube, where 30,000 nematodes and 5 *H. abietis* were placed into Site A and 5 *G. mellonella* were placed into Site B (Treatment A). After 7 days, 100% of *G. mellonella* were found to be infected with *S. carpocapsae* and 78% for *H. megidis* in the presence of *H. abietis*. In the absence of *H. abietis* no *G.mellonella* were found to be infected for both species (Treatment B). Thus, we show *H. abietis* could be a source of facultative phoretic dispersal for nematodes, and they must be capable of leaving *H. abietis* and infecting new hosts.

Exploring Environmental Impacts of Use of the Nematode-trapping Fungus *Duddingtonia flagrans* for Sustainable Management of Gastro-intestinal Nematode Parasites of Livestock

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Breakdown of control of gastro-intestinal nematode parasites of livestock due to resistance to various classes of anthelmintic drench requires alternative strategies. The nematode-trapping fungus *Duddingtonia flagrans* (= *Arthrobotrys flagrans;* Fungi, Hyphomycetes) has been widely investigated for its ability to contribute to sustainable parasite management. Experimental studies have shown parasite control, and financial benefit on a farm scale, following regular oral administration of *D. flagrans* chlamydospores to livestock. These spores are eventually deposited on soil within dung where their prey includes bacterial-feeding stages of gastro-intestinal nematodes such as trichostrongylids. Beneficial soil-inhabiting nematodes are also potentially exposed as prey following the prolonged inundative release of chlamydospores and this poster reviews a series of studies which have revealed no detectable impact of *D. flagrans* on soil nematodes. Clades of nematode-trapping fungi have existed since at least the Cretaceous and typically exploit small, early stage juveniles. The relatively constant size of hatchlings partly reflects the relative uniformity of embryonic material in all nematode eggs, however, it means that they are potential prey for nematode-trapping fungi. As soil-inhabiting nematodes contribute to nutrient cycling and other ecosystems services, there was concern that use of *D. flagrans* may compromise these activities. In field trials in Australia, Denmark, Sweden, Wales and New Zealand, under a range of soil and climatic conditions, *D. flagrans* failed to establish in mineral soil and no detectable effect of *D. flagrans* on soil nematode populations has been found. Although it is widely distributed in pockets of decaying organic matter such as compost and dung heaps, evidence to date indicates no threat to non-target organisms arising from repeated inundative release of *D. flagrans* for management of gastro-intestinal nematodes.

Evaluation of Six Iranian Strains of *Pseudomonas fluoresens*, Bacterial Antagonist to *Meloidogyne javanica*

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The effects of six Iranian strains of *P*seudomonas *f*luoresens (P10, P4, P60, P96, P76, P75) on the mortality of juveniles of the root- Knot nematode *Meloidogyne javanica* and production of metabolites was studied *in vitro*. After 48 hr exposure, cell suspension containing 10^9 colony forming units per ml of *P. fluorescens*, obtained from nutrient agar medium, caused mortality of *M. javanica* juveniles. Bacterial strains did differ significantly in their effect on mortality of the root knot nematode. Strains P4, P10, P75 and P76 caused significantly ($P \leq 0.1$) more nematode juvenile mortality than other strains and the control. Most of the strains produced hydrogen cyanide and protease which are probably involved in nematode mortality.

TOPIC EIGHTEEN – CHEMICAL AND INTEGRATED MANAGEMENT

Cross-degradation of Novel Non-fumigant Nematicides by Soil Biotic Factors

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Inconsistent efficacy of non-fumigant nematicides is a world-wide problem, especially in tropical agriculture with intensive cultivation, high temperatures and frequent use of pesticides. Due to the large scale application of nematicides soil-inhabiting microorganisms have in several cases become capable of rapidly degrade the active ingredients. In soil with no nematicide-application history no nematicide metabolization is observed. The objectives of our study are to further investigate the role of microorganisms in the rapid degradation of non-fumigant nematicides and to determine the biodegradability of novel nematicides in known nematicide-biodegradable soils. Metabolization studies using high-performance liquid chromatography (HPLC) are conducted in this investigation. Results of cross-biodegradation are discussed. Cross-biodegradation may easily occur since some new nematicides belong to the same chemical family as the previously used biodegradable ones. The nematicide degrading microorganisms and the mechanism(s) of metabolization are characterized. The impact and implications of 'cross-degradation' of nematicides by soil microorganisms are discussed.

Observations on the Nematicidal Activity of 1,3,7,-trimethylxanthine (caffeine)

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A nematicidal activity was discovered for caffeine (1,3,7-trimethylxanthine) through *in-vitro* and pot tests. In a first assay, *Meloidogyne incognita* eggs were placed in watch glasses with caffeine solutions ranging from 300 to $1.27 \cdot 10^4$ ppm. Hatching was measured after two weeks at 25°C. A juvenile (J2) mortality assay was carried out in watch glasses with caffeine solutions from $0.075 \cdot 10^4$ to $1.0 \cdot 10^4$ ppm. Mortality was measured after two weeks at 25°C, adding 50 µl of lactic acid and counting the J2 reacting to the pH change. Three replications were used for both tests, with distilled water as control. Data showed a progressive dose-dependent hatching reduction and a significant J2 mortality increase. No hatching was observed at 7500 ppm or higher concentrations (DL50 = 2500 ppm). Highest J2 mortality (100%) was at $1.27 \cdot 10^4$ ppm (DL50 = 1400 ppm). In pot tests, caffeine was applied to UC82 tomato plants kept at 25±2 °C, transplanted to soil containing 533 J2 · litRE^{-1} of *M. hapla*. Treatments (six doses) consisted in 20 ml additions of caffeine solutions from 1500 to 10^4 ppm, in five replicates, with water as control. At 1500-3000 ppm plant growth was higher than control, with a marginal effect decrease at 5000 ppm and higher levels. Height increase was 70% at 1500 and 3000 ppm, and 40% at 5000 ppm. No difference was found between control and highest dose. Similar trends were observed for root and leaves weights, highest at 1500 and 3000 ppm, at which less galls and eggs were found. At more than 5000 ppm, galls

and eggs increased and root weight decreased, due to phytotoxicity. However, at 10^4 ppm, the total number of eggs per plant was 35.7% lower than control. Caffeine was known for its activity against snails, but not for phytoparasitic nematodes.

Resistance of Pasture Grasses and Legumes to *Radopholus similis*

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There is increasing pressure on banana farmers to maintain ground cover around plants to prevent the movement of soil, nutrients and pesticides from the farm. However, in banana plantations there is potential for ground cover plants to host *Radopholus similis*. Therefore, plants chosen as ground covers need to be agronomically suitable, resistant to *R. similis* and hinder the migration of *R. similis* to the roots of bananas. Screening of potential shade tolerant grasses and legumes for resistance to *R. similis* and companion planting with bananas was conducted in glasshouse experiments.

Selected pasture species were established in pots and inoculated with 500 motile *R. similis*. Ten weeks later, nematodes were extracted from the roots. Pasture species, identified as resistant, were selected for an assay to determine their suitability to prevent *R. similis* migrating to the roots of bananas. Mung beans (*Vigna radiata*) infected with *R. similis* were grown in a pot on the outside of the selected pasture species and an *in vitro* banana plant grown in the centre of the pot.

All grass species tested were found to be more resistant to *R. similis* than bananas with Argentine Bahia (*Paspalum notatum*) more resistant than signal grass (*Brachiaria humidicola*), Rhodes grass (*Chloris gayana* cv. Katambora) and Sabi grass (*Urochloa mosambicensis*). Of the pasture legumes, Butterfly pea (*Clitoria ternatea*) and Pinto peanut (*Arachis pintoi*) were more resistant to *R. similis* than banana.

Butterfly pea, grown alone or in combination with carpet grass (*Axonopus affinis*) or Bahia grass, resulted in lower nematode populations in the roots of bananas. However, the banana root weight was significantly reduced. Pinto peanuts and Bahia grass, grown separately, reduced the number of nematodes recovered from the roots but did not reduce the weight of the banana roots making them better companion plants as ground covers in banana plantations.

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Evaluation of Pre-plant Treatments of Yam Setts for Production of Nematode Free Planting Material

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Healthy seed yam (*Dioscorea* spp.) availability in Nigeria and West Africa is poor with high proportions of available seed yam infected with nematodes, which perpetuate from season to season through use of infested seed. Cut setts (~100 g) of four local cultivars of *D. rotundata* were treated with a fungicide and insecticide mix, neem leaf slurry and hot water treatment (20 min @ 53°C) (HWT). Untreated and wood ash coated setts served as control and farmers' normal treatment respectively. Harvested seed yams were assessed for damage and losses during storage, with surviving tubers used for ware yam production during the following season. Pesticide treated setts sprouted faster and in higher proportions than other treatments. Application of the pesticide mixture prior to planting reduced nematode damage to yam at harvest and during storage, compared with other treatments. Pesticide and HWT setts yielded healthier seed yam, which had lower disease levels and reduced losses during four months of post-harvest storage. Ware yam grown from surviving seed yams (two cultivars only) had relatively higher yield per plant and per plot (26.1 kg) when derived from the pesticide treated setts, than wood ash (22.4 kg), HWT (19.6 kg), neem (19.1 kg), or untreated (18.3 kg). Taking two seasons of production into consideration however, including seed storage losses, the potential total increase in yield for pesticide treated setts was 214% for pesticide pre-treated tubers of cv Imola and 700% greater production for cv Ajimokun, compared with the untreated control. A simple, easily applied pesticide treatment of setts for seed yam production can therefore result in higher quality and quantity of seed material, that subsequently provides massive differences to ware yam production.

