

001 *Bursaphelenchus xylophilus* and *B. mucronatus* in Japan: where are they from?

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Geographical distribution and speciation of *Bursaphelenchus xylophilus* (pinewood nematode) and *B. mucronatus* were inferred from molecular phylogenetic analysis and chromosomal number. Several isolates of *B. xylophilus* and *B. mucronatus* in Japan and from some other countries were used for DNA sequencing of the ITS regions in ribosomal DNA. Published research on the number of chromosomes of selected isolates was used to identify a relationship in speciation of these nematodes. We identified three (or four) groups of *B. xylophilus* and two groups of *B. mucronatus* based on molecular phylogenetic information. These groups corresponded to differences in chromosomal number. We hypothesised a route of geographical migration and speciation of these two nematodes by estimating the chronology of speciation. The results support a previous hypothesis that *B. xylophilus* and *B. mucronatus* diverged with the separation of the European and North American continents. In conclusion, our results confirmed that Japanese *B. xylophilus* was introduced recently to Japan (*ca* 100 years ago) from North America, and suggested that both Japanese *B. xylophilus* and *B. mucronatus* might have two origins in North America and Eurasia, respectively.

002 Molecular taxonomy as a method for describing populations and species within the pinewood nematode species complex

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Shipment of untreated pinewood from North America and several Asian countries into Europe has been banned. This ban has arisen because of the potential for introduction of species of *Bursaphelenchus* pathogenic to pine into Europe with the consequent effect comparable to that seen in Japan, China and Korea. This ban is based on the lack of identification of *Bursaphelenchus xylophilus* in Europe

until the recent identification in Portugal. It is felt that if introduced the nematode would establish populations or interbreed with endemic non-virulent species. This ban has had major consequences on the North American forest industry. Recently many new species of *Bursaphelenchus* have been described from dead or dying pines throughout Europe. Because morphological characters are limited in usefulness for species descriptions and cannot be used to differentiate populations, molecular taxonomy has become important. We will look at the accuracy of methods used for species identification and at what criteria might be used to define and differentiate species of *Bursaphelenchus* when considering import and export bans.

003 Mitigating the pinewood nematode and its vectors in transported coniferous wood

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The pinewood nematode (*Bursaphelenchus xylophilus*) (PWN) has been intercepted in pine chips, unseasoned lumber and packing-case wood. Likewise, PWN insect vectors, *Monochamus* spp., have been found in pallets, crates, and dunnage. As a result of these interceptions and the known consequences of the introduction of the PWN, many countries regulate the import of coniferous wood. Science-based procedures are needed to ensure that globally transported wood is free of the PWN and its vectors. Mitigation measures that have been investigated include prevention, host selection, and treatment by fumigation, irradiation, chemical dips, pressure treatment with preservatives, and elevated temperature. A mill certification program for lumber (no bark, no grub holes) is rational, but has not got much support. Air-drying wood to its equilibrium moisture content will eliminate the PWN. Switching to hardwood lumber for wood packing material is a short-term solution. Irradiation and dipping wood in borates have shown little promise. The efficacy of pressure-treating green pine lumber with chromated copper arsenate has been shown. Pine chips can be fumigated with phosphine. Logs, lumber, and wood packing material can be fumigated with methyl bromide. Heating coniferous wood to a core temperature of 60°C will eradicate the PWN and its vectors.

004 Occurrence of the pinewood nematode, *Bursaphelenchus xylophilus* in Portugal and perspectives of the disease spread in Europe

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In 1999, the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, was found and identified for the first time in Portugal and in Europe. Following detection, Portuguese authorities initiated the implementation of eradication measures during 1999 and 2000, following an alert provided to European Community officials; as a result, the nematode was confirmed to be confined in the Setúbal region, near Lisbon. A task force from the follow-up group (GANP) created by the Secretary of State for the Rural Development established a national eradication programme (PROLUNP) to *i*) contain PWN within the initial geographic limits; *ii*) implement eradication measures; and *iii*) monitor PWN at a national level. Research is presently being conducted both at universities as well as research institutes, focusing on the characterisation of *Bursaphelenchus* species associated with maritime pine, as well as on the insect vector, *Monochamus galloprovincialis*. Recent reports indicate that the nematode may be present in Siberia (Russia), which would present a threat to Eastern European forestry. Efforts are presently being developed by several European countries to establish a research consortium to detect and study the possible presence of PWN, for a new PRA. A recent workshop held in Portugal in 2001 was an opportunity for sharing experiences and techniques on detection and control. There is clearly a greater awareness of this issue in Europe.

005 Mathematical models for spatial pattern of the spread of pine wilt disease

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An epidemic of pine wilt disease has been spreading in wide areas of Japan for nearly a century. The disease

is caused by the pinewood nematode, *Bursaphelenchus xylophilus*, with the pine sawyer, *Monochamus alternatus*, as vector. We present a mathematical model to describe the host-parasite interaction between pines and pine sawyers carrying nematodes on the basis of detailed data taken from the incidence of pine wilt disease at a study site located on the northwest coast of Japan. With this model we simulate the temporal change in the incidence of the disease and predict how the epidemic could be controlled by eradication of the pine sawyer. Furthermore, the model is extended to study the spatial spread of disease on a large scale, by incorporating short-range dispersal of the pine sawyer, together with long-range dispersal through air convection or transportation of logs infested with nematodes. We estimate that more than 10% of sawyers undergo long-distance dispersal in areas where rapid spread of the disease is observed.

006 Fluctuation in genetic structure and virulence of pinewood nematode in host pine trees and insect vectors at the end of a pine wilt epidemic

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The pinewood nematode, the causative agent of pine wilt disease, is harboured as a subpopulation in each dead pine tree and then in each vector beetle of *Monochamus alternatus* in Japan. The transmission is limited during early and mid-summer. To determine a temporal change in genetic structure and virulence of pinewood nematode, the nematode was sampled from dead *Pinus densiflora* trees and from *M. alternatus* beetles appearing in a *P. densiflora* stand between 1996 and 1998 after a heavy infestation. Alleles of 30 nematodes from each sample were determined for four microsatellite loci. A nematode isolate was established from each sample and inoculated on 30 *P. densiflora* seedlings to determine the virulence. Consequently, the gene frequency was similar between a nematode subpopulation in a dead tree and those in beetles emerging from it in many cases. Average gene diversity between nematode subpopulations was smaller than the average gene diversity within subpopulations. Genetic distance between nematode subpopulations harboured in beetles emerging from the same trees was often smaller

than that between those from different trees. The virulence of nematode was low. Multiple infection may not induce a large variation of virulence including high virulent strains at the end of infestation.

007 Nematode candidates for the biological control of pine wilt disease

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Environmentally friendly control methods are being demanded for pest control. There is undoubtedly the same demand for the control of pine wilt disease. The disease is caused by the pine wood nematode, *Bursaphelenchus xylophilus*, which is transmitted by the Japanese pine sawyer, *Monochamus alternatus*. In this presentation, recent studies on three kinds of beneficial nematodes are reported for the biological control of the disease; the entomopathogenic nematodes, *Steinernema* spp.; the tylenchid insect parasitic nematode, *Controtylenchus genitalicola*; and avirulent isolates of *B. xylophilus*. Steinernematids can kill the vector insects in the dead pine logs by spraying nematode suspension on the surface of logs. *Controtylenchus genitalicola* has the potential to reduce the fecundity of the vector insects and propagates by feeding on a fungus as well as in the host insects. This mycetophagy could allow mass production of the nematodes. Avirulent isolates of *B. xylophilus* can induce the resistance of pine trees against the disease by inoculation of pine trees with the nematodes. However, there are also many barriers to develop the practical control of the disease using these nematodes. The direction of further studies to cross these barriers is discussed.

008 Suppressiveness of soils against pathogens: biodiversity and nematology

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Scientific recognition of the existence of soils suppressive of plant diseases is over a century old and was based originally on the *Fusarium*-root-knot complex in Alabama cotton fields. Prior to this, researches into

the phenomenon of ‘tired soils’ [*Boden Muedigkeit*] in Germany led to the isolation of fungal species from within cysts and females of *Heterodera schachtii* from sugarbeet fields. It was thought then that some of these fungi were pathogens of the nematodes. Throughout the 20th century additional examples of soils suppressive to other pathogens were described. This led to the idea that the phenomenon of suppressiveness was linked to the presence of one or a limited number of microbial species antagonistic to the pathogens. Research efforts during the century led to the successful development of several schemes and cultural practices that generate or increase suppressiveness of soils against individual pathogens. These efforts evidenced that the phenomenon of suppressiveness is closely linked with biological diversity of soils. Also, that suppressiveness or ‘resistance’ of soils against pathogens is a continuum. It is demonstrable that a soil severely affected by disease can be converted gradually into a suppressive soil where disease incidence becomes unimportant. There are in soils degrees of ‘resistance’ against pathogens. Non-phytopathogenic nematodes play an integral role in the phenomenon of suppressiveness of soils against plant pathogens. These nematodes can be directly active against pathogens and can also disseminate microbial species throughout the soil and increase microbial activity, hence ‘resistance’ to pathogens.

009 Molecular diagnostics: going towards new frontiers

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Molecular diagnostic tests developed and used in the past few decades have been relying on antibodies for techniques such as ELISA and on DNA based assays, in particular PCR. A new revolution is in the making which is generated by the synergy between genomics and microfabrication, two of the most important research fields worldwide. Genomics tools can already be tapped directly for environmental research by using existing DNA microarrays, e.g., a gene expression profile of a soil sample can be determined by using bacterial microarrays. Several molecular and computer companies have made ‘bio-chip’ and are already producing a completely new generation of instruments for the life sciences. It is already

possible to combine all the steps of complex molecular biology protocols within devices of the size of a credit card. The competition is fierce to see which company will come up with the first hand-held device to perform standard molecular diagnostic tests for genetic disorders or infectious diseases at the point of care. The competitive research on these molecular diagnostic devices is going at a fast pace which has been accelerated further by recent bioterrorism events. In the near future, soldiers are likely to carry specialised miniature molecular diagnostic labs as part of their equipment against biological warfare. When DNA or biomolecules meet the microchip *in silico*, the possibilities are endless and science fiction movies might be outdone soon.

010 Progress in developing nematode diagnostics

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Distinguishing pathogenic nematodes at the species or subspecies levels is relying increasingly on molecular tools. Morphological differences may be absent or difficult to observe. PCR-based diagnostics offer possibilities for precision, sensitivity and quantification. For example, ribosomal DNA has been widely exploited to distinguish many species of plant-parasitic nematodes, to investigate intraspecific variation and to examine evolutionary relationships. Further refinements are in progress to use rDNA to quantify and determine the relative proportions in mixtures of species for use in risk assessments. Mitochondrial DNA size polymorphisms have also been used to distinguish many of the economically important root-knot nematodes; however, for many genera this molecular target remains unexplored or may be problematic. Where the 'conventional' targets have lacked sufficient variability to distinguish closely related species, randomly amplified polymorphisms from fingerprinting patterns have been exploited. Challenges remain, however. Honing molecular diagnostics to distinguish between pathogenic and non-pathogenic variants within species will require identifying molecular targets which are either closely linked to or define the functional pathogenicity. Incorporating diagnostics into management programmes to more effectively deploy resistance, pesticide use or crop rotation will require improving the simplicity, reproducibility and portability of these diagnostics to encourage their uptake.

011 Real time PCR for quantitative detection

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PCR assay for the simultaneous detection and identification of *Globodera rostochiensis* and *G. pallida* (PCN) has been developed but cannot be used to determine the level of infestation in the soil as the standard PCR signal is not quantitative. Direct single step quantitative detection is possible using Taqman technology as the intensity of the fluorescent signal generated during the PCR reaction is quantitative. Using this system, simultaneous detection and quantification of the target DNA of both *G. rostochiensis* (Ro) and *G. pallida* (Pa) was optimised. Quantitative PCR of PCN DNA template concentrations demonstrated that pure DNA from both Ro and Pa gave regression co-efficient of the standard Ct curve against template concentration on average better than 0.95. This result was repeated using second stage juvenile individuals and whole PCN cysts. Duplex primers for Ro and Pa were developed and we were able to detect both species and also determine the ratio of both species in the mixture. Other applications of this technology, problems and limitations will be discussed.