A Susceptible Weed Host Can Compromise Suppression of *Meloidogyne incognita* by Resistant Cotton

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Weeds can support nematode reproduction when a non-host or resistant host crop is grown. *Meloidogyne incognita*, the dominant nematode pathogen of cotton in many areas in the US, reproduces well on prickly sida (*Sida spinosa*), which is a significant weed in some cotton-producing areas. The development and use of *Meloidogyne*-resistant cotton in the future seems likely, so microplot experiments were conducted in 2006 and 2007 to study the extent to which infestation of cotton fields with prickly sida would affect the nematode-suppressive effects of growing *Meloidogyne*-resistant cotton. A single row (76 cm) of seven cotton plants (cv. M-120 RNR) was grown per microplot. A factorial arrangement of treatments with five replications was used: prickly sida seedlings were transplanted at 0, 1, 2, or 8 plants per microplot, and *M. incognita* eggs were either added (2,000 eggs to each cotton and prickly sida plant) or not added. Root-galling (0 to 10 scale) on prickly sida following cotton harvest in 2007 averaged 1.7 in non-inoculated plots and 5.5 in inoculated plots. Years were pooled for a combined analysis of variance, and nematode counts (juveniles/150 cm^3 soil) at cotton harvest increased with prickly sida density: mean counts in inoculated plots were 122, 299, 699, and 974 for 0, 1, 2, and 8 prickly sida, respectively. In non-inoculated plots, counts did

not differ ($P \leq 0.10$) but generally increased numerically with prickly sida density: mean counts ranged from 1 for 0 prickly sida to 147 for 8 prickly sida. Prickly sida, and any other equally susceptible weed, will greatly reduce the nematode-suppressive effect of growing nematode-resistant cotton, so weed control must be a component of nematode management.

Potential of Methomyl Soil Applications for Early Control of Root-knot Nematode in Vegetable Legumes

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Methomyl (Lannate® LV, a.i. 29 % methomyl) is a widely-used carbamate insecticide, but not much is known about its potential to control nematodes when applied as a soil drench. The nematicidal activity of methomyl and the potential of soil applications to provide short-term control of root-knot nematode (*Meloidogyne incognita*) in pea and bean were evaluated in a series of lab and greenhouse tests. Methomyl showed rapid knock-down of *M. incognita* in aqueous assays with effective concentrations, EC 50 and EC 90 values, averaging 4.9 and 15.2 mg/l (as compared to 4.0 and 11.5 mg/l respectively for oxamyl). In the greenhouse, soil applications of methomyl ranging from 0.56 kg a.i./ha to 4.0 kg a.i./ha provided significant root-knot nematode control during early growth of pea and bean, up to 25 days after planting. Higher application rates and split applications improved nematode control, but also increased the risk of phytotoxicity. Methomyl soil applications showed good potential for early control of root-knot nematode on pea and bean. More tests are required to verify activity under field conditions.

Efficacy of Bionematicides Derived from Different Plant Organs on a Nematode Population under Microplot and Field conditions

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Cissus cactiformis, Cassia abbreviata, Senna pertesiana, Ipomoea kituiensis, Maerua angolensis, Stylochiton natalensis, Tabernaemontana elongens and *Cucumis myriocarpus* are indigenous plants with known toxic properties. They are widely distributed in the Mopani and Vembe istricts of Limpopo Province. Powdered products of these toxic plant organs were tested for their effectiveness in controlling *Meloidogyne incognita* race 2 on tomato microplot and field experiments. A randomized complete trial layout was used with nine treatments and an untreated control, replicated 10 times. Each plant was inoculated with ca. 3000 *M. incognita* race-2 eggs and juveniles, followed three days later by planting of the tomato seedling (*L. esculentum* L. var. Floradate) and the same day the treatment was applied around the base of the plant. Following earlier trials with dosage rates that showed phytotoxicity effects, dosage rates per pot in this experiment were reduced to 4 g for *I. katuiensis,* 3 *g* for *C. cactiformis* and *S. pertesiana* and 2 g for *C. abbreviata.* Results in the microplot and field experiments showed that four plant species could be identified as being an effective bionematicide namely *C. cactiformis, C. abbreviata* and *T. elongens.* These treatments reduced nematode juvenile and egg numbers/g roots significantly and stimulated plant growth. *C. cactiformis* reduced nematode juveniles by 98-100% and eggs 90-98%

compared to the untreated control. For *C. abbreviata* and *T. elongens* these figures were 83-96% and 94-97%; and 96-99% and 95-99%. This compared well with the Nemacur (femaniphos) treatment. In addition to the observed nematicidal effects of these indigenous plant products, the variable effects on eggs and juveniles warrant further in-depth investigation.

Use of Remote Sensing for Estimating In-field Reniform Nematode Numbers in Mississippi, Alabama and Louisiana

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The reniform nematode has increased from a relative unknown nematode pest to a major constraint to cotton production in the southeast United States. This nematode is the most economically important nematode in Alabama, Louisiana, and Mississippi. In these three states the reniform population numbers are higher on average compared with the other southeast cotton growing states. Over 328,073 bales of cotton are estimated to be lost due to the reniform nematode valued at over $128 million dollars in these three states alone. On a per farm bases it has been estimated that this nematode costs our producers 100 to 500 lb of lint per acre or $55 to $275 per acre each year. The primary means of management includes rotations with crops that are a non-host and nematicides. The applications of nematicides have proven effective at lowering reniform numbers and increasing yields but can be expensive and offer short term protection. To implement a successful reniform management program, the cotton producers must first identify that the nematode is present and determine the numbers present in each location. This requires collecting numerous nematode samples from the soil and submitting them to a laboratory for identification and enumeration which is time-consuming and costly. Currently nematicides are applied as a single rate across the field without regards to the spatial in-field. The spatial distribution of the reniform nematode is in a scattered pattern across a field and areas exist where there are no nematodes present. Other areas of the field may seem uniformally infested with the nematode but the population or numbers will vary. This is ideal for site-specific applications of nematicides using variable rate technology. The application of the chemical only where needed and at rates necessary to manage the nematode is more efficient and environmentally safe.

The collection of a representative number of soil samples is a limiting factor for site-specific placement of nematicides. In Mississippi, remotely sensed hyperspectral imagery has been correlated with reniform nematode population levels to obtain an accurate estimation of the infield nematode distribution without taking a soil sample. This will provide the producer with a more accurate nematode distribution across the field.

In 2008, to test remote sensing on a larger scale, cotton production fields naturally infested with the reniform nematode were selected in Alabama, Louisiana and Mississippi. Hyperspectral reflecrance data was collected and accuracies in estimating nematode numbers with actual counts. Relationships between estimated counts are determine to examine the robustness of this method to maximize cotton yields.

Relationships between Soil Electrical Conductivity and NDVI for Site-specific *Rotylenchulus reniformis* Nematode Evaluations and Yield Potential in Cotton

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The test was conduced on a 20 acre producer cotton field naturally infested with the reniform nematode in north Alabama. Before planting, soil electrical conductivity data (EC) was collected utilizing the Veris 3100 mapping system measuring both shallow (0-1foot) and deep (0-3feet) electrical potential. The field was placed on 0.25 acre grids (160 targets per field) at 30 days after planting (DAP) using the GIS Site Mate system. Corresponding nematode soils samples were collected at each grid intersect 30 and 45 DAP. Real-time NDVI was determined using the Greenseeker technology. Yield estimates were collected with a yield monitor system and placed into four low to high yielding categories. EC shallow and deep, NDVI, nematode numbers, and cotton root mass were then analyzed with the SAS® CANDISC procedure and the resulting phenotypic correlations (r) were used to determine the relationship of these response variables with yield classes. There was a significant separation of the highest yielding areas from the lower three yield classes along the 1st canonical axis, which accounted for 50% of the multivariance. High yielding areas were distinguished from the low yielding regions by cotton root mass (r = -0.80), *R. reniformis* per gram of root (r = 0.93), and NDVI at 45 DAP (r = -0.93). The 2nd canonical variate discriminated among the three lower yielding areas. These differences were best described by EC values and the *R. reniformis* extracted from the soil. Regression analysis indicated a significant (*P* = 0.06) relationship of reniform counts with NDVI at 45 DAP, although the coefficient of determination was very small.

Agri-Terra: A New Low-rate Nematicide

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2007 was the seventh consecutive year of trials evaluating the efficacy of Agri-Terra against nematode species associated with major crops in Louisiana. Trials with soybean, cotton, rice and carrot were conducted in microplots. Concentrations of 0.5 and 1% and rates of 0, 5, 10, 20, 40 and 80 gallons per acre were applied to microplot soil infested with one or a combination of five nematodes. The 10GPA/1% concentration was the most optimal treatment for soybean, resulting in harvest plant weights, pod numbers and pod weights that were significantly greater than those of controls. This concentration/rate combination of Agri-Terra was also the best treatment for cotton, producing statistically significant increases in plant growth and boll production while providing nematode control. The 0.5% concentration of Agri-Terra produced slight to moderate increases in the overall growth of rice and the 1% concentration produced moderate growth inhibition. All Agri-Terra treatments of soil in microplots of rice resulted in marginal control of ring, spiral and stubby-root nematodes. Agri-Terra treatments also resulted in significant reductions in populations of root-knot nematode on carrot. Residual nematode populations, however, caused marked root galling and plant damage at harvest.

Commercial vegetable production protocols were employed in field trials with tomato, cucumber and bell pepper. With tomato, Agri-Terra successfully managed reniform nematode populations and resulted in significant increases in yields of tomato fruit in the Extra-Large and Large size categories. With cucumber, treatment of soil with Agri-Terra reduced reniform nematode populations significantly and increased yields of fruit in the Super-Select and Select size categories. Results of field trials with cotton conducted in 5 consecutive years showed that the application of Agri-Terra to soil as an at-planting, in-furrow (fine mist) spray treatment produced highly significant decreases in nematode populations and highly significant increases in yield.

MCW-2: A 'True' Nematicide Belonging to the Fluoroalkenyle Group

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Nematicidal efficacy of MCW-2 (Makhteshim Chemical Works, Beer-Sheva, Israel), which belongs to the fluoroalkenyle group, was studied in laboratory, growth chamber and in fields. MCW-2 showed an irreversible nematicidal activity after exposure of *Meloidogyne javanica* second-stage juveniles to 0.5 μg/ml solution for 48 hr and rinse in water for another 24 hr, in contrast with fenamiphos or cadusafos, which had only reversible nematostatic effect at the same or higher concentrations (~ 8 μg/ml). An EC formulation of MCW-2 inhibited the nematode hatching at a high concentration (8 μg/ml), but the hatching recovered after rinse in water. In pot experiments, the compound at a concentration as low as 0.25 mg/l soil showed the same or better control efficacy against *M. javanica* than those of fenamiphos or cadusafos at the same concentration. The duration of the nematicidal activity of the EC formulation lasted at least two weeks after application into an alkaline sandy soil; however, fenamiphos showed a tendency of longer nematicidal activity in the soil than that of MCW-2. In microplot experiments, soil drench with an EC formulation at 2.0 kg a.i./ha had the same control level of fenamiphos at 4.0 kg/ha or cadusafos at 3.0 kg/ha, based on galling index of tomato roots caused by *M. javanica*. No phytotoxic symptoms were observed on tomato plants at the concentration. The compound has a far lower toxicity to rats (acute oral LD_{50}: >500 mg/kg) and non-target organisms (non-toxic to bees and earthworms) comparing with organophosphate or carbamate nematicides. MCW-2 has also a low leaching potential in the soil. The results indicate that MCW-2 has a great potential as a nematicide, which belongs to a new chemical group, and probably has a novel mode of action. Results from field experiments will be also presented.

Crop Rotation for the Management of Root-lesion Nematodes in the Northern Grain Region of Australia

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Root-lesion nematodes, *Pratylenchus thornei* and *P. neglectus* are widely distributed in the northern grain region of Australia which encompasses inland northern NSW and south-east Queensland. This sub-tropical region has a summer dominant rainfall and fertile clay soils. A broad range of both summer and winter crop species are grown and it is notable for its production of Australian Prime Hard wheat (high protein milling wheat) and grain sorghum for stock feed.

P. thornei is the most commonly found root-lesion nematode in this region and poses a significant economic threat to susceptible crops, such as wheat and chickpea. For example, intolerant wheat varieties can suffer yield losses as high as 70%. Marked differences are observed in the virulence of *P. thornei* and *P. neglectus* not only for crop species but also between crop varieties. For example, wheat cv. EGA Burke has a moderate level of resistance to *P. thornei* but is susceptible to *P. neglectus*; grain sorghum is susceptible to *P. neglectus* but resistant to *P. thornei*; mungbeans are susceptible to *P. thornei*, but resistant to *P. neglectus.* We have identified the resistance/ susceptibility of common crops grown in this region to *P. thornei* and *P. neglectus* in both glasshouse and field experiments. This information is complemented by work on tolerance/ intolerance of susceptible crop species of wheat and chickpea.

So, the management of root-lesion nematodes depends firstly on correct identification to species level, then tailoring crop rotation plans so that resistant crops and tolerant cultivars are dominant in the farming system. When this is combined with farm hygiene to limit the spread of nematodes between paddocks and on farm machinery, the production of valuable, but susceptible crops, such as wheat, can be optimised and root-lesion nematode populations will remain at low, manageable levels.

Secondary Metabolites Present in *Canavalia ensiformes* Seeds are Toxic to Juvenile Plant Parasitic

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Plant parasitic nematodes of the genera *Meloidogyne* sp. cause remarkable losses to important world-wide crops reaching up to 120 billion dollars annually. Currently, their control is based on synthetic nematicides that, beyond expensive, cause risks to the human health and environment. Consequently, search for new and safer strategies for managing these pathogens are urgently required. Plants are source of natural products, which may possess nematicidal compounds. In this context, a screening for specific and effective molecules towards second stage juvenile (J2) of *Meloidogyne incognita* was carried out using aqueous seed extracts of *Canavalia ensiformis*, *Crotalaria juncea*, *Crotalaria paulinea, Crotalaria espectabilis*, *Tagettes minuta*, and *Mucuna pruriens.* Amongst all the tested extracts, those from *C. ensiformis* showed the highest nematicidal activity (85%). Dialysis fractionation of the crude components from *C. ensiformis* allowed us to separate molecules smaller than 3.5 kDa (External dialysis) which are very effective against J2 nematodes but innocuous to phytopatogenic fungi, saprophytic nematode, protozoan, larvae of *Spodoptera frugiperda* and *Antonomus grandis*. Mass spectrometry revealed the existence of wide variety of secondary metabolites in this fraction. Hemolysis tests showed that these components were not able to disrupt bovine red blood cells. When submitted to 50°C for 24 hours nematicidal activity reduced only 20% illustrating their thermostability. Green house bioassays carried out on tomato roots employing the dialysis fraction reduced the number of egg masses with 82.5% corroborating our results on nematicidal activity. Metabolites were further purified by HPLC resulting in a more pure and very effective fraction causing 98% mortality of J2 nematodes. Microscopic analysis revealed that this fraction induces the complete disruption of the J_2 intestine due to an abundant vacuolization of epithelial cells. Mass spectrometry and NMR analysis are currently being performed in order to elucidate the molecular structure of these compounds.

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Non-target Effects of Methyl Bromide Alternatives: Statistical Comparisons and Use of Non-target Nematodes as Indicators

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In intensive agriculture, the use of pesticides and soil fumigants is necessary to reach economically viable crops worldwide. However, this practice may involve undesirable effects on human health and the environment. The first objective of this study was to compare environmental and toxicological properties of eleven active substances with nematicide properties, some of them recognized as methyl bromide alternatives.

Four groups of active substances were discriminated by a series of Principal Component Analyses (PCA): a) High toxicity to non-target fauna, humans and animals and medium persistence in the environment (cadusafos, ethoprophos and fenamiphos), b) high toxicity to humans, animals and non-target fauna, high persistence (carbofuran, fosthiazate), c) low toxicity to non target fauna, humans and animals and low persistence (carbosulfan, benfuracarb, oxamyl), and d) low toxicity to humans, animals, and non-target fauna and medium persistence in the environment (1,3-dichloropropene, chloropicrin, methyl bromide).

To evaluate the non-target effects of plant protection products on the environment in field conditions, effects of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) are being evaluated in strawberry crops in Southern Spain. Preliminary results show that, as expected, the nematode assemblage was deeply disturbed by treatments. Effects of 1,3-D and Pic on non-target nematodes in wetlands and pine forests, probably affected by contaminated lixiviates, will be further evaluated.

In conclusion, our results show that statistical comparisons of the multiple aspects of toxicological and environmental properties of active substances help us to understand their complex toxicological behavior, but further field evaluations are required to determine the behavior and the real effects of such products on specific scenarios.

Management of Nematodes in Plantation Crops

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Phytonematodes are extremely important limiting factors in plantation crops especially pepper, cardamom, tea, coffee, coconut and arecanut. The damage due to nematodes is more severe in intensive production systems such as nurseries of coconut, tea, cardamom etc. Although a sizeable number of plant parasitic nematodes have been reported from plantation crops, only burrowing (*Radopholus similis*), root-knot (*Meloidogyne* spp.) and lesion (*Pratylenchus* spp.) nematodes can be regarded as economically important.

In pepper, *R. similis* and *Meloidogyne* are involved in 'slow wilt or slow decline' disease almost identical to 'pepper yellows' in Indonesia. Though there was an effective management measure the average yield reduction due to this disease was 10-30% in different cropping situations. Pepper being a perennial crop trailed in live standards, any management measures need to be repeated every year under Indian conditions. The high density multiple cropping system does not permit use of nematicides ,as most of the crops are export oriented and tender pepper berries and ginger rhizomes are consumed without any processing or cooking or as pickles. When coconut and arecanut are used as live standards the situation becomes further complicated because they are good hosts of *R. similis*. Several other live standards and weeds have been reported as hosts of root-knot nematode. Mulching and biofumigation ameliorate symptoms of slow wilt. Application of chopped leaves of *Glyricidia maculata*, neemcake are the ecofriendly methods in existing pepper plantations. Denematization is another good method to eliminate nematode in rooted cutting for establishing new plantations. It can be either by soil fumigants or by fortifying the soil with biological control agents like *Paecilomyces lilacinus, Pochonia chlamydosporia, Pasteuria penetrans, Bacillus macerans and Pseudomonas fluorescens* Arbuscular mycorrhizal fungi (AMF) and endophytic fungi are other options . An integrated nematode management package was developed by Kerala Agricultural University.