012 Quantifying nematodes and other root diseases in the Australian wheat belt

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Root lesion and cereal cyst nematodes are the major plant-parasitic nematodes to infest grain crops in Australia. They rarely occur in isolation and several major fungal pathogens such as take-all, *Rhizoctonia* and crown rot contribute to significant soil borne disease. A comprehensive testing system has been developed that quantifies DNA extracted from soil using a plate based hybridisation assay to identify the major pathogens present in a pooled soil sample. This data is then fed into a Decision Support System that takes into account the pathogen levels, environmental and other agronomic factors and provides advice on risk categories to growers.

013 Nematodes, quarantine pests and pathogen detection using rapid automated sampling of bulk cargo shipments

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Nematodes are usually not evenly dispersed. Therefore, testing of bulk cargo for the presence of nematodes is only as good as the methodology and sampling strategy applied. Limiting factors for taking lots of samples are the produce bulk size, laborious and costly processing. While there have been significant advances in procedures for invertebrate pest identification, the methodology and tools for sampling these organisms are less well developed. Effective sampling procedures are critical to successful implementation of quarantine measures. The *Discovery*TM CERT technology was originally developed by RAY for detection of explosives and chemical-biological warfare agents in bulk luggage, mail and cargo. Inspection process lasts 8 min for an ULD aviation pallet 2449 kg checked as a whole in one batch. The system allows selective collection of ultra trace and particles of a distribution size of 0.5-800 μm . Tests carried out by DANI/ QUB and NRCL using spiked bacterial/fungal and quarantine pests, combinations of invertebrate/substrate e.g., non-viable nematode cysts (*Globodera rostochiensis*) placed in boxes, lemons infested by live thrips (*Heliothrips haemorrhoidalis*), specimens of leafminer (*Liriomyza sativae*) and whitefly (*Trialeurodes vaporariorum*), inoculated onto bulk cabbages, have shown that *Discovery*TM can successfully recover the target organisms for identification.

014 Precision agriculture: tools of the trade

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Precision agriculture is the optimisation of management of agricultural crops. Crop management is often affected by physical, chemical, and biological factors which are non-uniform in their impact. Therefore, tools have been

developed and are increasingly utilised to manage crops in zones which are smaller than the total field area. The first tool was the yield monitor. Yield maps provide growth patterns that may either be consistent over time (often based on soil physical properties), or be variable because of an unusual or sporadic stress. Grid soil sampling for fertility based on global positioning systems (GPS) was another early tool. However, this has been superseded by zone sampling in some areas. Variable rate application equipment has been most heavily utilised in fertiliser application. However, other uses include seeding rates, pesticides, and plant growth regulators. Guidance systems are now used to improve precision of applications and row positioning. Precision agriculture is highly dependent on information and its success may depend on the ability to gather inexpensive, yet accurate information.

015 Using electrical conductivity to predict nematode distribution in cotton fields

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Meloidogyne incognita and *Hoplolaimus columbus* are controlled in cotton by the uniform application of nematicides across a field. In this system a significant portion of the nematicide is applied where nematode densities are below treatment thresholds. This is due to the spatial aggregation patterns these nematode species typically exhibit in agricultural fields. Variable rate applications of nematicide would allow applications only to areas of the field where treatment thresholds are exceeded. However, generating the nematode distribution maps required for variable rate applications by grid or zone sampling is cost prohibitive. Soil composition, especially percentage of sand, is one of the primary factors influencing nematode distribution. Soil electrical conductivity (SEC) can predict the sand content of a sandy loam soil with a very high degree of accuracy. A 9% drop in sand content can result in a 57% drop in population densities of *H. columbus*. Use of a commercially-available SEC meter coupled with a GPS system allows mapping

of a ten ha field in several hours. These maps can then be used for cost-effective variable rate applications of nematicides.

016 Remote sensing and precision nematicide applications for reniform nematode management in Mississippi cotton

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Site-specific management of cotton pests is practised in Mississippi using remote sensing to direct spatially variable pesticide applications. Remotely sensed imagery is correlated with crop growth parameters using Normalized Difference Vegetation Index (NDVI) values to create prescription maps for bi-modal or multi-modal distributions. This technology is being examined using the spatial distribution of the reniform nematode. Reflectance properties exhibited by reniform-infected cotton plants are being acquired with hyperspectral spectroradiometers using both airborne and ground platforms. Specific reflectance curves, collected with the spectroradiometers, can be correlated with nematode numbers and used to generate nematode contour maps for global positioning system (GPS) directed variable rate technology (VRT) nematicide applications. Hyperspectral data may provide useful remote-sensing tools reducing the time consuming and costly grid sampling process required in precision nematicide applications.

017 Economies in nematode management from precision agriculture – limitations and possibilities

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The potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, represent a severe constraint to potato production in the United Kingdom. Management of PCN depends heavily on nematicides, which are costly. Of all the inputs in UK agriculture, nematicides offer

the largest potential cost savings from spatially variable application, and these savings would be accompanied by environmental benefits. The potential benefit:cost ratios for such a strategy will be considered, along with some of the risks. Maps of real PCN infestations in potato fields in which susceptible potato crops were grown will be used to demonstrate some of the principles and risks. The inverse relationship between population density before planting and multiplication rate of PCN makes it difficult to devise reliable spatial nematicide application procedures, especially when the pre-planting population density is just less than the detection threshold. Also, the spatial dependence found suggests that the use of sampling grids that are too coarse is likely to produce misleading distribution maps. Coarse grids can miss patches of PCN, but an economic analysis of the risks of missing patches can be made.

018 Management strategies for nematode control in Europe

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The improvement of food and environment quality is a priority of the European Union. Therefore, funds for agricultural research and management aim at achieving this goal and alternative strategies to chemicals are promoted. In Europe, the most severe nematode pests are cyst, root-lesion, root-knot (RKN), stem and bulb, citrus and virus vector nematodes. Classical control of field crop nematodes relies on crop sequences, resistant cultivars when available and clean planting materials. Nematicides are used to control nematodes of high-value crops (vegetables, flowers, nurseries) for which the ban of *e.g.*, methyl bromide is a concern. Soil solarisation, new resistant cultivars or rootstocks and soil-less crops are gaining interest as alternative methods. In perennials, management strategies may be preventive by using nematode-free plant material obtained through certification schemes or curative with tolerant rootstocks. Pre-plant soil fumigation is limited to severe nematode attacks. Research for genetic solutions is in progress for various crops, such as for RKN in *Prunus* spp. and *Xiphinema index*/GFLV in grape. For this latter problem, an original alternative to nematicides is the application of

systemic herbicides to kill roots before pulling out the crop combined with fallow to eliminate the virus from surviving nematodes.

019 Nematode management practices in African agriculture

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The African continent is mostly exposed to tropical climates, which favour the reproduction and feeding activity of plant-parasitic nematodes all the year round. In various parts of Africa, many nematodes are parasitic on economic and food crops, but the three most important plant-parasitic nematodes are the root-knot nematodes (*Meloidogyne* spp.), the root lesion nematodes (*Pratylenchus* spp.) and the cyst nematodes (*Heterodera* spp.) which attack and cause severe yield reductions in crops such as cowpea, soybean, maize, yam, cotton, coffee, sugarcane and several vegetables. Agriculture in Africa is characterised by small sized farms and low-level technology. As a result of these, the most commonly adopted nematode management practices in several parts of Africa are cultural control measures which are not capital intensive and which require little or no technical know-how on the part of the mostly illiterate peasant farmers. To a large extent, the use of synthetic nematicides is restricted to large and experimental farms, but the recent awareness of environmental safety coupled with the prohibitive costs of synthetic pesticides has encouraged research into safer and cheaper alternatives. The most commonly used cultural methods of nematode management in Africa are: *i*) shifting cultivation and bush fallowing (until very recently due to land limitation, this method has played a very significant role in the control of many economically important plant parasitic nematodes); *ii*) mixed cropping; *iii*) use of soil amendments; *iv*) soil tillage; *v*) use of resistant varieties and healthy propagation materials; *vi*) crop rotation; *vii*) roguing and farm sanitation; and *viii*) soil solarisation.

020 Nematode management in Russia and neighbouring countries of the former USSR

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In Russia and neighbouring countries, of ten important genera of plant parasitic nematodes, six have a great economic impact. They are *Heterodera*, *Globodera*, *Meloidogyne*, *Ditylenchus*, *Pratylenchus* and *Helicotylenchus*. The division of USSR into many independent states and recent economic reforms in Russia led to deep changes in agricultural practices. These changes increase damage of main crops caused by plant-parasitic nematodes, and provide the opportunity for some dangerous nematodes to come back and new species to appear and spread. These countries are characterised by zones specialising in monocrop growing (*e.g.*, cereals, potato, sugar beet, grapevine and cotton-sowing areas). Hence a close relationship exists between the degree of crop concentration in high-specialist collective farms and the acuteness of nematode problems. Damage caused by nematodes is one of the limiting factors in crop production. Annually farmers lose a significant part of crop yields due to poor control of the seed materials, low agricultural technologies and absence of nematicides. Traditional nematode management includes the following measures: prophylactic (prevention of the nematode establishment in agricultural fields due to quarantine regulations and cleaning of propagation materials), physical (control by heating and vapour), chemical (nematicides), crop rotations, resistant cultivars and some cultural practices (soil amendments). At present, alternative nematode control strategies are being developed. They are based on the use of biological products, wastes from logging and the timber industry, new resistant cultivars with complex resistance to plant pathogens, and stimulation and modulation of host resistance by using of phytohormonal and adaptogenic phyto regulators and phyto-control technologies.

021 Nematode management strategies in East Asian countries

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East Asian countries have diverse agricultural systems. However, root-knot nematodes, cyst nematodes and root-lesion nematodes are important pests throughout this area. Extremely intensive farming systems are prevalent in Japan. Many farmers continuously cultivate high

value crops such as tomato or melon for years in the same plastic greenhouses. Currently similar farming practices are increasing in Korea and other countries. In such intensive farming, control of soil-borne pests is inevitable to maintain profitable yields. Fumigation with nematicides is the most reliable and practical control measure. 1,3-dichloropropene is often applied with granular nematicides. Methyl bromide or chloropicrin is applied in fields where both nematodes and soil-borne diseases occur. The prohibition of methyl bromide is a serious problem for pest control. Solarisation of sealed plastic greenhouses for about 30 days in mid-summer is an effective method with few adverse environmental effects. Cover crops such as *Crotalaria* spp. are also used to reduce nematode populations and improve soil conditions. Tomato cultivars with resistance to root-knot nematodes are commercially available. However, resistant breaking races are present in many fields in Japan.

022 Management practices in South Asian agriculture

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Southern Asia comprises very diverse and intensively cultivated areas, characterised by small farm holdings and economically poor, and often illiterate or poorly educated, farmers. The nematode management practised over large areas, often inadvertently, depends upon crop rotation, tillage, solar heat and organic amendments. Use of nematicidal pesticides is very limited. Some organophosphates and carbamates are applied for the control of insect pests and rarely against nematodes. Small doses of pesticides are advised for application to nursery-beds or to seed of direct seeded crops. Research efforts have been made for finding sources of nematode resistance in crops and a few fairly resistant varieties or crops like rice, tomato, chillies, cowpea, mungbean, cotton, grapes, etc., have been selected or specifically bred. Use of genetic engineering for nematode resistant crops is being attempted. Biological control agents including imported and indigenous strains of *Paecilomyces lilacinus*, *Trichoderma viride*, *T. harzianum*, *Aspergillus niger* and VAM, *Glomus* spp., *Pasteuria penetrans*, *Pseudomonas fluorescens*, *Bacillus subtilis* etc., have been found effective but their production and use are still very limited. Combinations of more than one practice have been designed for

certain cropping systems to provide integrated nematode management modules to fit into plant protection packages.

023 Nematode management strategies in major crops in the Australasian region

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The Australasian region encompasses a huge range of agricultural systems, from subsistence to highly mechanised commercial farms. There are many different nematode pests and management strategies, but similar approaches in broad agricultural systems. *Heterodera* has been the major pests of broadacre low intensity farming of grains in the region, but its successful management by crop rotation and resistance have seen *Pratylenchus* emerging as major pests of this cropping system. In pasture, nematode management is directed largely towards resistance, with lesser efforts in agronomic practices and a range of emerging techniques including mulching and soil amendments. In intensive horticulture, *Meloidogyne*, *Radopholus*, *Heterodera* and, in New Zealand, *Globodera* are managed by phytosanitary controls, chemical methods, and emerging techniques. In woody crops, *Meloidogyne*, *Tylenchulus*, *Xiphinema* and criconematids are all important, and controlled mostly chemically. In small-scale horticulture *Meloidogyne*, *Radopholus* and *Pratylenchus* are most important, and controlled by enforced crop rotations. Quarantine and phytosanitation are also common themes in nematode management because a number of pest species common in the rest of the world are absent from countries or certain areas. Trends are for increasing use of plant resistance, but allied with emerging techniques and greater knowledge of the pest species systematics and biology.