Root-knot nematodes are very serious in nurseries than in plantations, recorded about 50 % reduction in germination of seeds and 40% damage in seedlings. Bio intensive management measures can be recommended for nematodes in the nursery. In cardamom also nursery treatment is the most effective strategy. Nurseries are ideal for practicing biological control using the above agents . In ginger neemcake and AMF are found effective in managing nematodes

Many different nematodes have been found in diverse forms of association with living coconut palms, the major nematode diseases affecting the crop is red ring disease caused by *Bursaphelenchus cocophilus.* The only nematode known to cause severe damage in our situation is burrowing nematode, *R. similis*. It infests a number of crops in India including arecanut ginger, turmeric, banana, betel vine, medicinal and ornamental plants. Even one nematode in 35, 640 cm^3 of soil or 100 nematodes per seedling reduced the yield by 77% (Koshy and Sosamma, 1994 and 1996). Control of the nematode on a perennial palm like coconut and arecanut with a massive root system is difficult, especially under high density multispecies cropping system involving susceptible crops. Hence an integrated approach is highly warranted suitable to the agro ecosystem. Introduction of biological control agent in the nursery and at the time of transplanting in pits was also reported as effective

Control of *R. similis* in arecanut also it can relay on cultural practices, biological agents and use of resistant varieties, as arecanut is chewed directly by many consumers. For the use of non hazardous chemicals the dosage, frequency and time of application have to be studied carefully to avoid residues in the nut. Judicious selection of intercrops should also be a primary concern in this case. In betel vine green leaf mulching and application of organic amendment can only be recommended for controlling root-knot and reniform nematodes *P. brachyurus, P. coffeae, P. flakkensis* and *P. zeae* are commonly occurring nematodes in the traditional coffee areas of S. India. Management can be achieved by addition of soil ameliorants like oil cakes, coffee cherry husk or organic matter. *Robusta coffee* is fairly tolerant to nematode attack hence can be recommended for planting or use as root-stocks. Root- knot nematodes are most frequently encountered in tea plantations in India especially in nursery and young plants. Management of nematodes in the nursery, cultural practices like fallowing, intercropping with marigold can be recommended apart from soil application of nematicides.

The Effect of Organic Amendments for Nematode Control in Rural Vegetable Gardens in South Africa

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A survey conducted in rural areas in the eastern and northern parts of South Africa revealed serious problems with nematodes, especially *Meloidogyne* spp. Due to the high nematode numbers, vegetable growing has become a problem and many gardens have been abandoned. Although these people have no funds available for nematicides, other practices could help reduce the problem and allow them to produce reasonable yields.

To determine the effect of organic amendments on yield and nematode numbers, two 2-year trials were done with vegetables at Nelspruit Experimental Farm. The treatments included cattle manure, chicken manure, compost, plastic filled with sterilized medium, permaculture, an untreated control, fenamiphos, oilcakes of soy and sorghum, sorghum, *Tagetes* and plastic cover (solarization).

After two years, results showed that permaculture delivered the best results for all the vegetables, even with very high nematode infestations in the tomatoes and spinach. Although nematodes are not affected directly, the effect on yield is huge. After two years and three crops, yield was enhanced by 60 - 80% compared to the control. Permaculture is a technique where a trench is dug and filled up with garden waste including green and brown material, old papers, rotten fruit and vegetables and than covered with soil. The second best treatment was chicken manure, followed by several other amendments. It is obvious that most of the amendments had a positive effect on the crops and although the direct effect on nematodes was insignificant, the benefit in terms of growth and yield were substantial.

Development of a Management Strategy for the *Meloidogyne incognita/Cyperus rotundus/C. esculentus* Pest Complex

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Pest complexes limit profitable crop production in many of the intensively-managed irrigated fields in the arid southwestern USA. These persistent interactions plague producers who must manage limited irrigated acreage in an economically efficient manner without the use of nonselective biocides like methyl bromide. One complex that threatens the sustainability of vegetable and cotton production involves *Meloidogyne incognita* (southern root-knot nematode) and the perennial weeds *Cyperus rotundus* (purple nutsedge) and *C. esculentus* (yellow nutsedge). These pests do not disseminate readily and are well adapted to a mutually beneficial coexistence that sustains and enhances the pest complex. Both nutsedges are hosts of *M. incognita* and can propagate vegetatively via tubers in which the nematode over-winters and survives spring application of 1,3-dichloropropene, the main fumigant nematicide in the region. Vegetative nutsedge growth shows little pathogenic response to nematode infection, and tuber production is often enhanced. All annual rotation crops that would compete successfully with these nutsedges are excellent hosts for *M. incognita,* and therefore unacceptable. Research utilizing a three-year alfalfa rotation sufficiently suppressed the pest complex such that chile pepper (*Capsicum annuum*) yield without 1,3-D fumigation was twice that recovered following a three-year cotton rotation (*Gossypium hirsutum*) with fumigation. Research was conducted to determine if a two-year rotation with a perennial, nondormant *M. incognita*-resistant alfalfa (*Medicago sativa* 'Mecca II') effectively suppresses the pest complex while reducing the irrigation demands of a third year of alfalfa. Following the two-year alfalfa rotation, 32% and 47% of yellow and purple nutsedge root systems, respectively, showed galling by *M. incognita* 26 days after chile was planted, indicating inadequate suppression of the pest complex compared to that achieved with the three-year rotation. Economic return, efficient water use and effective suppression of the root-knot nematode/nutsedge pest complex must all be considered when choosing rotation schemes.

TOPIC NINETEEN – DETECTION AND SAMPLING METHODOLOGY OF PARASITIC NEMATODES

Development of a Sensitive PCR Test for Detecting the Potato Cyst Nematode (*Globodera rostochiensis*) in Large Volume Soil Samples

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Potato Cyst Nematode (PCN), *Globodera rostochiensis* & *G. pallida,* impacts potato production and affects market access. *G. rostochiensis* was detected in Western Australia in 1986. Six farms (15ha) were immediately quarantined and an eradication program implemented. There have been no new detections of PCN from state-wide surveillance since 1989. Since PCN cysts can remain dormant in soil for at least 20 years, demonstration of Area Freedom is required before locally grown potatoes can be exported to areas that impose restrictions for PCN.

Our aim is to develop a sensitive PCR test to enable presence/absence of PCN to be determined directly from soil. This will provide data to demonstrate Area Freedom from PCN for Western Australia. PCR offers an alternative to traditional microscopic detection of PCN, which is time-consuming and prone to operator error, particularly if cysts are present in low numbers. There are numerous technical challenges when amplifying DNA extracted directly from soil (e.g. incomplete cyst/egg lysis, DNA absorption to soil, co-purification of PCR inhibitors, degradation of target DNA). To reduce inputs, it is necessary to develop methods that maximise sample area per test without compromising assay integrity. PCR analysis of soil has usually been done with samples of 1 to 15g. In contrast, we are developing a novel strategy to test 20kg pooled soil samples (representing assessment of 1ha sampled on a 5 x 5m grid) for presence/absence of PCN.

Due to quarantine against the use of PCN, we are developing methodologies using Cereal Cyst Nematode, *Heterodera avenae* (CCN). The goal is to develop the technology for routine detection of 5 cysts in a 20kg soil sample. Preliminary results are encouraging, with detection of as little as 1 CCN cyst in 20kg of soil. Once optimised, detection methodologies will be validated in blind studies using PCN-infested soil in New Zealand.

Healthcare Assessment Methodology in a Developing Country

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The health care assessment system in Nepal is still taking a long time and the cost is high. Collection of data about prioritization of community health problems by the community is essential for planning and monitoring of programs and interventions for improving community health status. Scientific Rapid Community Health Assessment Methodology needs to be validated in rural communities in a developing country like Nepal which is economic and less time consuming.

To validate the Rapid Community Health Assessment Methodology (RCHA) for prioritization of community health problems in a rural community including nematodes (or Helminthes) control programs.

Study area: 14 Villages of rural areas. *Study population*: 34 Primary School Teachers from 10 randomly selected Primary Schools in above villages. (One Teacher each from Class I to V, in each School). *Validation*: Heads of households from 500 Households (50 per village, systematically sampled). *Data collection technique*: Self-Administered Questionnaires for Teachers, Interview Schedules for Heads of Households. *Data analysis*: with the help of EPI info program.

There was significant correlation between the responses of the school teachers and heads of households on community health problems viz., (a) the prioritization of ten village problems($r=+0.77$, $p<0.02$), (b) prioritization of utilization of services of various health functionaries for treatment of and advice for children's illnesses($r=+0.75$, $p<0.05$), and (c) prioritization of households using water from different sources($r=+0.975$, $p<0.02$). The method was also found to be more rapid (3.3 times) and less costly (6.3 times) compared to the traditional household survey method.

Rapid Community Health Assessment Methodology (RCHA) for prioritization of community health problems in a rural community is validated. The information thus obtained can be utilized for purposes of health policy and program planning, monitoring and evaluation. This is especially relevant for micro planning/evaluation of health services in developing countries. Repeated use of questionnaires for monitoring disease control programs must be carefully considered. Further studies to confirm and reconfirm the results of this study may be done before wider application of above methodology.

A Method to Estimate the Population Density of Viable Potato Cyst Nematodes in Soil, Using Potato Plants Grown in Closed Plastic Containers

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Potato cyst nematode (PCN), *Globodera rostochiensis*, causes severe damages to potato, and has spread every year to uninfested land in Hokkaido, the northern part of Japan. To detect and estimate the population density of viable PCN precisely and easily, a simple detecting method was developed by using potato plants grown in closed plastic containers in dark, producing root systems on which PCN can reproduce. Assessment was conducted by counting numbers of developing females/cysts visible through the transparent container walls with four replications per sample. The best results were obtained as follows. The containers are 6 cm in diameter and have a volume of 85 ml, filled with 33g of dry soil + 10 ml (at the beginning) + 8 ml (during the assay) of water, incubated at an average temperature of 18 deg C for 8 or 9 weeks. Tubers are from 10 to 20 g of susceptible cultivars, budding and fully basked in sunlight. This method was tested against a conventional method of counting eggs/juveniles in cysts recovered by flotation from air-dried soil. PCN was detected at 60.5% in the new method, while at 60.8% in the conventional method from 397 tested soil samples collected from fields in two different districts of Hokkaido. Correlation coefficients between the numbers of visible females/cysts per 85 ml container and the numbers of eggs/juveniles per g of dry soil were r=0.93, p<0.001, n=16 in A district, whereas r=0.89, p<0.001, n=87 in B district, except the highly infested samples containing more than 100 eggs per g of dry soil.

Detection of the Golden Nematode *Globodera rostochiensis* in Canada 25 Years after Quarantine

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The golden nematode, *G. rostochiensis* was first detected in 1965 on a commercial field in the Saanich peninsula, British Columbia, Canada, one of only three sites in all of Canada known to be infested with the nematode. Extensive surveying during subsequent years identified 150 infested acres and another 300 acres associated with the infested lands. Eradication efforts including regular soil fumigation were unsuccessful. In 1980, the movement of plants, soil and farm equipment was restricted, and in 1982, a ban on the production of potato, tomato and eggplant in the region was enacted, with both restrictions remaining in force today. Since that time, a single survey for golden nematode was undertaken in 1992 on a limited number of fields, only one of which was found to be positive. In 2006, we decided to undertake a survey of ten well defined sites which had either tested positive for golden nematode in the past, or were likely to be infested with the nematode, to determine whether viable golden nematode cysts could still be detected 25 years after the absence of a suitable host plant. The sites were intensively sampled and the collected soil collected in 7 gallon pots and brought into a contained green house. In order to biologically amplify any viable cysts still present in the soil before mechanical testing, potato plants were grown in each pot for 100-120 days. The plants and potatoes removed and the soil was dried and allowed to remain dormant for another 120-140 days. After three successive rounds of amplification, the soil was processed using standard (Fenwick can) methods, and analyzed for the presence of cysts.

Phytoparasitic Nematode Infestations of California's Grape, Citrus and Stone Fruit Crop

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California provides almost fifty percent of the United States' fruit and nut production, as well as exports of significant percentages to supplement productions and food demands of various countries worldwide. Production losses due to phytoparasitic nematodes can be devastating if left unregulated. For this reason, the California Department of Food and Agriculture's (CDFA) regulatory programs provide for the detection of economically important phytoparasitic nematodes associated with stone fruit, citrus and grape production, amongst other crops. Periodically, surveys for new and exotic target nematode species are also conducted. During 2005-2007, CDFA conducted surveys of California's grape, citrus and stone fruit major production sites. Over 4,500 samples were collected for seven stone fruit (almond, apricot, cherry, nectarine, plum, peach and prune), citrus (including grapefruit, lemon, mandarin, orange and tangerine), and grape, through the survey and CDFA's nematode regulatory program. Approximately, 5,081 total diagnostics included 3,091 negative detections with no plant parasitic nematodes present in samples, and 1,990 positive nematode detections. Thirty-six phytoparasitic nematode species representing 13 genera were identified collectively for all stone fruit hosts, 15 species representing 12 genera for citrus, and 27 species representing 16 genera for grape. Most species were found in all three host groups and already known to exist in California agricultural production sites. Nematode groups included, *Helicotylenchus* spp., *Hemicycliophora* spp., *Macroposthonia xenoplax, Meloidogyne incognita, M. javanica, M. arenaria, Paratrichodorus* spp., *Paratylenchus* spp., *Pratylenchus* spp. *(P. brachyurus, P. hexincisus, P. neglectus, P. penetrans, P. scribneri, P. thornei, P. vulnus, P. zeae), Scutellonema conicephalum, S. clathricaudatum, S. brachyurus, Tylenchorhynchus* spp., *Xiphinema americanum sensu lato* and *X. index*. *Hemicycliophora arenaria*, *Longidorus africanus* and *Tylenchulus semipenetrans* were detected only in citrus. No quarantine (new or exotic) phytoparasitic nematode species were detected.

Quantitative Recovery of Anhydrobiotic Nematodes from Dry Soils in Mediterranean environments

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Plant parasitic nematodes were recovered form dry soil in several surveys from Mediterranean cereal and grassland agrosystems after the summer fallow season. A high percentage of nematodes in soil (73-94%) were observed in a coiled anhydrobiotic state, which could lead to underestimation of the real nematode population in soil. To estimate real population densities and viability of these nematodes they were exposed to a hydratation process and recovery in Withehead-Baermann trays. Time of reactivation and ability to migrate varied with the species of nematode. A series of ecological experiments showed that presence of water or moist air, time of moistening and oxygen availability were the factors that trigger reactivation from the anhydrobiotic resting stages. Different survival strategies were defined according the biological parasitic behavior of nematodes. *Ditylenchus dipsaci* became rehydrated and active before than any other, followed by ectoparasites (*Merlinius* *brevidens*, *Amplimerlinius magnistylus*, *Paratrophurus loofi*) and then migratory endoparasites (*Pratylenchus thornei*). No sedentary endoparasites (*Heterodera avenae*) were found in anhydrobiotic stages, but egg hatching in water or root exudates was slower than reactivation of migratory endoparasites and ectoparasites. Ecological implications regarding ability to survive in dry soils and fitness to invade roots when these become available to plant parasitic nematodes are discussed. A seasonal adaptation in reactivation of nematodes adjusted to soil moisture and host plant germination seems to benefit migratory endoparasites as select them as the prevailing nematodes in European Mediterranean cereal and grassland agrosystems.

Interception of Plant Parasitic Nematodes by the Quarantine Station of Embrapa, Brazil, in Imported Plant Material

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The Plant Quarantine Station (PQS) of Embrapa Genetic Resources and Biotechnology (Cenargen) does phytosanitary analyses for most of the plant material imported by Brazil. During the last two years, the Nematology laboratory analysed 22,000 samples of these materials, coming from different countries. The majority of accessions is germplasm, and the others are commercial products. In 2007, being 2,549 germplasm accessions and 87 of commercial products were found infected with economic imported nematodes such as *Ditylenchus acutus*; *Ditylenchus africanus*; *D. emus*; *D. myceliophagus*; *Ditylenchus thornei*; *Helicotylenchus bambesae*; *Pratylenchus penetrans*; *Scutellonema brachyurum*. The majority of these species are exotic to Brazil. Others nematode species also were detected, but some already occur in Brazil or some were not plant-parasitic nematodes, as listed in alphabetic order: *Aphelenchoides abyssinicus*; *A. asterocaudatus*; *A. besseyi*; *A. bicaudatus*; *A. blastophthorus*; *A. pusillus*; *A. subtenuis*; *Aphelenchus avenae* and *Paraphelenchus* sp. Some specimens also were detected and it waspossible to identify only in their Ordem: Araeolaimida and Tylenchida. The infected plant materials were: *Arachis*. *Bismarckia*, *Bromélia*, *Glycines*, *Gossypium*, *Helianthus*, *Hordeum*, *Lilium*, *Oryza*, *Sorghum* and *Zea*. All these data, refer to the nematological analysis, are storage in a database, denominated ‘Sistema de Informação de Germoplasma’, that is administrated in Cenargen. Part of these germplasm was submitted to the thermal treatment, dry and humid, for the parasite eradication, such as maize; peanut, soybean, and sunflower. With those procedures the PQS of Embrapa collaborates actively to reduce the risk of introduction of new plant parasitic nematode species in Brazil.

Quantitative Detection of the Major Plant-parasitic Nematodes in Japan using Real-time PCR

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Four primer sets in real-time PCR were designed for the quantitative detection of the root-lesion nematode (*Pratylenchus penetrans*), the root-knot nematode (*Meloidogyne incognita*), the potato cyst nematode (*Globodera rostochiensis*) and the soybean cyst nematode (*Heterodera glycine*). These primer sets were highly specific to the corresponding species according to the previous sequences in database, except for the one for *M. incognita*, which will detect *M. arenaria* and *M. javanica*. Different numbers of the second-stage juveniles (J2) of *P. penetrans* were mixed with various stages of free-living nematodes and then DNA was extracted from the nematode mixture. There was a significant correlation between the *Ct* values and the number of *P. pentrans* added. Highly significant correlations were also observed in *M. incognita*, *G. rostochiensis* and *H. glycine*. Real-time PCR sensitively detected only a single plant-parasitic nematode in 200 to 1000 individuals of free-living nematodes. This study demonstrated that real-time PCR assay for the major plant-parasitic nematodes provides a sensitive and reliable means for the rapid quantification of the vermiform pests

Application of Taylor's Power Law to Sample Statistics of Nematodes Associated with Cucurbits in Egypt

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Taylor's power law could be fitted ($P \leq 0.01$) to most of the common nematode genera extracted from the rhizosphere of cucurbitaceous crops in Egypt. These nematode genera were arranged in an ascending order according to their index of dispersion (represented by slope values of the power law) in soil as follows: *Tylenchus*, *Pratylenchus*, *Tylenchorhynchus*, *Heterodera*, *Meloidogyne and Hirschmanniella.* The spatial distribution of most nematode genera was aggregated which can confound parametric statistical analyses necessary to study their effects on the different cucurbitaceous hosts. Yet, approaches based on estimate of such dispersion indices may be used to solve these problems and in the development and evaluation of control measures. Also, the law was used for sample size optimization. For example, to sample Tylenchus spp. and Meloidogyne spp. from one feddan (4200 m2) cucurbitaceous area with a 0.25 standard error to mean ratio and 5 nematodes/100 gm soil, one could collect one and twenty samples, respectively.