024 Nematode management in North American crops

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The crop and the associated nematodes in North America influence the type of management used. Control of nematodes in low cash value crops depends heavily on

host plant resistance and crop rotation. For example, the soybean cyst nematode is largely controlled with a combination of crop rotation, often the non-host corn, and nematode race specific cultivars. In contrast, fields designated for planting with high cash value crops are frequently fumigated or treated with a nonfumigant nematicide. Treatment with dichloropropene is a common practice for many vegetable crops and fruit crops. Methyl bromide fumigation is used on crops such as strawberry because benefits beyond nematode control, such as control of numerous other pathogens and weeds, are achieved with a single treatment. For nematode management in some perennial tree crops, resistant rootstocks are employed. A case in point is the grafting of peach scions onto 'Nemaguard' rootstocks for areas where root-knot nematode is a problem. Sanitation is a crucial management component for potato production in the Pacific Northwest in the USA. Cash value of the crop and the longevity of the crop are major factors in the selection of management tactics.

025 Nematode management practices in MesoAmerica

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The crops of greatest socioeconomic importance in MesoAmerica (southern Mexico and Central America) are coffee, banana, cucurbits and tomato. Various phytoparasitic nematodes are associated with them but without doubt the gall inducers (*Meloidogyne* spp. and *Nacobbus aberrans*), along with nematodes causing root lesions (*Pratylenchus* spp. and *Radopholus similis*), are widely considered by producers to be factors limiting productivity. In this talk, we will discuss efforts that are being made to attack nematode problems by crop and by geographic zone. We will try to distinguish that which is academic from that which has been adopted by producers. In this process, we will try to put into perspective the difficulties of making integrated nematode management (INM) a reality in the field. Based on reports in the literature the following generalisations can be made: *i*) there are few studies; *ii*) most deal with chemical control; *iii*) non-chemical alternatives for the most part are based on excellent investigations but have not been adopted by producers; and *iv*) there are very few demonstration studies on non-chemical alternatives

at the commercial farm level. The distinct socioeconomic world of MesoAmerican producers is a goal for future generations of nematologists and through INM techniques they should develop different INM systems appropriate to the needs and socioeconomic realities of producers.

026 The Mediterranean agrosystem as an agroecological model

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The necessity of designing a new agricultural model is proposed, which would take into account the demands of consumers, who are concerned not only about greater food safety, but also about the environmental and social impact of agricultural production. New models of agriculture are emerging, which use the ecological management of agricultural systems as a reference, based on the management of biological, environmental and cultural diversity among territories. Environmental and social aspects of Mediterranean agriculture are analysed as a diversified agroecological model, which could serve as a reference for the development of new productive systems, integrating proposals from the European Community agricultural policy. It is also indicated that the environmental characteristics of the Mediterranean environment permit the design of an integrated production system based fundamentally on the use of non-chemical alternatives for nematode control, which prevent pests and disease from becoming serious problems. The definition of 'Mediterranean' refers to a concept that describes a region with its own identity, emerging with characteristics from a specific climate and environment and high agroecological values produced when various cultures interacted and the outcome of integration by the people of the Mediterranean basin. Moreover, the contributions must be emphasised of the great biotechnological revolution and the impact of the discovery of America on diversification in the production of high quality agricultural systems.

027 Reflections on the systematics of the Dorylaimoidea, a group of terrestrial and freshwater nematodes

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The system of Dorylaimoidea is (some well-defined families excepted) unsatisfactory. It is difficult to establish groups on the basis of synapomorphies, the morphological characters being distributed erratically, many species were discovered and described in the 19th century when knowledge of the morphology was almost nil (it remained in this state until 1936); moreover the importance of tail shape (very easy to observe) has been overrated strongly. Furthermore, the literature is full of misidentifications and should be consulted very critically. The author gives some suggestions that in future might lead to development of a better system.

028 Maintenance of biodiversity through predation and competition in freshwater nematodes?

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Why does a particular habitat harbour a certain number of species and how is this species diversity maintained? Theory predicts that biotic interactions may give rise to an arms race that favours variability. Nematodes are ideal to study α -diversity. They show high abundance and species richness and play a central role in soil food webs. Species within a genus (*e.g.*, *Eumonhystera*) show differences in the width of their buccal openings, which could be interpreted as mutual exclusion through competition. In this study we review the species richness of freshwater nematodes and ask whether predators and competitors can explain species richness in the field. The number of freshwater nematode species in 28 different lakes ranges between three and 120. Within 1 year, α -diversity remains at a constant level within a habitat in Lake Brunnsee and Königssee. To study the effect of predators we started mesocosm experiments with different macrobenthic taxa and analysed soil samples of 12 alpine lakes. Plathelminthes, chironomids and annelids do not influence nematode abundance. The influence

on nematode diversity through macrobenthic predators, predacious nematodes and the diversity of trophic groups will be presented. We discuss the relevance of these results for the maintenance of biodiversity.

029 Status quo of limno-nematology: how close are we to understanding the ecology of nematodes in inland water bodies?

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Despite an early start, limno-nematology can safely be called a science in its infancy compared to marine nematology or other aspects of limno-benthology. Past limited interest in free-living limnetic nematodes has given way to an upsurge of interest in the past two decades. Most nematological research in limnetic habitats on free-living forms still has a lot to achieve compared to studies in the marine meiobenthos. Lotic free-living nematodes are relatively less well studied than those from lentic habitats. Taxonomic reports continue to appear, but a noticeable proportion of the recently published work was devoted to their ecology, *i.e.*, mainly to distribution, spatial and temporal variation, abundance, diversity, and the relation of these phenomena with some biotic and abiotic factors. Experimental data that revitalises our notion of their ecological role has started to appear. Their potential use as indicators of water pollution was also strengthened. Hitherto ignored tropical habitats were studied, albeit to a limited extent. Lentic nematodes seem not to conform to the general understanding of biodiversity gradient with latitude. Gaps are indicated and recommendations made.

030 Molecular studies of nematode diversity: past, present and future

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Marine nematodes are an important component of the ecosystem and their ecological and biodiversity properties make them a potentially ideal taxocene for testing ecological theory and biomonitoring. Work at the species level will yield significantly greater information than

work at higher taxonomic levels. However, identifying samples of nematodes is difficult, time consuming and expensive. In addition, the morphological species concept used for classification may be unreliable, and known species, especially those used for biomonitoring, may be complexes of siblings with different functional responses, especially when animals are examined across wide geographic ranges. Molecular methods offer a more objective estimation of species identity. These techniques range from sequencing informative segments of DNA and using them to define 'molecular operational taxonomic units' (M-OTU) to assessment of species diversity in samples through techniques such as denaturing gradient gel electrophoresis (DGGE). Identification of species in bulk samples offers a rapid and comparative means to analyse samples from different sites, even over large geographic ranges. In the future, genomic technologies may offer the prospect of developing 'identification chips' or means of identifying specific gene expression patterns associated with activities of nematodes related to environmental monitoring.

031 Comparative nematode genomics

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The completion of the sequencing of the *Caenorhabditis elegans* genome in 1998 was a milestone in biology. The genome sequence has spurred discoveries in basic biomedical science, but is of added importance to us as nematologists because, of course, *C. elegans* is a nematode. We, and others, are engaged in large scale projects which aim to gather genomics data from a phylogenetically and biologically diverse selection of nematodes. Due to funding constraints, most of this effort is focussed on parasites of humans and our domestic animals. This presentation highlights findings from these ongoing nematode genome projects, illustrating topics such as the conservation of synteny and linkage between *C. elegans* and other nematodes, the nematode-specific and parasite-specific genes we have identified, and the prospects for using comparative genomics to identify new

control methods. Our data and analyses are available at <http://www.nematodes.org>.

032 On the evolution of parasitism genes

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In the last 5 years considerable effort has been put into the identification of parasitism genes from sedentary plant-parasitic nematodes. Thousands of (partial) DNA sequences are now available from several nematode species, which allows comparison of gene expression in plant-parasitic and other nematode species. From this comparison it is evident that plant-parasitic nematodes have evolved a set of genes that have no homologues in other nematodes or even in other phyla. Similarly, plant-parasitic nematodes have genes in common with other plant-pathogens, which are absent in other groups of nematodes. And finally, plant-parasites and animal parasites share a unique set of homologues, too. Pieces of this evolutionary puzzle will be illustrated with examples.

033 Parasitism gene discovery in sedentary phytonematodes

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Parasitism genes expressed in phytonematode pharyngeal gland cells code for the stylet secretions that control the process of plant parasitism. In sedentary endoparasitic nematodes, the products of parasitism genes induce the elaborate modifications of plant cells into feeding cells required to support growth and development of the nematode. Efforts to identify and characterise parasitism genes have been accelerated by several molecular approaches, including direct analyses of secreted proteins and cloning of genes differentially expressed in the pharyngeal gland cells. For differential gene expression, comparative analyses have been conducted among nematode life stages and among nematode tissues. The most direct approach for cloning parasitism genes, however, involves the microaspiration of pharyngeal gland cell cytoplasm from multiple parasitic stages for cDNA library construction followed by expressed

sequence tag sequencing and bioinformatic analyses. Current results reveal a large number of novel parasitism genes and paint a complex picture of cellular events under specific control by the nematode. Furthermore, only very few parasitism genes have homologues in *Caenorhabditis elegans*, limiting the usefulness of this model nematode in studying nematode adaptations for plant parasitism.

034 Adaptations of parasitic nematodes to mammalian host immunity

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Nematode parasites of man and animals are typified by their longevity in the mammalian host, although little is known about molecular mechanisms of establishment and survival of these organisms in the face of aggressive immune responses. In addition to a robust arsenal of anti-oxidants, there is growing evidence that parasite-secreted products include a panel of anti-inflammatory agents which act in concert to suppress the recruitment and activation of effector leukocytes. These products may constitute enzymes which hydrolyse pro-inflammatory signalling molecules such as chemokines, lipid mediators, and nucleotides released from damaged tissues, or proteins which antagonise receptors involved in this process. In addition, a number of cytokine homologues are expressed which have the potential to modulate immune responses. Recent EST projects have highlighted abundantly expressed genes which encode secreted proteins of unknown function, although the lack of genetic knockout techniques has resulted in a bottleneck in the assessment of these proteins as potential mediators of persistence and the mechanisms involved. RNA interference (RNAi) offers an opportunity to address some of these problems, and we are applying this technique to address this specific question. Progress and current data in this area will be reviewed in this presentation.

035 Biosystematics of entomopathogenic nematodes: current status and future directions

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Assimilation of molecular approaches into nematode systematics has escalated dramatically in the last few years. With respect to entomopathogenic nematodes (EPN), various molecular techniques have been used for diagnostic purposes and for separating cryptic species, populations and strains. Another valuable contribution to EPN systematics has been the discovery and description of new species in a phylogenetic context. This new approach has been applied in taxonomic decisions, including re-evaluation of previously described species and their relationships. In this presentation we will discuss the importance of several molecular markers and their current use for diagnosis and identification of EPN at different taxonomic levels. Additionally, we will discuss the application of combined evidence datasets (*i.e.*, morphological + molecular) to interpret phylogenetic relationships among EPN species and as tools to make predictions of ecological traits that will have significance for the application of EPN in biocontrol programs.

036 Advances in molecular biology of entomopathogenic nematodes

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Entomopathogenic steinernematid and heterorhabditid nematodes have been used for almost a decade as biological control agents of insect pests. Despite the impressive progress in propagation and application little has been done, so far, to utilise the advances in molecular biology techniques to advance the use of these nematodes. Molecular tools have been used mainly for identification and classification of entomopathogenic nematodes. In recent years, molecular approaches have been employed to study key processes in development and survival of steinernematids and heterorhabditids. These included: *i*) isolation and characterisation of genes involved in the initiation of development from the infective juvenile stage and *ii*) using cDNA subtractive hybridisation to identify genes that are differentially expressed during exposure to desiccation stress. The advances in the molecular research are reviewed.