Detection and Quantification of *Phasmarhabditis hermaphrodita* using Real-Time qPCR

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The identification of soil nematodes is a time consuming and highly skilled process. Quantification of nematodes is also extremely difficult, with soil extraction, dilution and manual counting techniques rarely achieving either consistency or accuracy. Advances in molecular biological techniques have made species detection and accurate quantification possible using Real-Time Quantitative PCR. This technique monitors an increase in fluorescence generated during the reaction and relates the rate of increase to the quantity of initial DNA template thus quantifying an unknown template amount when compared to a standard concentration series of identical DNA. Primers and a dual labelled fluorogenic probe were designed based on the sequenced 18S gene of *Phasmashabditis hermaphrodita,* and BLAST searched to ensure species specificity. These were used to produce the rapid and accurate quantification of known numbers of *Phasmarhabditis hermaphrodita* which had been spiked into field soil samples containing natural populations of various other nematode species.

TOPIC TWENTY – DIAGNOSTICS OF PARASITIC NEMATODES

Insights into Virulence of Portuguese *Bursaphelenchus xylophilus* Isolates by ITS-RFLP Analysis

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There is great variability in virulence among isolates of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Morphometrics and morphological studies were carried out on females and males of sixteen Portuguese PWN isolates obtained from maritime pine, *Pinus pinaster*. The morphological characters were typical of *B. xylophilus* but, the morphology of the female tails varied from rounded, digitate to mucronate. The rDNA containing the internal transcribed spacer regions (ITS) of these isolates was analysed by ITS-RFLP using the restriction endonucleases *Afa*I, *Alu*I, *Hhae*III, *Hinf*I and *Msp*I. The Japanese PWN isolate (J10) was used as a reference. The Portuguese PWN isolates presented specific restriction patterns identical to the restriction patterns presented by J10. However, it was possible to differentiate the Portuguese isolates from J10 using ITS-RFLP with the restriction endonuclease *Hha*I, which discriminates virulent from avirulent isolates of PWN. The *Hha*I restriction pattern presented by the Portuguese isolates was identical to the pattern described for virulent isolates of PWN and the pattern presented by J10 was identical to that described for avirulent isolates. Further studies on the reproductive and dispersal ability of these isolates on selected hosts should help to understand the virulent potential of Portuguese PWN isolates.

EPPO Work Programme on Diagnostics

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What is EPPO? Within EPPO (European and Mediterranean Plant Protection Organization) 49 member countries cooperate to prevent the introduction of dangerous pests into the EPPO region and limit their spread if introduced. EPPO prepares recommendations to be approved by EPPO's Council and which are then internationally considered as 'regional standards'.

Development of diagnostic protocols. For many years the need for a harmonized approach to diagnostic methods for regulated pests has been recognized. In 1998, EPPO started a programme to prepare diagnostic protocols. The Panel on Diagnostics conducts this work in collaboration with specialized Panels (Bacterial Diseases, Nematodes and the European Mycological Network). Panels are composed of specialists proposed by their National Plant Protection Organizations. The author(s) prepares the first draft of the diagnostic protocol according to a common format. Each protocol is intended to contain the information necessary to detect and identify a particular pest. Relevant Panels and other EPPO bodies review them, thus basing the protocols on the experience of specialists. 81 protocols have been approved since 2000 (see EPPO website) and are considered regional standards, 3 general standards have also been adopted. Approximately 20 protocols are currently in preparation.

EPPO programme on quality assurance and accreditation. In 2003, a Panel was created to identify critical elements in ISO Standard 17025[1], and has developed a standard which includes basic requirements for quality assurance in plant pest diagnostic laboratories (PM 7/84). This standard is being revised to include elements on method validation.

EPPO database on diagnostic capacities in the EPPO region. In 2004, EPPO members stressed that the decrease of expertise in plant protection could jeopardize the implementation of phytosanitary regulations for quarantine pests. It was then decided that an inventory of diagnostic expertise should be made. EPPO has developed a database on diagnostic expertise which is available on its website: www.eppo.org).

[1] ISO/IEC Standard 17025 on "General requirements for the competence of testing and calibration laboratories"

The PineWood Nematode *Bursaphelenchus xylophilus*: The Result of Five Years Survey in France

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Bursaphelenchus xylophilus is a nematode of world wide concern and a major threat for pine stands. In many countries it is listed as a quarantine pest. In the European Union, it has been considered as such since 1986 (directive 86/546/EEC amending the annexes of directive 77/93/EEC, now replaced by directive 2000/29/EC). And the non-European populations of its vector *Monochamus* spp. are also regulated since 1990 (directive 90/490/EEC). The recent detection of *B. xylophilus* in Portugal (1999) resulted in supplementing the EU legislation: in particular, since 2000, official annual survey are required within the EU members states to allow early detection in the event of introduction of this nematode. In France, forests represent around 15.5 millions hectares (approximately 28% of territory) including 4.4 millions hectares of conifers (33% of forestry area). A current objective of 350 samples is taken each year in declining pines stands or risk areas (locations around points of import, wood processing industries, where coniferous trees grow). They are analysed according to EU recommendations (sampling regimes, handling, extraction and identification). Different pine tree species are monitored: *Pinus sylvestris, Pinus pinaster, Pinus nigra, Pinus halepensis*… The nematodes' identification is mainly based on an initial morphological identification confirmed by molecular test (PCR, PCR-RFLP). Among the 1.773 samples collected from 2002 to 2006, *B. xylophilus* has never been identified, although some other non-pathogenic and endemic *Bursaphelenchus* species (*B. mucronatus, B. tusciae*…) were sometimes detected in the pine samples. The specific status of the sampled *Bursaphelenchus* populations was also tested using new innovative molecular tools developed by INRA (Sophia Antipolis, France) in the framework of the EU research projects PHRAME and PORTCHECK, which confirmed the absence of *B. xylophilus* in French forests.

A Real-time PCR Assay for Detection and Quantification of the Burrowing Nematode, *Radopholus similis*

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A real-time PCR method using SYBR Green was developed for the detection and relative quantification of the burrowing nematode, *Radopholus similis*, a major pest in banana-growing regions worldwide. Primers were designed to amplify a portion of the internal transcribed spacer (ITS2) region of the rDNA and used for both conventional and real-time PCR. A standard curve was generated using three replicates of independent DNA extractions from 2000 mixed stages of *R. similis* and ten-fold serial dilutions from 1 to 0.0001. Precise numbers of nematodes were used to determine the sensitivity and accuracy of the assay. This real time PCR SYBR green method can be used to accurately detect and quantify 1 to 2,000 individuals of *R. similis* extracted from root or soil samples. The assay described is highly specific and has several advantages over conventional PCR.

Genetic Diversity in Portuguese Potato Cyst Nematode Isolates assessed by AFLP and Massive Parallel Sequencing

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Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are widely distributed among potato production areas in Portugal, where infestations are often associated with significant economic losses. As both species and populations within species have different spectra of virulence towards host resistance genes, it is important to know the genetic diversity that is present in the field populations. Among molecular methods, AFLP, a DNA fingerprinting technique based on selective PCR amplification of restriction fragments from a total digest of genomic DNA, has been widely used to provide molecular markers for organisms that still lack genome sequence information. The discrimination power of AFLP technology, in combination with massive parallel sequencing, was used to obtain sequence profiles for PCN isolates in order to find molecular markers of known sequence that might later be used with simpler methodology to assess the virulence of the PCN field populations. Genomic DNA was extracted from cysts of eight isolates of *G. rostochiensis* and two of *G. pallida*, adaptor-ligated, and pre-amplified. Selective amplification was performed with primer set E63-M33 and subjected to 454 sequencing, following the standard protocol. Overall sequencing yielded 58,180 reads, which clustered into a minimum of 63 and a maximum of 408 sequence markers. Comparative analysis of the sequences is under way to determine differences among isolates.

PCN: Dead or Alive, That is the Question

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PCN, or potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), are quarantine organisms in the EU and due to this fact it is compulsory to sample the soil and find free of PCN before growing propagation material. When cysts are found the contents of these cysts are checked, as only live cysts form the basis for officially recording a field to be infested with PCN. Hence the determination of juveniles being dead or alive is crucial. In most countries the viability of cyst contents is determined visually by the microscope. In the Netherlands a table describing visual characteristics is used in a attempt to standardize the determination between laboratories. Proficiently tests however show that deciding whether eggs and juveniles are dead or alive still causes much variability. To minimize the variability between laboratories the table was extended with pictures and descriptions of characteristics were adapted, based on comparative research and personal experience. The results of the research and the proficiency test performed with the new extended table will be discussed. A proposition is made to put forward the new table as an addition to the EPPO protocol of PCN.