037 EPN product development: from strain discovery to commercial field application

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Entomopathogenic nematodes (EPN) have an enormous potential to serve as biocontrol products in protected and outdoor crops. There are several examples of the successful introduction of EPN products into the ornamental and turf care market. However, promising EPN strains often sit on the shelf of highly motivated scientists and cannot easily be transferred to product development. After the control potential of a strain has been demonstrated under field conditions, research into biotechnology has to solve the problem of mass production, down- and upstream processing, storage and formulation. Quality control procedures are needed to predict field performance of production batches. Some countries require registration for EPN causing significant costs and delays in product introduction. Prior to this development, information is needed on alternative control strategies, the commercial significance of the target pest and an approximate cost-benefit analysis for the use of EPN. Marketing needs to motivate potential users to try alternative, environmentally friendly control strategies. Product introduction is accompanied by intensive trials under different production and environmental conditions in order to investigate the ecological potential and limits of the EPN strain.

038 Investigating mutualism between entomopathogenic bacteria and nematodes

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The entomopathogenic nematode *Steinernema carpocapsae* forms a mutualistic association with the bacterium *Xenorhabdus nematophila*. At least one nematode developmental stage, the infective juvenile (IJ), is colonised at a discrete intestinal location by multiple bacteria and is responsible for transporting bacteria between insect hosts.

We are using microscopic and molecular biological techniques to investigate the cellular and molecular interactions between nematode and bacteria with the following goals: *i*) developing a detailed model of nematode-bacteria interactions throughout the course of their association and *ii*) defining bacterial factors that are important to this mutualism. Achieving these goals will yield general insight into mechanisms of microbe-host associations, as well as provide information important to the further development of these organisms as biocontrol agents. Construction of a GFP-labeled *X. nematophila* strain has allowed direct observation of bacteria-nematode associations, resulting in the description of distinct stages of the mutualistic interaction. Additionally, bacterial mutants have been generated that are either completely or partially defective in the ability to colonise nematodes. We are now characterising the genetic lesions in these mutants to identify genes and their products that are necessary for colonisation.

039 Nematode management in subsistence farming in Africa with emphasis on bananas, plantains and yam cropping systems

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Bananas (*Musa* AAA), plantains (*Musa* AAB) and food yams of the genus *Dioscorea* play a major role in the diet of many people in Africa. These crops are also an important source of revenues to farmers in rural areas. World production is estimated at about 86 m t for bananas and plantains and about 34.5 m t for food yams. Nematodes are among the major biotic constraints to these crops in Africa, causing significant yield losses. The major species attacking bananas and plantains in Africa are *Radopholus similis* and *Pratylenchus* spp. (*P. goodeyi* and *P. coffeae*). *Scutellonema bradys*, *P. coffeae* and nematodes of the genus *Meloidogyne* are the damaging species on yams. This report presents progress on the management of these pests on banana, plantain and yams in subsistence farming systems where these crops are often grown in association with tree crops such as coffee and cocoa and other food crops, including cassava cocoyams and maize.

040 Nematode management in subsistence farming in Mexico

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Factors such as geography, climate, vegetation, biogeography and human settlement have contributed in defining Mexican rural and socio-economic scenarios. Mexican agriculture has two main approaches: the campesino and the agro-industrial, both with contrasting characteristics related to production and management strategies against pests and diseases. In campesino and subsistence systems, low external-input strategies that have proved effective in the management of the most common and important plant-pathogenic species of nematodes in Mexico (*i.e.*, *Meloidogyne* spp., *Nacobbus aberrans*, *Pratylenchus* spp. and *Punctodera chalconensis*), include production of plant and tree seedlings free of nematodes, physical control methods, rotations, organic amendments, antagonistic plants, suppressive soils and maintenance of biodiversity. Most of these practices are considered to be environmentally friendly and, although subsistence agriculture can produce enough food to feed families or small communities, it may be adversely affected by socio-economic factors such as availability of good land, adequate water, migration, pressures on land use, shortened rotations, crop value and changes in cultural patterns, thus compromising sustainability.

041 Nematode management in subsistence farming in the Caribbean

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The key characteristics of Caribbean islands are insularity and diversity: diversity in size, climate, vegetation, crops, economy and human history. Subsistence farming also reflects the diversity from island to island. It is possible to look at these differences in considering some important subsistence crops and related nematode problems *e.g.*, small grain cereals, roots and tubers,

banana and plantains, fruits and vegetables. From a strict nematological viewpoint, all these crops can suffer severely from nematode damage *e.g.*, *Ditylenchus dipsaci*, *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis*, *Scutellonema bradys* but often the expertise of farmers, the extensive way the lands are cultivated, and the mixed-crop production systems let these crops escape nematode damage. Numerous nematological studies have been conducted in the Caribbean with different levels of knowledge, technical skill and success in order to improve nematode management on subsistence crops through fallow and crop rotation, organic soil amendment, use of resistant cultivars, non-host and antagonistic plants. The experience of Cuba, Dominican Republic, Guadeloupe, Jamaica and Martinique would be a good example of what can be achieved with low inputs within the scope of sustainable agriculture.

042 Nematode management in subsistence farming in Bangladesh, Nepal and Pakistan

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The economy of Bangladesh, Nepal and Pakistan is agriculturally based. The main crops of the region are wheat, rice, sugarcane, cotton, maize, fruit orchards and vegetables. In Bangladesh, *Ditylenchus angustus*, *Meloidogyne graminicola*, and *Hirschmanniella oryzae* are all major pests of rice. Management of ufra is by the burning of stubble. Root-knot can be prevented on rice by the transplanting of healthy seedlings and suitable rotations. No methods to successfully manage *Hirschmanniella* are known. The important nematodes of vegetables are root-knot species. In Nepal, *M. graminicola*, *H. oryzae* and *H. mucronata* are associated with rice when followed by wheat, winter legumes, corn and summer legumes. In upland systems, where corn, wheat, different legumes and vegetables are grown, different nematode species are reported. *Meloidogyne* is reported from almost vegetables, legumes, fruit, ornamentals and weeds. *M. aranaria*, *M. incognita* and *M. javanica* are limited to Terai (plains) and *M.*

hapla to the hills. *Tylenchulus semipenetrans* is found associated with citrus crops causing slow decline. Soil solarisation and long-term use of farmyard manure show encouraging results, while compost prepared from manures of different animal species, host resistance and chemicals in brinjal, tomato, gram and pigeon pea are used to control *Meloidogyne species*. In Pakistan, root-knot is the major problem. Root-knot nematodes are damaging vegetables and *M. graminicola* is associated with rice and wheat. Potato cyst nematode (*Globodera rostochiensis*) is restricted to northern Pakistan. Some success in the management of this nematode is achieved by growing turnips, carrots and peas before potato crop, thereby lowering the nematodes population almost 70%. Trap cropping is tested and tobacco is reported to induce significant amount of hatch. Other important nematodes are *R. similis* in banana and *T. semipenetrans* in citrus. In vegetables root-knot is one of the major problem in almost all the vegetable growing areas of Pakistan, *M. incognita*, *M. javanica*, *M. arenaria* are found in the plains whereas *M. hapla* is found in the hills. Most farmers have small land holdings and cannot afford chemical controls. *Paecilomyces lilacinus*, *Pasteuria penetrans*, and *Verticillium* sp. are being tested, as well as neem products for the management of plant-parasitic nematodes, particularly root-knot nematode.

043 Shared signal transduction pathways lead to nematode parasitism, rhizobial symbiosis and programmed plant development

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During the compatible parasitic interaction, root-knot nematodes (*Meloidogyne incognita*) face many challenges to successfully infect their host, including avoiding host defences, and redirecting host gene expression to establish the complex structures of giant cells, central to the parasitic relationship. Conceivably, rhizobial bacteria (*Sinorhizobium meliloti*) symbionts face similar challenges during establishment of nitrogen fixing nodules. Our findings of similar pattern of gene expression in giant cells and nodules of two transcription regulators required for the establishment of shoot and root meristems (PHAN and KNOX) of *ccs52*, which

encodes a mitotic inhibitor responsible for nuclear endoreduplication, and *ENOD40*, which encodes an early, nodulation mitogen, suggest a model in which inputs from nematodes and rhizobia elicit a developmental pathway. This developmental pathway converges at PHAN and KNOX to modulate phytohormones, and subsequently diverges to form giant cells, nodules and meristems. Gene ablation experiments in tomato further showed that *PHAN* is necessary for giant cell formation, and is epistatic to *KNOX*. Our recent finding of a strong homologue of *S. meliloti nodL* in root-knot nematode suggests similarities between the endosymbionts themselves, and an intriguing possibility is that nematode and rhizobia produce similar signalling molecules to suppress host defence responses and/or to induce novel plant structures.

044 Syncytium development: the result of a sophisticated manipulation of plant cells by cyst nematodes

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Potato cyst nematodes have evolved a sophisticated way to parasitise their host plants. They migrate intracellularly through the root, and an outer cortex cell is selected as a starting point for syncytium formation. The recruitment of plant cell wall-degrading enzymes by the nematode results in an expansion of the syncytium towards the vascular bundle via a so-called cortical bridge. A local accumulation of auxin is crucial for syncytium development. Analysis of cellulase expression patterns in tomato revealed that two auxin-inducible members (*LE-Cel7* and *LE-Cel8*) were specifically up-regulated during the onset of syncytium development. Intriguingly, nodule formation in legumes starts in the cortex, proliferates towards the stele and is also accompanied by a local accumulation of auxin. This process is preceded by the expression of *ENOD40*. To see whether this gene is also essential for syncytium development, the role of *ENOD40* was investigated in the non-legumes tomato and *Arabidopsis* upon cyst nematode infection. To unravel the

molecular mechanisms that underlie syncytium induction more thoroughly, a high throughput approach is needed. cDNA-AFLP was used to monitor the expression of auxin-regulated genes in cyst nematode-infected tomato roots, and some preliminary results will be presented.

045 Cell cycle genes as markers to study the ontogeny of nematode feeding sites in plant roots

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Research on the ontogeny of feeding sites induced by sedentary plant endoparasitic nematodes has been in focus already for many decades. Nematodes induce root cells to de-differentiate into large multinucleated and cytoplasm-dense feeding cells. We have used *Arabidopsis thaliana* as a model host to study cell cycle progression in nematode feeding sites induced by the root-knot nematode *Meloidogyne incognita* and the cyst nematode *Heterodera schachtii*. Cell cycle markers were used to monitor how nematode feeding sites develop and what the initial signals are that trigger certain root cells to evolve into giant cells embedded in a gall, or into a syncytium. Tritiated thymidine incorporation experiments were applied to monitor DNA synthesis, which was used as a marker for the S phase of the cell cycle. Extending our analysis, the expression pattern of two CDKs and two mitotic cyclins were examined. The *cycB1;1* gene was used as a marker for the G2 phase, the *cdc2bAt* and *cycA2;1* for the S and G2 phases whereas the *cdc2a* gene was used as a marker for all cell cycle phases. Nematode infected seedlings were also treated with two cell cycle inhibitors (hydroxy-urea and oryzalin) to investigate the relevance of DNA synthesis and mitosis on the development of these feeding sites. A strong correlation was observed between initiation of feeding cells by both root-knot and cyst nematodes and the induction of DNA synthesis and the expression of particular cell cycle genes.

Our results show that endoreduplication and mitotic cycles play a role during the root cell de-differentiation process caused by nematode infection.

046 Characterisation of plant genes involved in giant cell formation induced by root-knot nematodes in *Arabidopsis thaliana* and *Medicago truncatula*

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To identify plant genes involved in giant cell formation induced by the root-knot nematode *Meloidogyne incognita*, a promoter trapping strategy was developed in *Arabidopsis thaliana*. We identified a gene, *NEC*, early up-regulated in the giant cells. During root development, this gene is expressed in meristems including initiation sites of lateral roots. This gene encodes a calcium-binding protein with an EF-hand motif. In order to investigate its function, analysis of homozygous *nec* plants and plants over-expressing sense or anti-sense gene were carried out. The role of *NEC* in signal transduction will be discussed. Secondly, we investigated whether nitrogen-fixing nodules and giant cell formation induce distinct or overlapping regulatory pathways in *Medicago truncatula*. We studied expression patterns of nodule-expressed genes after infection with *M. incognita*. Analyses of promoter-GUS fusions indicate an accumulation of the cell cycle gene *CCS52a* in giant cells while the early nodulin gene *ENOD40* expression was detected only in cells surrounding the giant cells. Interestingly, on transgenic plants over-expressing *enod40*, a significantly higher number of galls was formed. In addition, results obtained on microarray analysis will be discussed. Taken together, these results suggest that certain events might be common between giant cell formation and nodule development.

047 Molecular markers for resistance to cyst nematodes in potato

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A mapping strategy based on catalogued, chromosome-specific AFLP markers facilitated the localisation of genes for resistance against potato cyst nematodes. The genes *Gpa2*, *Grp1*, *Gpa3*, *Gpa5* and *Gpa6* could relatively easily be mapped with this AFLP catalogue. Fine mapping showed that most of these genes are located in genomic regions harbouring also resistance genes for other pathogens. Especially, *Grp1* and *Gpa5* on chromosome V and *Gpa3* on chromosome 11 seem to be located in 'hotspots' for resistance. Remarkably, both qualitative and quantitative resistances map to these resistance gene clusters indicating that they might be controlled by similar molecular mechanisms. Dissecting resistance in potato will in the near future be accelerated by the availability of an ultra dense genetic map. Using an offspring of about 100 individuals, approximate 10 000 AFLP markers have been placed on the map of potato. The possibilities for marker-assisted breeding in potato will be discussed.

048 The identification and development of resistance to *Meloidogyne arenaria* in groundnut

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Highly effective resistance to *M. arenaria* in groundnut was first discovered in diploid wild *Arachis* spp. Development of a synthetic tetraploid interspecific hybrid (*A. batizocoi* × (*A. diogoi* × *A. cardenasii*)) that was cross-compatible with cultivated groundnut was the first step towards achieving a resistant cultivar. A backcross breeding programme was used to introgress the resistance into agronomically acceptable groundnut genotypes. The first cultivar (COAN) released from this breeding programme was selected from the fifth backcross generation. Yields of COAN in fields infested with *M. arenaria* were 20-120% greater than those of susceptible

cultivars, but in non-infested fields yield of COAN was only 80-90% of the susceptible cultivars. The cultivar NemaTAM was released from the seventh backcross generation and has yields equal to that of susceptible cultivars in non-infested fields. In fields infested with *M. arenaria*, yields of NemaTAM are greater than those of COAN. Resistance in COAN and NemaTAM is inherited as a single dominant gene. The resistance in the synthetic triple species hybrid is inherited as one dominant gene and one recessive gene. Thus, at least one additional resistance gene appears to exist in a genotype cross-compatible with cultivated groundnut that can be used to increase the durability of resistance.

049 Global importance of cyst (*Heterodera* spp.) and lesion nematodes (*Pratylenchus* spp.) on cereals: a review of yield loss studies, populations dynamics and progress of the use of host resistance for nematode control using traditional methods with the application molecular tools

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The sedentary cereal cyst nematode (CCN, *Heterodera* spp.) and the migratory endoparasitic root lesion nematode (RLN, *Pratylenchus* spp.) have a global distribution and are economically important to cereal production systems worldwide. Several species of CCN and RLN cause yield loss on cereals; however, morphologically these genera are difficult to identify at the species level. The population dynamics and plant damage thresholds in relation to yield loss will be discussed and compared for both types of nematodes. Resistance is one of the major control measures and offers the possibility to reduce populations of nematodes below damaging thresholds. Through extensive screening work, several sources of plant resistance at various levels have now been identified for both

CCN and RLN from landrace materials and conventional germplasm. CCN has been more extensively studied and most of the resistant sources currently identified possess single dominant genes. The introgression of this material into wheat breeding programmes will be discussed, particularly with reference to the application of molecular tools. With further understanding of the distribution and importance of both CCN and RLN, the usability of these resistant sources should reduce losses caused by these nematodes.

050 Resistance in soybean to soybean cyst nematode, *Heterodera glycines*

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Soybean cyst nematode (SCN), *Heterodera glycines*, was found in the USA in 1954, and breeding programs soon were initiated. The source of resistance in the first resistant cultivars was Peking, which was obtained in China. Subsequent sources of resistance incorporated into commercial cultivars include, PI88.788, PI90.763, PI209.332, all of which also originated in China. The high level of genetic variability within and among populations of SCN has proved problematic to soybean breeders and to farmers. In 1991, germplasm was released that incorporated resistance from PI437.654, which was collected in the Amur region of Russia and highly resistant to most populations of SCN. Classical breeding techniques were used exclusively until the late 1990s when marker-assisted selection using PI437.654 resistance was developed and is now being utilised in several breeding programs. Genes, *rhg₁–rhg₃*, and *Rhg₃–Rhg₅*, have been reported to be involved in expressing resistance in soybean to SCN. Some genes are linked and multiple alleles may be involved in expressing resistance. Monoculture of resistance has not proven durable, but gene deployment may offer long-term sustainable control of SCN.

051 Sustainability and mechanisms for durable use of resistance to control sedentary nematodes

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Evidence from three cyst nematode crop pests (of small grain cereals, *Heterodera* spp.; soybeans, *H. glycines* and potatoes, *Globodera* spp.) will be used to emphasise the significance of the relationship between the zone of plant nematode co-evolution and the cropping areas in which resistance is used. The impacts of these relationships on durability of resistance provide important lessons for the two approaches likely to be of value in enhancing durability in agriculture. These are either seeking to imitate sustainable natural metapopulation dynamics or an intensive on-going management of gene deployment. Both are technically feasible with modern plant breeding technologies, but the latter requires substantial continuing R&D investments, only likely to be justifiable in intensive agricultural systems where nematodes cause prominent and economically significant crop loss. This latter situation is likely to be more widespread in relation to root-knot nematodes, and an attempt will be made to consider how the biology and co-evolutionary histories of these nematodes may indicate approaches to use and management of resistance so as to enhance its durability.

052 Breeding for durable resistance to RKN in perennials: a European initiative for *Prunus* rootstocks

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Breeding for durable resistance in *Prunus* rootstocks is a challenging task since the long generation time increases the risk for resistance breaking. The breeding strategy is based on the selection and pyramiding of major genes conferring when possible a high-level, wide-spectrum and heat-stable resistance. Screening among *Prunus* species and accessions for appropriate resistance sources was performed through a first EU project from 1993 to 1997. Some Myrobalan (M) plum accessions resisted all RKN species, even under durable inoculum pressure and high temperatures, due to single major genes with a complete spectrum, termed *Ma*, recently considered as allelic. By contrast, peach (P) and almond (A) sources expressed near-complete or incomplete spectra. In a second EU

project (1999-2003), breeding for a new generation of interspecific rootstocks, of the type $M \times P$, $M \times A$ and $M \times (P \times A)$, based on the pyramiding of one *Ma* allele and *R* genes from peach or almond is being performed for all *Prunus* species. In order to develop marker-assisted selection (MAS), location of the *R* genes using SSR markers from a European reference *Prunus* map and a BSA positional approach are carried out. *Ma* (LG7) and at least one peach gene (LG2) are independent. Several SCAR or AFLP markers located less than 0.5 cM or cosegregating with the *Mal* allele are available for MAS of all interspecific crosses.

053 Molecular phylogenies of plant and entomoparasitic nematodes: congruence and incongruence with morphological and biological data

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One of the exciting developments in nematology during the last years has been the application of nucleic acid data analysis to problems of systematics of certain groups. In many cases, molecular data confirm the monophyly of species groups recognised by morphological studies. In other cases, the molecular data reveal taxa of which the phylogenetic relationship based on the morphology seems to be problematic. Molecular data put more light on problems of species boundaries and co-evolution of nematodes with their hosts. The early presumption that phylogenetic trees based on molecular data would more precisely reflect the true phylogeny than morphological data seems to be wrong. Analyses of influence of alignment or tree building methods on phylogenies showed that molecular data could be subjected to many problems. Congruence and incongruence of results of analyses of molecular and morphological data sets and the possible reasons for conflicts are discussed with examples of plant-parasitic nematodes from the family Heteroderidae, Anguinidae, Longidoridae, and entomoparasitic nematodes of the Tylenchida and the families Steinernematidae and Heterorhabditidae.

054 Effect of Oxycom on growth of tomato and reproduction of *Meloidogyne incognita*

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Single or multiple applications of Oxycom, peroxyacetic acid plus various biocontrol agents, were compared on 15-day-old susceptible tomato inoculated with 1500 second stage juvenile (J2) *M. incognita*. Forty pots were dipped into 2500 ppm (v/v) Oxycom, and 20 in water. Twenty of the 40 pots received four additional Oxycom applications at 10-day intervals. Plants were harvested at 60 days after inoculation to assess the effect of treatments on plant growth, nematode development and reproduction. A single treatment of Oxycom just prior to nematode inoculation significantly increased tomato top weight but not other growth parameters. Associated with plant growth benefit was a significant increase in the number of root-knot females. Multiple treatments of Oxycom significantly reduced leaf area, top weight and root weight while significantly increasing the number of galls, females and J2 per plant or per g root. This study demonstrates that Oxycom stimulates plant growth and earlier fruiting while hastening nematode development. Proper timing and frequency of applications is important. No treatment reduced nematode population levels in this 60-day trial.

055 A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa

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We recently proposed a new classification for the phylum Nematoda, based primarily on phylogenetic hypotheses resulting from small subunit rDNA sequence analysis. The strength and weaknesses of this new system are discussed with particular regards to ranks and names of nematode clades. As almost 2 years have passed since we finished writing the original manuscript, we also provide a timely update on the implications of phylogenetic analyses conducted in the past 2 years, and improve

or correct our system where appropriate. We emphasise the importance of disentangling the earliest branches of the nematode tree, and discuss various scenarios with some tantalising implications for our understanding of the morphology and ecology of the earliest nematodes.

056 Measures to avoid new introduction of quarantined nematodes into Brazil and the spread of non-regulated quarantine nematodes

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The Brazilian National Plant Protection Organization is issuing a number of new actions and regulations to avoid introductions of quarantine pests in the country and among them quarantine nematodes. A new quarantine pest list based on technical justification and pest risk analysis is available; an integrated electronic system to detect pests is starting to be used in the main points of entry in the country; new laboratories, quarantine stations and centres for conducting pest risk analysis are in the process of accreditation to facilitate inspections at the points of entry. All the 27 rules with general requirements to import commodities from all countries are in the process of being replaced by measures that will be based on pest risk analysis related to the origin and to the product. Besides that, an expert group has been working for more than 3 years to determine the tolerance levels for non-quarantine regulated pests for plants for planting, and new regulations on that matter will be in force soon.

057 Dilemmas for the quarantine diagnostician and taxonomist in the 21st Century

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During the last century, many nematologists were involved in the golden era of identification and many type specimens were stored in key institutes to serve as reference points for future diagnosticians and taxonomists. The basis of identification was the ability to distinguish a new species from all others that had preceded it by recognising significant variation of critical morphological characters. The demise of such skills and the lack of re-

sources for curation have led to concerns for the whole basis of the quarantine identification, despite the recent development of molecular techniques which have been hailed as the resolution to this problem. However, there remains a lack of robust, reliable methods using DNA for the immediate distinction of listed species from all others that might reasonably occur in world trade. Increasing demands for quality accreditation procedures and the refinement of computer-based imagery are stimulating new ways of providing evidence of statutory findings, and may prove to be the salvation of many national collections, turning many into a world-wide resource.

058 Quarantine nematodes or invasive alien species?

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The Convention on Biodiversity (CBD) has recently been developed as a global agreement with the overall aim of ensuring the maintenance of biological diversity throughout the world. One of the main thrusts of the CBD is to prevent the transfer, by human activity, of species of plants or animals from one part of the world to another. Species that are transported (or transportable) are referred to as 'invasive alien species' and can be any type of plant or animal species that could disrupt ecosystems, habitats or species. As such, the concept of invasive species overlaps with the long-established 'quarantine pest'. Plant quarantine services in many countries are reacting to these developments by expanding the range of organisms for which they have responsibility, beyond the pests of economically important crops. The consequences for nematology will be discussed and could include the need for study of nematode pests of wild plants, the ecology of non-plant-parasitic nematodes and the effects of biocontrol nematodes on natural ecosystems.