Pathotypes of *Globodera* spp. as Detected by Superoxide Dismutase Isoelectrofocusing Patterns

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Twelve populations of *Globodera rostochiensis* and 3 populations of *G. pallida* were collected from different States of Venezuela, and different Chilean and Italian regions. Standards of Ro1, Ro2, Ro3, Ro4, Ro5 pathotypes of *G. rostochiensis*, and of Pa2, Pa3 pathotypes of *G. pallida* were provided by the Czech University of Life Sciences in Prague. About one hundred cysts of each population were used to extract proteins. Proteins were separated according to their charge by isoelectrofocusing (IEF) on mini-gels inserted into the automated Phast-System® equipment. Gels were stained for superoxide dismutase (SOD) activity. A high SOD polymorphism was found among the tested samples with the presence of up to 12 enzyme activity main bands at different isoelectric point (pI range 8.7-4.4). By means of a matrix built up on the presence/absence of these 12 bands, considered as variables, a cluster analysis was carried out and a UPGMA dendogram was obtained. Groups including populations of the 2 different species were well discerned by this analysis, as predicted. Interestingly, the group including Ro1 standard was markedly distant from those including all the other *G. rostochiensis* populations and standards. Ro2, Ro4, Ro5 standards grouped together in a cluster different from that including Ro3. However, Ro2 belongs to a subgroup which can still be differentiated from that to which Ro4 and Ro5 belong. The provided standards of these latter pathotypes actually show almost identical IEF patterns. Standards of Pa2 and Pa3 pathotypes were easily differentiated by this analysis. Also if this method may be improved by using IEF patterns from other enzymes, this study indicates that isozyme IEF phenotypes may be utilized to identify pathotypes or virulence groups of these cyst nematodes in a manner easier, faster and more economic than that using the differential *Solanum* clones.

Plant Parasitic Nematodes in Australian Turf

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A total of 1106 soil samples from turfs (mainly golf and bowling courses) of Victoria (VIC), New South Wales (NSW), South Australia (SA), Queensland (QLD), Western Australia (WA) and Tasmania (TAS) were tested for plant parasitic nematodes over the last 10 years (1997-2007). The data were recorded in diagnostic database of crop health services of DPI, Victoria. Thirteen types of plant parasitic nematodes were detected from NSW [e.g. spiral (*Helicotylenchus* spp., *Rotylenchus* spp., *Scutellonema* spp.), stubby-root (*Paratrichodorus* spp.), Sheath (*Hemicycliophora* spp.), sting (*Morulaimus* sp.), root lesion (*Pratylenchus* spp.), root knot *Meloidogyne* spp.), cyst (*Heterodora* sp.), ring (*Macroposthonia* spp., *Hemicriconemoides* spp. *Ogma* spp.), lance (*Hopolaimus* spp.), dagger (*Xiphinema* spp.), needle (*Longidorus* spp.), pin (*Paratylenchus* spp.) and stunt (*Tylenchorhynchus* spp and *Merlinius* spp.)] followed by 12 types from VIC (same as NSW except lance), 12 types form SA (same as Victoria), 12 types from QLD (same as NSW except pin), 11 types from WA (same as NSW except lance and needle) and 10 types from TAS (same as NSW except sting, lance and needle). The severities of infestations were highest in turf courses of NSW followed by VIC, SA, QLD, WA and TAS. The information is useful to study the pattern and types of incidence of plant parasitic nematodes in turfs of Australia.

Comparison of the Morphology, Host Range and DNA Sequences of Single Female Isolates of *Pratylenchus* cf *neglectus.*

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Over 500 single female isolates of *Pratylenchus* cf *neglectus* were grown in carrot culture from specimens obtained from different geographic regions of the Western Australian cereal cropping zone. These isolates were similar to the described species *P. neglectus,* although there were differences in morphological and morphometric characters. Since isolates were reared on carrot disk under controlled conditions, the influence of host and environment was minimised. The isolates were compared by measurement of 17 morphological characters, host range tests and DNA sequencing. Significant morphometric differences were found between the Western Australian isolates and also when compared with a known standard population of *P. neglectus* from South Australia. There were also differences in DNA profiles between selected isolates, but these were not considered significant at the species level. While there were host differences between isolates, these were only significant for 2 of the 8 hosts tested. These results indicate that while morphological variation between populations of *P. neglectus* can be identified, this does not necessarily equate with differences in host range. The differences in DNA profiles between populations of *P. neglectus* may reflect the start of speciation due to selection pressures imposed by hosts and environment.

Real Time SYBR GREEN1 Based Detection Technique for *Globodera rostochiensis* of Potato Cyst Nematodes

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A new DNA primer (ITS1H) was designed based on broader sequence information to distinguish *Globodera rostochiensis* (*Gr*) from *Globodera pallida* (*Gp*). This primer (ITS1H) along with universal primer (ITS5) will amplify a short piece of DNA (174 bp) for *Gr* in both conventional and real time PCR. This amplified DNA product effectively differentiated *Gr* from *Gp* in both types of PCRs. The melting peak for *Gr* with ITS1H primer was 85^{O}C against 85.7^{O}C for *Gp* with primer ITSp4. The primer ITS1H has been produced consistent melting peaks compared to inconsistent peaks with published primer (ITSr3) during validation of new primer across a number of isolates of *Gr* from different locations of Victoria, Australia. The new primer gave significant positive relationships between threshold cycles and DNA concentrations in quantitative real time PCR. The method described in this article could be an efficient tool in identification and quantification of DNA from *Gr*.

Molecular Confirmation of a *Xiphinema americanum* Complex (Virus-vectoring Nematode) from New Zealand

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Species in the *Xiphinema americanum* complex have been subject to much study, as they transmit at least four plant pathogenic nepoviruses. The identity, taxonomy and worldwide distribution of nematodes in this species complex remain much debated due to their close morphological similarity. Virus vectoring nematodes pose biosecurity and a market access threats for New Zealand. At least three *X. americanum* species have been described previously from New Zealand, but no molecular techniques have been used in these identifications. We describe the first use of DNA barcoding to identify *X. brevicollum*, a nematode from the *X. americanum* complex, in New Zealand. We used the prepGEM enzyme system for DNA extraction from nematode isolated from soil and DNA fragments were amplified with the LSUD3B/LSUD2A primer pairs. DNA sequences obtained from the New Zealand nematode were used for comparison against GenBank sequences. BLASTN comparison indicated that the LSU fragment was most similar to sequences from *X. brevicollum*. Phylogenetic trees inferred from the collected nematode sequences show this close relationship as well as the clustering of the *X. brevicollum* with *X. diffusum* and *X. taylori* sequences. The prepGEM enzyme system for DNA extraction and the barcoding techniques were simple to apply and gave excellent species identification of longidorid nematodes in our sample.

Diagnostics and Management to Protect California Agriculture from Invasion of False Root-knot Nematode (*Nacobbus* spp.)

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As a potential invasive plant nematode, *Nacobbus* spp., the false root-knot nematode, poses a significant, urgent threat to California crops and agricultural trade. While not yet established in California agriculture, *Nacobbus* is established in neighboring areas, including other parts of the western United States, Mexico, and Latin America where it causes serious crop loss. The threat is exacerbated by diversity in pathogenicity and host range and by lack of quick, reliable diagnostic tools to support routine regulatory operations. Herein we present the analysis of the ITS-rRNA gene sequences of several populations of *Nacobbus* spp. originating from North and South America. PCR-ITS-RFLP diagnostic profiles and PCR with specific primers were developed as practical tools to distinguish some isolates. Presence of several cryptic species and species boundaries within *Nacobbus* are presented based on molecular, morphological and morphometrical studies. Diagnostic characterization of *Nacobbus* isolates is a first step toward testing these isolates against specific crop and weed hosts to recognize potential natural reservoirs as well as to identify sources of crop resistance. A combination of diagnostic profiling with host and resistance specialization will be used to support the design of management strategies.

Study on Determination of Population Density of Fruit Trees from Karadj of Iran

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In order to study nematodes of fruit trees in Karadj, 30 soil core samples were collected in 2006-2007. The nematodes were extracted by centrifugal flotation technique (Jenkins, 1964). Nematodes Population were counted by counting slide and fixed, they were mounted on slide for other investigation. Collections show that 26 species belonging 18 genera were identified according to morphology characters:

1)*Aphelenchoides centralis* 2)*Aphelenchoides limberi* 3)*Aphelenchus avenae* 4) *Boleodorus thylactus* 5)*Cephalobluides sp.* 6)*Cephalobus sp.* 7)*Criconemella parvus* 8)*Discolaimus major* 9)*Ditylenchus destructor* 10)*Diphthrophora commonis* 11)*Geocenamus affinis* 12)*Geocenamus brevidens* 13)*Geocenamus stegus* 14) *Helicotylenchus digonichus* 15)*Helicotylenchus vulgaris* 16)*Mesocriconema antipolitanum* 17)*Mesocriconema xnoplax* 18)*Mesolaimus sp.* 19)*Mylonchulus brachyuris* 20)*Mylenchulus sigmatarus* 21)*Pratylenchus neglectus* 22)*Pratylenchus thornei* 23)*Pratylenchus volnut* 24)*Tylenchus davanei* 25)*Xiphinema Pachtaicum* 26)*Zygotylenchus guevaraii.*

Counting of population density shows that 10 nematodes<100, 14 nematodes <300 and 3 nematodes >300. Maximum populations are about non parasitic nematodes because of physical soil conditions with manner and good penetration. *Pratylenchus spp.* is important in this study.