059 Organic amendments for the management of phytonematodes: enhancing natural biological control

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Organic amendments are useful in plant disease management because they suppress pathogen populations through production of toxic substances during decomposition, and by alterations on soil microbial ecology. However, little information is available on the mode of action of these materials, especially how they affect activities and populations of soil microorganisms. Interactions between antagonistic microorganisms and plant pathogens are widespread in nature and organic amendments may be useful to enhance naturally occurring biological control and reduce diseases caused by nematodes. Experiments were conducted during the last 5 years in Puerto Rico that showed the value of organic matter to suppress nematode populations under tropical conditions. Changes in populations of microorganisms associated with use of organic matter were studied. The use of organic amendments improved the activity of microorganisms associated with the rhizosphere of tropical crops. We are focusing on the exploitation of microorganisms promoted by organic amendments as sources of biological control agents for phytonematodes. This approach may be a practical tool to reduce the dependence on synthetic pesticides and to mitigate the environmental contamination produced by the intensive use of chemicals.

060 Biofumigation and nematode control in the Mediterranean region

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Biofumigation is defined as the effect from the gases produced during the biodecomposition of organic matter for controlling plant pathogens. Biofumigant action is indicated as a general property of organic matter, of animal manure as well as green manure or agroindustrial remains. Its effectiveness is similar to conventional fumigants, at the same time as it improves soil chemical, physical and biological characteristics. The scientific bases for biofumigation are established, differentiating it from the use of organic matter as an amendment, with regard to the characteristics of the biofumigant

and the method of application. Cost of transportation is shown as its principal limiting factor. Differences are established between it and other control alternatives such as solarisation, flooding and biosterilisation, although they can be considered complementary. The effectiveness of biofumigation increases with time, when included in an integrated production system, which should take into account the specific characteristics of areas with a Mediterranean climate. It is concluded that biofumigation is a control alternative based on the use of local resources and that it reduces environmental impact from agriculture and increases the quality of agricultural production.

061 Soil solarisation, nematicide and chicken compost for root-knot nematode control in carrot crops in Brazil

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A field experiment was performed in sandy soil, naturally infested with *Meloidogyne incognita* and *M. javanica*, in Piracicaba, SP, Brazil, to assess the effectiveness of soil solarisation, chicken compost (20 t/ha), carbofuran 100G (40 kg/ha), and combinations of these treatments for the control of root-knot nematodes in carrot cv. Aline. Soil solarisation was performed over 69 days during the warmest months of summer of 2000/2001, using a 100 µm polyethylene plastic film as soil covering. The soil temperature in solarised plots was 8-12°C higher than in the non-solarised. Maximum soil temperatures were recorded in solarised soil combined with chicken compost and reached 77, 56 and 46°C, at depths of 5, 10 and 20 cm. Population densities of *M. incognita* and *M. javanica* were controlled by both soil solarisation alone and combined with chicken compost or carbofuran. Carrot cv. Aline yield was significantly increased by combination of solarisation and chicken compost plus nematicide application before planting.

062 Green manure amendments and management of root-knot nematode on potato in the Pacific Northwest of USA

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Meloidogyne chitwoodi, a serious pest of potatoes in the Pacific Northwest (PNW) of the USA, blemishes potato tubers and renders them unmarketable. Currently, this nematode is effectively controlled by an environmentally hazardous and costly fumigant, Telone II. As an alternative to fumigation, green manure cover crops including rapeseed, white mustard, and sudangrass, and the organic amendments canola seed meal, and milkweed seed meal, have been used to control *M. chitwoodi* in the PNW. Green manures and seed meals alone have reduced the nematode impact on potatoes by 50-80%. In addition, the above amendments have been used successfully in combination with contact nematicides, such as ethoprop, and provided control against *M. chitwoodi* at a level comparable to soil fumigation. Our studies demonstrated that the use of green manures together with 2 years of crop rotation controlled *M. chitwoodi* to acceptable levels without the use of a nematicide. However, studies from other regions of the USA have shown that the amendments failed to control other species of root-knot nematodes. These conflicting results indicate that amendments should be utilised according to local environmental conditions. Management with green manures may be more successful in short or cool growing seasons where nematode damage potential is lower. In addition, we found that green manures improve soil physical characteristics, especially water infiltration and penetration resistance.

063 Impact of paper mill residual amendments on *Pratylenchus penetrans* in a vegetable rotation

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Amending soil with paper mill residuals is a strategy to improve soil fertility and minimise the loss of nutrients and pesticides. We initiated an experiment in 1998 to study short- and long-term changes in the chemical, physical and biological properties of soil after adding paper mill residual amendments to the plots every spring. The treatments were two rates of fresh paper mill residuals, paper mill residuals composted alone, paper mill residuals composted with bark, and a non-amended control. The experiment, a randomised complete block with five replications of each amendment treatment, was planted with potato in 1998, snap bean in 1999, cucumber in 2000, and potato in 2001. Population densities of *Pratylenchus penetrans* were 88 nematodes per 100 cm³ soil at the start of the experiment. Root lesion population densities declined in the control plots and stayed the same or increased in amended plots from 1998 to 2001. In November 2001 *P. penetrans* was most abundant in the 'high rate-composted alone' treatment. Incidence of symptoms indicative of the potato early dying disease were also most severe for this treatment. Amended plots had a higher capacity for water retention and increased organic matter.

064 Nonchemical control of root-knot nematodes (*Meloidogyne complex*) in glasshouse conditions and *Xiphinema index* in the vineyard

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A group of scientists from the university of Sofia and the Experimental Station in Septemvri investigated more than 10 years plan residues and manufacturing industries waste products as means to avoid agrochemical for control of soil pathogens (nematodes). The last 3 years we continued the experiments with substrates and extracts from waste products of microbial industry (Biovet factory – Peshtera). The investigated products are obtained on the base of an original recycling technology. The results showed a very favorable effect and advantages for *Meloidogyne complex* as a significant reduction of L₂, a later and weaker infection of the tomato and the cucumber roots and the total yield increased up to 62%. These substrates reduced

very strongly the population density of *Xiphinema index* and stimulated several times the plant crowing and the yield. The investigated products pointed to the new possibilities of the nonchemical control of the parasitic nematodes.

065 Organic amendments as therapeutic treatment of guava trees (*Psidium guajava* L.) infested with *Meloidogyne incognita* in Zulia State, Venezuela

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The biofumigant effect of organic amendments as a therapeutic treatment on 6-year-old guava trees infested with root-knot nematode was evaluated at the Centro Frutícola del Zulia-CORPOZULIA. Treatments included control (T1), 30 kg (T2), 60 kg (T3) of goat manure per tree, 30 kg (T4) and 60 kg (T5) of composted sugarcane bagasse, and combinations of 15 kg (T6) and 30 kg (T7) of each, applied every 3 months. Results after 10 months of evaluations indicated no statistically significant differences in *Meloidogyne incognita* populations. However, comparing the first and third sampling dates, a reduction in populations could be seen, with T7 having the fewest (827 second stage juveniles (J2)/100 g soil and 10 g roots) and T1 having the most. Regarding phenological characteristics, the highest value for leaf presence was observed in T3 (60%), branching and flower buds in T7 (25 and 14%, respectively), and fruiting in T5 (24%). Preliminary results of this study suggest we should continue the evaluation of these organic amendments as an efficient alternative for integrated pathogen control in fruit horticulture.

066 The symbiosis of *Wolbachia* bacteria and filarial nematodes

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Wolbachia bacteria appear to have evolved a mutualistic symbiosis with their nematode hosts. This is in contrast to the closely related *Wolbachia* endosymbionts of arthropods, which display a diverse range of associations. Within the Nematoda, *Wolbachia* appear to be restricted to filarial parasites, and molecular phylogenetic analysis suggests nematodes probably acquired their symbionts due to their intimate association with their arthropod vectors some 100 million years ago. Since then, however, there is no evidence for recent horizontal transmission between nematodes and arthropods. *Wolbachia* have so far been found in more than 20 species of filarial nematode, with only two species appearing to be uninfected. Of those with *Wolbachia*, the infection appears to be ubiquitous in all individuals, developmental stages and populations throughout their global distribution. The bacteria reside in vacuoles and are restricted to the lateral cord cells and developmental stages within the female reproductive organs and intrauterine developmental stages as a consequence of their vertical transmission via the egg. As filarial nematodes are major pathogens of humans throughout the tropics, our research has focused on the contribution of *Wolbachia* to disease pathogenesis and as a novel target for antibiotic therapy. These studies show that *Wolbachia* endotoxin-like activity is the principal cause of inflammatory filarial disease. Antibiotic therapy of filarial nematodes and removal of *Wolbachia* results in a complete and long-term block of embryogenesis and the eventual death of the nematode. Recent studies in our laboratory suggest that the symbionts also contribute to the evasion of immunity in mammalian and vector hosts and so may be responsible for the long-term survival of filarial nematodes.

067 Coevolution between *Fergusobia* and *Fergusonina* mutualists

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The contemporary associations between *Fergusobia* nematodes (Neotylenchidae) and *Fergusonina* flies (Fergusoninidae) represent the only putative example of nematode and arthropod-associated mutualism. The nematode appears to induce a bud or leaf gall that both organisms use while the fly provides gall maintenance, dispersion, and sustenance for the nematode. Based upon molecular analysis, this is a potentially large monophyletic radiation of more than 50 mostly undescribed species of nematodes and flies that exhibit a high degree of host specificity within the Australasian Myrtaceae (mostly Lepidospermoideae; e.g., *Eucalyptus*, *Corymbia*, *Angophora* and *Melaleuca*). The Agromyziidae (with no known nematode associates) is the putative sister group to the Fergusoninidae. *Fergusobia* could have evolved from parasitic nematodes similar to present day *Howardula* that parasitised the cyclorrhaphan stem ancestor of *Fergusonina* flies and developed a plant-parasitic association that provided a mutual benefit to fly host and nematode. Alternatively, *Fergusobia* could be related to present day anguinids that produced aboveground galls and developed an association with an agromyzid ancestor. In either case, the evolution of the host-parasite interaction requires that host resistance and virulence be moderated. Contemporary examples of commensal and parasitic associations between nematodes and insects are used to generate hypotheses about how such an association could have evolved and is maintained.

068 Evolutionary attenuation of virulence in *Drosophila*-parasitic nematodes

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Virulence is of central importance in host-parasite interactions, yet little is known about how it changes over extended evolutionary periods. Four species in the *testacea* species group of *Drosophila* were experimentally infected with sympatric and allopatric nematodes in the *Howardula aoronymphium* species complex, and the effect of parasite infection on host fitness was quantified. The fertility of infected *Drosophila* females appears to be determined solely by the host, with some species being sterilised and others not, regardless of the source of the nematodes. Reductions in host adult lifespan and

male fertility, however, depend on both the host and the parasite. In particular, survival of two *Drosophila* species was drastically reduced when infected with an allopatric parasite. Thus, virulence is evolutionarily labile in associations between *Drosophila testacea* group species and their *Howardula* parasites. The high level of virulence manifested in some host-parasite combinations is due to a lack of resistance in the hosts, perhaps as a result of recent host colonisation by *Howardula*. These results provide evidence for a continuum of host-parasite interaction, with virulence initially high, followed by evolution of the parasite to reduce the rate of host mortality, and evolution of the host to resist parasite-induced sterility.

069 R-gene homologues in potato confer resistance against distinct pathogens: a virus and a nematode

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Genome analysis of a single R-gene cluster in potato revealed two highly homologous resistance genes, *Gpa2* and *Rx1*. The two genes confer resistance to fully unrelated pathogens, namely the potato cyst nematode *Globodera pallida* and the potato virus X. To get more insight into the evolutionary relationship between the two resistance genes, three new homologues mapping to the same cluster on chromosome 12 of the diploid potato clone SH83 were identified by using a single LRR-based primer pair. cDNA analysis resulted in the identification of the ORFs for two out of the three new homologues. To investigate the diversification process within this cluster, DNA sequence data were analysed; the remarkable sequence conservation between the effector domains of *Gpa2* and *Rx1* points at the activation of a similar resistance mechanism. On the other hand, infection studies and histological observations revealed a relatively slow resistance response for *Gpa2*, while *Rx1* results in an extreme resistance. To gain more insight in the mechanism underlying nematode and virus resistance structure-function analysis of GPA2 and RX1 are currently under investigation.

070 Resistance to soybean cyst nematode: an *Xa21*-like gene family that requires possible dimerisation for signal transduction

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In the soybean (*Glycine max* (L.) Merr.) cv. Forrest, disease resistance inheritance to the soybean cyst nematode *Heterodera glycines* Ichinohe (SCN) race 3 (Hg₀) was demonstrated to be digenic, and conditioned by the presence of both the *Rhg1* and *Rhg4* genes. A high-density genetic map was constructed for the two chromosomal regions carrying resistance loci to SCN. An integrated physical genetic map was constructed around the *Rhg1* and *Rhg4* loci. Candidate gene sequences for *Rhg1* and *Rhg4* have been isolated from soybean cv. Forrest BAC clone 73P6 and 100B10. Shotgun gene sequencing and cDNA hybridisation identified two genes with high homology to the *Xa21* rice disease resistance gene and to an *Arabidopsis* receptor-like kinase family, which contain three functional domains each. Domains within the 854 residues of *Rhg1* show the presence of 12 extracellular leucine rich repeats, a trans-membrane spanning domain and a kinase domain. *Rhg4* shares the same structure, with a long peptide sequence of 894 residues and has low amino acid homology to the *Rhg1* peptide. An active-dimerisation form of the *Rhg1*-*Rhg4* protein is suggested from the interaction studies for the activation of the signal transduction pathways for resistance to SCN.

071 Functional analysis of the *Mi-1* gene

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The tomato gene *Mi-1* confers resistance against several species of root-knot nematodes and against specific isolates of potato aphid. *Mi*, like many plant resistance genes, encodes a protein with an apparent nucleotide binding site and a C-terminal string of loosely conserved leucine-rich repeats. Resistance is characterised by a localised necrosis or hypersensitive response. The phenotypes of domain swaps and single nucleotide

changes between *Mi-1.2* and *Mi-1.1*, a homologue that does not confer resistance against nematodes, have been tested in two assays. Nematode resistance was tested in transgenic roots, and ability to induce a hypersensitive response was tested after transient expression in *Nicotiana benthamiana* leaves. Analysis of reciprocal swaps has identified regions of the gene that are important for nematode recognition and for signaling the defence response. Our results indicate that intramolecular interactions of the *Mi*-protein are involved in its regulation. We have also used the transient expression system to investigate the role of signaling molecules and plant hormones in the resistance response.

072 Understanding the genetical and molecular basis of (a)virulence in the root-knot nematode *Meloidogyne incognita*

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Plant resistance is currently the most effective and environmentally safe method to control root-knot nematodes. Resistance genes generally act by inducing an hypersensitive reaction at or near the infection site, that prevents the parasite installation and/or reproduction. However, the emergence of virulent biotypes able to overcome the plant resistance genes may constitute a severe limitation to this control strategy. To investigate the molecular determinants of (a)virulence in root-knot nematodes, we have been developing differential strategies based on the selection and subsequent comparative analysis of pairs of *Meloidogyne incognita* near-isogenic lines, just differing in their ability to reproduce or not on tomatoes bearing the *Mi* resistance gene. The objective of these approaches is to identify genes differential or differentially expressed between the avirulent and virulent lines. Recent results will be presented, and discussed in relation with the mode of reproduction of the nematode, *i.e.*, mitotic parthenogenesis. Elucidating the genetical and molecular mechanisms involved in the selection process of virulent nematodes from avirulent ones should have important consequences for the management and durability of natural resistance genes in the field.

073 Nematode biodiversity research – current status and future promise

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The presentation attempts an evaluation of the current status of nematode biodiversity research. Starting from an overview of how nematology is integrated in international biodiversity programmes, and from a synopsis of biodiversity-related information which has been made generally accessible to the scientific public, three major questions are addressed: *i*) what are the drivers of nematode diversity; *ii*) how tightly is nematode diversity linked to environmental factors; and *iii*) what can we therefore expect to be the information content of nematode diversity? Knowledge gaps and research priorities are specified and discussed.

074 Nematode survey in Costa Rican conservation areas

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The Instituto Nacional de Biodiversidad (INBio) is a non-profit scientific institution with social orientation, created in 1989, whose main mission is to promote a new awareness of the value of biodiversity and, thereby, achieve its conservation in Costa Rica. The nematode subprogram was initiated officially in 1998 in close cooperation with the Universidad Nacional of Heredia and financed by the Netherlands government. We conducted a nematode inventory in five conservation areas of Costa Rica, chosen because they contain the most representative and diverse ecosystems of the country. Several hundreds of samples were collected and referenced with a GPS navigator. All contrasting morphotypes from each sample were represented and stored permanently on Cobb's slides. The reference nematode collection of INBio contains 17 757 specimens, distributed as follows: Dorylamida (39.3%), Rhabditida (18.7%), Araeolaimida (10.1%), Tylenchida (9.3%), Enoplida (9.1%), Mononchida (8.2%), Chromadorida (2.1%), Aphelenchida (1.9%), Monhysterida (1.0%) and Mermithida (0.2%). Most of the collection is identified at genus level; only 7% is identified at species level of which

14 are new to science. The interaction with international taxonomists is essential to both train INBio staff and generate knowledge related to tropical nematode diversity.

075 Nematode diversity in Dutch soils, from Rio to Dutch Soil Quality Network (DSQN)

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Analysis of nematode fauna has been part of a Dutch monitoring network since 1993. It has the advantage that available comprehensive abiotic measures can be combined with biological characteristics. The monitoring network consists of 200 locations, representing ten major types of soil type/soil use combinations. Sampling locations are chosen in proportion to the form of land-use because the network aims to give a general picture of biological soil quality in the Netherlands. Seventy percent of the Dutch soil area is in agricultural use. Therefore, 180 locations are situated on different types of farms, with 40 sampled per year on a cycle of 5 years. After the biodiversity conference of Rio de Janeiro, the Dutch government ratified the treaty, and concluded that functional diversity aspects of non-natural soils need more attention and quality standards. In 1999, the biological soil monitoring was extended to a foodweb-based programme. The nematode fauna is an important component due to the ecological (feeding) groups that can be distinguished. In the second round of the monitoring programme, 100 biological farms were added to serve as a reference for regular farming systems. A method for comparison and quality assessment expressed in a Soil Quality Index is under development.

076 Nematode component of the Fauna Europaea project

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The Community Biodiversity Strategy of the European Commission provides a framework for the development

of Community policies and instruments to comply with the Convention of Biological Diversity. Fauna Europaea contributes by identifying and cataloging components of European biodiversity to serve as a basic tool for science and conservation policies. Fauna Europaea will assemble a database of scientific names and distribution of all living multicellular European land and fresh-water animals, including nematodes. Marine nematodes are included in a 'sister' project, the European Register of Marine Species (ERMS). In anticipation of a *Key to the European Nematodes*, the basal species list (*Nematoden van Nederland*) was extended to include the whole of Europe. Currently, the nematode database includes a total of 2500 species, including the full name, author, date, original genus, main synonyms and geographic distribution. Classification is based on the most recent, generally accepted standards. Published records or personal communications for material deposited and available in a public nematode collection must support database entries. Geographic information will be provided for each species according to ISO-TDWG standards; the reporting region will include Europe, Macaronesian islands, Cyprus, Franz Josef Land and Novaya Zemlya. Species recorded from non-field locations including glasshouses and aquariums will not be included.

077 Past and present status of nematode community indicators

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Differences in sensitivity of nematodes towards some chemicals have been recognised for nearly four decades. Initially, single species or taxonomic groups were considered, followed by parameters of whole communities to measure different properties of natural and disturbed terrestrial ecosystems. A short overview of nematode community indices will be presented, progressing from those considered traditional, relatively simple, and widely applied in environmental studies (*e.g.*, abundance, richness, diversity, similarity and constancy) to those considered more sophisticated (*e.g.*, Maturity index and its modifications) and specific to nematodes (*e.g.*, proportions among trophic groups). Emphasis will be placed on indices used most often. Their contribution to understanding the functioning of soil ecosystems (*e.g.*, nature of decomposition pathways, N-mineralisation) in different situations (*e.g.*,

enrichment, stress and succession) is critiqued. Applicability of indices to assessment of soil quality and associated sampling methods will be discussed relative to geographical scale, soil and vegetation type. Fundamental constraints and research priorities are identified: *i*) clarifying trophic affinity of some species; *ii*) integrating morphological, ecological and molecular approaches; and *iii*) intensifying long-term studies in natural and disturbed environments.

078 Nematode faunal analyses to assess food web enrichment and connectance

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The cp classification recognises that nematode taxa with anatomical and physiological commonalities are probably similarly adapted to specific environmental conditions. Functional guild analysis, which integrates cp-scaling with food sources, reveals that some guilds respond opportunistically to enrichment while others represent the presence of higher trophic connectance in the food web. This dichotomy was portrayed graphically in cp triangles as the proportional representation of enrichment opportunists (cp 1), general opportunists (cp 2) and taxa indicating higher connectance (cp 3-5). Confounded in the calculation of cp triangles is interdependence of the axes; proportionality of the three groupings to the whole nematode fauna requires that increase in food web structure (cp 3-5 taxa) concomitantly decreases the enrichment indicator (cp 1 taxa). The categorical separation of nematode taxa into five cp classes does not imply unit increments in *r* or *K* characteristics. We use body size and growth rates to weight the importance of enrichment indicators and estimates of corresponding food web connectance to weight the importance of structure indicators. We consider cp 2 taxa basal to both trajectories and calculate position along the enrichment and structure axes of a faunal diagram independently as the weighted ratios of the indicator and basal taxa.

079 Selection of sentinel taxa and biomarkers

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Nematode community indices would be more feasible for use in environmental monitoring programmes by reducing the number of genera that need to be enumerated and identified. This could be achieved by narrowing indices to include only sensitive or tolerant genera or species while eliminating ambiguous ones. Identification of sentinel taxa can be achieved by employing a combination of tools. For example, multivariate statistics can help identify taxa that demonstrate relative tolerance or sensitivity to physical and/or chemical/nutrient types of disturbance. Life history characteristics of taxa meeting these criteria can be verified in empirical studies to evaluate and recommend refinement in coloniser-persister weights employed in maturity indices. Verification of coloniser-persister values can be achieved using independent molecular biomarkers. Once sentinel taxa are identified, molecular diagnostic tools are possible. Availability of commercial kits then becomes approachable to non-specialists and cost-effective (time and labour) for implementing nematode bioindicators within large-scale environmental monitoring programmes.

080 A nematode reference database as an instrument for biological soil assessment: a case study from the Netherlands

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Traditionally, assessments of ecological risks of soil pollution are based on chemical analyses compared to a set of standards. Recently, in the Netherlands, investigations were initiated to explore the possibility of including biological indicators in ecological risk assessment procedures. Nematodes are among the soil fauna groups that have promising characteristics as indicators for soil quality (*i.e.*, ubiquitous, high species diversity, sensitive to various kinds of soil disturbances, specific nematode-based indicators). However, interpretation depends on comparison with undisturbed controls or references. As local reference sites or data are often unavailable, there is demand for a nation-wide reference system. In the present project, we built a database (DAWACO Nematodes) of 1600 nematode fauna samples from which all taxa were identified. The samples were collected across the country, representing a variety of soil-, vegetation- and land-use types between 1985 and 2001. Samples from undisturbed or reference locations were analysed with multivariate analyses techniques to select clusters of samples (14 from terrestrial soils, six from aquatic sediments) with similar nematode fauna and habitat characteristics. Based on the statistical characterisation of the composition of taxa from a reference cluster, the probability of membership of any sample requiring assessment can now be calculated and evaluated.