Collecting and Identifying Plant Parasitic Nematodes of Pine Trees in Central Iran

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In order to study the plant–parasitic nematodes of Pine trees in Tehran, Qom and Markazi provinces, 50 soil samples from rhizosphere depth were collected during 2007. The nematodes were extracted by centrifugal flotation technique, fixed and transferred to glycerin according to the De Grisse method (1969). Morphmetrical characters were determined under light microscope, for identification and description of nematode specimens, species keys and references were used. In this study 16 species belonging to 12 genera were identified as follows:

1)*Aphelenchus avenae* 2)*Boleodorus thylactus* 3)*Ditylenchus acris* 4)*Filenchus vulgaris* 5)*Geocenamus brevidens* 6)*Geocenamus rogusus* 7)*Helicotylenchus digonicus* 8)*Longidorella sp.* 9) *Mesocriconema xnoplax* 10)*Pratylenchus thornei* 11)*Pratylenchus neglectus* 12)*Rotylenchus glabratus* 13)*Tylenchus filiformis* 14)*Xiphinema americanum* 15)*Xiphinema index* 16)*Xiphinema pachtaicum*

To our knowledge, three genus *Xiphinema*, *Mesocriconema* and *Pratylenchus* cause economic damage to Pine trees.

Morphological and Molecular Characterization of *Rotylenchulus reniformis* Populations Occurring in China

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Reniform nematodes (*Rotylenchulus* spp.) are semi-endoparasites of roots and occur commonly in tropical and subtropical regions. One of the most economically important species in the genus is *Rotylenchulus reniformis*, which has widest geographical distribution and host range. The present research provided some morphological and molecular data of *R.reniformis* populations from three geographical different provinces, Hainan, Zhejiang and Chongqing, in China. All the populations are amphimictic, and the hosts include banana, corn, tomato, pumpkin, soybean, and pimiento, pepper, cabbage, carrot, etc. The morphology and morphometrics of males and immature females of typical populations were observed and measured, respectively. All the populations have similar morphology and some variability in morphometrics was detected. Sequencing of ITS region of ribosomal DNA (rDNA) was compared among populations from different province of China. Amplification of rDNA-ITS region yielded a single PCR fragment of approximately 800 bp (including primers) for each of the typically populations of *R. reniformis* from China. ITS sequencing enabled to be made of molecular intraspecific polymorphism in *R. reniformis* in which two ITS types were detected, type A, which only has 2 bp differences for most of the populations from Hainan, and type B, which has 3 bp differences and was typical for the population from Zhejiang and Chongqing, but there are 53 bp differences between the two ITS types. This is the first report of molecular studies with Chinese reniform nematode populations, and it provides some information from which, a robust molecular diagnostic protocol can only be developed if based on examination of more populations from widely disparate geographic origins.

TOPIC TWENTY-ONE – COMMUNICATION AND EXTENSION

Do Current Crop Growth Models Allow for Nematode Damage?

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With the rise of computer technology, modelling has become a useful tool for many fields of scientific research. Canegro, a crop growth model specific to sugarcane, has been under development at SASRI since the early 1990's, and simulates potential plant growth and development under various climatic conditions. The current model incorporates weather data and soil physical characteristics to simulate the growth and phenology of sugarcane. The model does not simulate several other yield-limiting factors such as the effects of weeds, flowering, nutritional deficiencies, ratoon yield decline and pests and diseases. Among the pests of sugarcane in South Africa, plant parasitic nematodes are a major limiting factor for sugarcane production, particularly on the sandy soils. The main species associated with sugarcane damage are *Meloidogyne javanica, Pratylenchus zeae* and *Xiphinema elongatum*.

Results shown here are from initial attempts to quantify the differences between yields simulated on Canegro and actual yields from field trials, with and without nematicide (aldicarb) application.

Data were collected for two cultivars from five cultivar x nematicide trials, representing 98 datasets. In these field trials, nematodes were controlled in half the plots. No other major pests and diseases were evident. Results showed that, in the control plots, the actual yields were 48% (+/- 16%) of that of the modelled yields. When nematicide was applied, the yields increased to 64% (+/- 17%). But at some sites, particularly where responses to nematicide were high, the average dropped to only 20% (+/- 3%) of the simulated yields.

To better integrate the 'nematode effect' into Canegro, more systematic measurements, such as crop growth (above- and below-ground), changes in nematode communities and differences in soil properties need to be collected and evaluated.

Creating Awareness of Nematodes is One of the Biggest Challenges in Rural South Africa

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Surveys conducted on vegetables in the rural areas of the eastern and northern parts of South Africa indicated serious problems with nematodes, especially *Meloidogyne* spp.. Nematode numbers were as high as 64000 *Meloidogyne* larvae per 30g roots of tomatoes. These numbers not only caused yield declines, but in several instances fields were abandoned due to complete crop failures. Since nematodes can be controlled effectively, the biggest challenge is to create awareness of nematodes and to provide the farmers with a viable control strategy.

While part of the project was to investigate affordable, easily available and effective control measures, an equally important part was to create awareness among rural farmers. Extension officers in both Limpopo and Mpumalanga provinces were trained during workshops, on the importance of nematodes and effective control measures. In collaboration with extension officers, sites were selected where demonstration trials were planted to show the effect of different Integrated Pest Management (IPM) strategies in controlling nematodes and the effect on yield. Farmers were involved in planting and applying all the treatments and were informed on nematodes. Clinics were identified and vegetable gardens were planted. Regular study groups were organized during which participants were trained in vegetable gardening and nematodes. Primary schools were identified in both provinces and vegetable gardens were established with the help of senior pupils. At the same time, pupils were educated in vegetable gardening, including nematodes and affordable control strategies available in rural areas. The project continues.

Journal of Nematology

Schaffer, R. & D. M. Bird

The Journal of Nematology (JON) is the official publication of the Society of Nematologists (SON) and publishes original papers on all aspects of basic, applied, descriptive, theoretical and experimental nematology. As the premier nematology publication, JON is indexed by the US Library of Medicine and papers are fully and freely accessible at the time of publication from PubMed (www.pubmed.gov). Reflective of its stature as an international journal, submissions are received from many countries, which in 2007 included Belgium, Brazil, Canada, China, France, Ghana, Italy, Japan, Korea, Malaysia, Mexico, New Zealand, Pakistan, Romania, Spain, Turkey and USA. More than 70% of these manuscripts were published in the 2007 edition of JON. Manuscripts submitted to JON are rapidly processed, with most acceptance/rejection decisions being communicated to the authors in 10 weeks or less. To further enhance JON as a tool for the nematology community at large, the JON Style Guide (http://www.pngg.org/jon_style/) is available on the web with detailed information for authors, reviewers and editors. In this poster, we present the salient features of the new, updated JON on-line Style Guide and highlight key journal processes.

NEMYS: A Digital Encyclopedia on Marine Nematodes

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NeMys offers since a few years the possibility to setup online digital catalogues on different groups of fauna and flora. One of the more important datasets in the system is the global database on free-living marine nematodes. This dataset, which is accessible through http://nemys.ugent.be/nematoda , combines all kinds of information on this taxon. The nematode dataset was initiated by nematologists working at the Marine Biology Research group of the Ghent University. It was believed that the many years of expertise and the large literature collections had to be shared with a much broader audience by means of a digital archive. Data are available for more than 8500 taxa, mostly based on published literature sources; the species are illustrated with images of the original description. Moreover, about 3500 references give information on the biology and another 5000 give a view on the biogeographical aspects. Morphological and morphometrical information is present for a high number of taxa and is reused in the online identification keys for selected genera.

More general data such as the most commonly used methods are documented and a glossary explains the nematological terminology, providing tools for research, teaching, workshops,

The database is linked to a wide variety of other internet resources and is shared with biodiversity portals.

Currently about 400 users from all over the world are using the system. It is hoped that in the future the dataset will be broadened also to non-marine nematodes and that the system will be maintained by different specialists.

Status of Plant-Parasitic Nematodes on Soybean and Corn Grown in Missouri, USA

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Soybean cyst nematode (SCN; *Heterodera glycines*) continues to be the most widespread and damaging plant-parasitic nematode on soybeans grown in the state of Missouri. Over the last fifteen years, three different surveys have shown that approximately 75% of Missouri soybean fields have detectable levels of SCN. A survey of growers also confirmed that SCN is considered among the three most serious disease problems encountered in Missouri soybean production. Growers throughout the state use SCN-resistant soybean varieties (primarily the PI88788 source of resistance) and crop rotation to manage SCN; however, recent HG-type tests suggest that 70% of SCN populations in Missouri can defeat the PI88788 source of resistance. Root-knot nematode (RKN; *Meloidiogyne spp.*) is also a significant problem in southeastern Missouri soybean fields. Growers in that area use root-knot resistant soybean varieties and crop rotation to minimize losses. Plant-parasitic nematodes have not been considered a serious problem on corn in most areas of Missouri. However, this perception is changing. RKN is becoming a significant problem on corn grown in southeastern Missouri. Stubby-root nematode (*Paratrichodorus spp.*) has been recovered from corn fields in many counties along the eastern edge of the state causing yield reduction in some fields. Lesion nematode (*Pratylenchus spp.*), stunt nematode (*Tylenchorhynchus spp.*) dagger nematode (*Xiphinema spp.*) and spiral nematode (*Helicotylenchus spp.*) were detected in a recent survey of corn fields in southeastern Missouri. A new cyst nematode on corn (identification not yet confirmed but resembling a *Cactodera spp.*) was detected in a soil sample submitted to the University of Missouri Extension Nematology Laboratory in 2006 from a field in Tennessee, but has not been detected in any samples submitted from Missouri corn fields in 2007. Emphasis is being placed on educational programs to increase awareness of nematodes damaging corn and soybean crops in Missouri.

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