081 Spermatogenesis and nematode phylogeny

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Data on sperm structure and spermatogenesis are frequently discussed in the analyses of the phylogenetic relationships inside many classes of the metazoan animals with the exception of the class Nematoda. One of the reasons for this situation is a lack of studies on the relatively primitive aquatic nematodes from several important orders. Our latest observations on sperm development in free-living nematodes from the orders Enoplida (suborders Enoplina, Oncholaimina, Tripyloidina), Chromadorida (suborders Chromadorina and Cyatholaimina), Desmodorida, Araeolaimida and Monhysterida, together with the data available on many parasitic species, allow definition of four main patterns of nematode spermatogenesis. Each pattern is distinguished

clearly by special way of development of aberrant organelles – membranous organelles (MO) and fibrous bodies (FB): *i*) MO and FB develop as the MO-FB complexes (most Rhabditia (Rhabditida, Strogylida, Ascaridida, Rhigonematida, Spirurida) and some of Chromadoria (Araeolaimida (including Comesomatidae) and Monhysterida); *ii*) MO and FB occur but their development is asynchronous and independent (some Enoplia: Enoplida (*Enoplus*, *Pontonema*); Dorylaimida (*Xiphinema*)); *iii*) MO do not appear, only FB are developed (Tylenchida from Rhabditia, Chromadorida and Desmodorida from Chromadoria); *iv*) FB do not appear, only MO are developed (Enoplia: Enoplida (*Anticoma*), Mermithida, Trichurida, Dioctophymida). Pattern of sperm development may be proposed as a new distinct cytological character for the analysis of the nematode relationships.

082 Phylogeny and ontogeny of free-living and parasitic nematodes

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Phylogenetic relationships between nematodes have always been discussed controversially. In recent years analysis of molecular data, particularly sequence data from genes coding for ribosomal RNA, have challenged some of the traditional taxonomic proposals based on morphological criteria. As a third means to identify characters of potential phylogenetic value, we are studying selected developmental processes during early embryogenesis. These include establishment of axial polarity, separation of soma and germline, assignment of cell fates and the pattern of gastrulation. Some of the existing differences can be readily identified by comparative analysis while others require experimental interference. A variety of free-living and also several parasitic species have been studied. Our results support the notion that the classical separation into Secernentia and Adenophorea is artificial, and that at least three supertaxa should be defined. In some cases we identified major differences between species considered as close relatives, questioning their present position in the phylogenetic tree. To reach a more lucent picture of nematode phylogenetic relationships, data from different

approaches have to be taken into account and the number of analysed nematode species must be increased.

083 Nematode embryology and its importance for the classification of higher nematode taxa

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Investigations on the embryology of nematodes of different taxa revealed the great variety of the development within the class. There are three principal patterns of nematode development. The first one is characteristic for nematodes of the order Enoplida. The cleavage of the enoplid eggs is variable. The bilateral symmetry forms relatively late, usually after the closing of the blastopore. The fate of the blastomeres is not determined strictly as is characteristic for other groups of nematodes. The second pattern is characteristic for nematodes of the Enoplia line of evolution with the exception of the Enoplida. This line includes the orders Monochida, Dorylaimida, Mermithida, Dioctophymida and Trichurida. The cleavage of the nematodes of the above-mentioned orders is bilateral with early determination of the fate of blastomeres. The key feature of the second pattern is the origin of the endoderm from the anterior blastomere of the two-cell stage. The third pattern is characteristic for the Chromadoria + Secernentia line. In this line, the egg cleavage is also bilateral and strictly determined, but the endoderm comes from the anterior blastomere of the two-cell stage. Three principal patterns of nematode development correspond to the main trend of nematode evolution and could be the base for a new nematode classification.

084 Division and fate of vulval precursor cells: is polymorphism an image of evolutionary divergence?

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In order to approach evolutionary processes of developmental mechanisms, we are studying development of different nematodes at the cellular level. The cell lineage of nematodes is mostly invariant for a given species, but varies between species. We have performed a microevo-

lutionary approach with the nematode vulva as a model system to define how a cell lineage varies during evolution. The vulva is formed by precursors in the ventral epithelium, called Pn.p cells. Each Pn.p cell has a specific fate, which is specified by cell interactions that are different in *Caenorhabditiselegans* and *Oscheius* sp. 1 CEW1. Two distinct networks of cell interactions can therefore lead to an identical pattern that forms a similar morphological structure. Moreover, the same vulva lineage characters that diverge between closely related species are polymorphic within a species (between laboratory strains). In order to understand evolutionary mechanisms of cell lineage variation, we performed a genetic analysis of P3.p division between strains of *C. elegans*. Recombinant inbred lines show intermediate levels for the lineage character. Polymorphism between two strains is therefore due to several loci.

085 Biocontrol – a route to market

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With nematicides declining in popularity for environmental reasons, and resistance not being a long-term solution due to the ability of nematode populations to overcome it, there is a need for an environmentally safe control measure that can give effective, long-term control of nematodes. Biocontrol has been studied for many years, with some encouraging results, and there is now mounting pressure to develop it towards practical application. I will be presenting the route I am taking to try and achieve this. It is notoriously difficult with biocontrol to obtain good, consistent data from small-scale experiments. Such data is best obtained from large, long-term trials, but to do this it is necessary to have large-scale production systems, preferably with industrial support. Most production companies, however, need to see good efficacy data from field trials before they will invest in new products. BioNem Ltd attempts to fill this gap between research and commercialisation by selecting effective agents (fungal parasites), developing suitable production systems, and conducting realistic field tests. The two fungi I am concentrating on are *Paecilomyces lilacinus* and *Verticillium chlamydosporium*.

086 The commercialisation of *Paecilomyces lilacinus* as an agent for the control of plant-parasitic nematodes

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Paecilomyces lilacinus is one of a number of fungi with nematophagous properties that have been examined over the past two decades for potential use as agents for the control of plant pathogenic nematodes in agriculture. While showing promise, their commercialisation has in the past been constrained by field results which have not delivered sufficient promise to attract commercial attention, as well as a certain lack of appreciation by farming communities at large of the economic significance of nematode infestation. The South African company, Biological Control Products (BCP) was established in 1995 with funding secured from private investors together with a substantial grant from the South African government. BCP's prime objective was to conduct the necessary applied research in areas of product formulation, field application and solid substrate manufacture which would lead to acceptance by the agricultural community and regulatory authorities of *Paecilomyces lilacinus* as an economically justifiable addition to strategies for the management of plant-parasitic nematodes. While the development of the manufacturing process and product formulation was largely conducted in-house, the research institutes attached to the Department of Agriculture were commissioned to assist with efficacy trials. BCP now has registration for the use of this biological agent sold under the brand name PI Plus, for the control of nematodes in bananas, papinos, tomatoes, tobacco and citrus. Further trials are in progress on other crops. A solid substrate manufacturing plant capable of processing up to 50 t per year has been in operation since 1997 and local farmers currently use PI Plus in integrated crop protection programmes.

087 *In vitro* culture of *Pasteuria penetrans*

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Pasteuria penetrans has been acknowledged worldwide as an effective biological control agent of plant parasitic nematodes. The inability to mass-produce this fastidious bacterium *in vitro* has been the barrier to making *P. penetrans* a marketable product. *Enterobacter cloacae*, a ubiquitous soil bacterium, has been found in close association with the cuticle of *Meloidogyne arenaria* females extracted from tomato roots. Co-cultures of *E. cloacae* and *P. penetrans* in simple nutrient broth have produced viable *P. penetrans* endospores. Filtrates from *E. cloacae* grown on simple nutrient broth are suitable culture media for both vegetative growth and sporulation of *P. penetrans*, producing viable, infective endospores. *In vitro* culture and therefore mass production of *P. penetrans* is now possible.

088 Development of multi-component transplant mixes for plant growth-promotion and disease suppression

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Research was undertaken to develop a biologically-based product that, when added to transplant mixes, would enhance plant growth, increase yield, and provide protection against pathogens. Studies at Auburn University in cooperation with Gustafson LLC tested organic components and plant growth-promoting rhizobacteria (PGPR) for effects on tomato growth and nematode viability. Organic components and PGPR were combined and evaluated. Gustafson LLC and USDA, ARS then entered into a Cooperative Research and Development Agreement (CRADA) for field-testing in Florida, where combinations of amended transplants and methyl bromide alternative soil treatments were evaluated. Significant increases in tomato and pepper growth, vigor, and transplant survival occurred with formulations of PGPR. One formulation reduced root-knot nematode galling and several improved pepper root condition. Four PGPR treatments reduced angular leaf spot lesions caused by *Pseudomonas syringae* pv. lachrymans, and gummy stem blight, caused by *Didymella bryoniae*, on watermelon. One PGPR treatment reduced root-knot nematode disease severity on muskmelon. The effects of bare root, plug, and PGPR amended plug transplants on growth and yield of strawberry showed that PGPR amended plugs had highest yields, and bare root transplants had lowest yields. This

cooperative research resulted in a commercial product, Bio Yield™, for use on transplanted crops.

089 Nematode movement through soil: simple responses in a complex environment

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In theory, similar responses to simple cues can guide ecologically different nematodes through the soil in completely different ways, when the unique characteristics of gas and solute diffusion, water movement, and temperature fluctuation in soil are considered in relation to subtle differences in response thresholds, rates of locomotion, and sensory adaptation. Validation of models of nematode movement within the diurnally fluctuating, vertical temperature gradients of soil confirmed that two root-parasitic species that respond similarly under static conditions on agar move in opposite directions in thermodynamically natural soil. Experiments on attraction to CO₂ in soil showed that gradients inducing maximal nematode accumulation at a point source are achieved at optimal soil moisture for nematode movement, are not generated in saturated soil, and can be sustained for 24 h in soil with a total gas volume that would not induce attraction on agar in most assays. Evidence from entomopathogenic and nematophagous nematodes suggests additional chemical stimuli modulate responses to CO₂ as observed for insects. Future progress will be accelerated by integration of molecular, anatomical, and physiological data from diverse nematodes, direct comparisons within soil, better conceptual preparation, and improved techniques to measure and simulate the conditions and stimulus gradients of soil.

090 Sensory responses of plant-parasitic nematodes to semiochemicals

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The understanding of nematode semiochemicals has improved in recent years due to incorporation of novel techniques to study nematode behaviour. Extracellular electrophysiological recordings from the cephalic region of plant-parasitic nematodes in conjunction with behavioural bioassays have revealed that chemoreception is essential

for nematode survival. The function of the amphids as a main chemoreception organ has been affirmed in all nematode behavioural studies. Principal semiochemicals that elicit nematode responses are plant host exudates, food stimulants, food deterrents, and homospecific sex pheromones. Several amino acids and plant root diffusates elicited positive responses from plant nematodes. In addition, sensory adaptation and chemical isomer specificity have been demonstrated. Recordings from the cephalic region of a potato cyst nematode, *Globodera rostochiensis*, verified the species-specific nature of sex pheromones. Biological activity associated with *G. rostochiensis* and *Heterodera schachtii* sex pheromones has been linked to a specific HPLC-derived fraction, and although the sex pheromones have not yet been identified, their physical properties have been reported. Sensory physiology is a new and promising field in nematology that will lead us to a better understanding of nematode biology and will provide a means to control nematodes by interfering with their sensory perception.

091 Neuropeptide signalling systems in parasitic nematodes and their potential as novel control targets

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The diversity of neuropeptide signalling molecules in nematodes is as great as that known for any organism. By far the largest and most multifaceted nematode neuropeptide family is the FMRFamide-related peptides (FaRPs). In *Caenorhabditis elegans*, this family comprises 60 distinct peptides that are encoded on 22 genes (designated *flp*-genes) and available evidence indicates that this complexity is mirrored in plant and animal parasitic nematodes. Neuropeptides are known to be expressed in all neuronal subtypes and to have potent and wide-ranging effects on motility, alimentation, reproductive function and sensory perception. Although the effects of FaRPs are widespread in nematodes,

individual *flp*-genes have restricted expression patterns indicating specific roles for encoded peptides. Sequence data reveal that a significant number of FaRPs are structurally homologous across a broad range of nematode species; a similar pattern of functional homology remains to be established. While information on FaRP receptors is limited, physiological data suggest that the diversity in FaRP structure is not reflected by endogenous FaRP receptors. The FaRP-signalling system likely harbours numerous potential targets for novel control measures. However, the selection and exploitation of these is made difficult by the complexity inherent within the FaRP signalling system and the potential for functional redundancy.

092 Chemoreceptor genes: what can we learn from *C. elegans* and how can we apply this information to studies on other nematodes?

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Soil dwelling nematodes encounter many types of volatile and water-soluble molecules in their environment. For free-living nematodes like *C. elegans*, successful foraging depends on the ability to detect a gradient in one odorant while ignoring extraneous odours. The infectious stages of plant and animal parasitic nematodes also rely on chemoreception as their primary host finding cue. Using a combination of genetic, molecular and bioinformatic approaches chemoreceptor genes have been identified in *C. elegans*. These *C. elegans* chemoreceptor genes encode seven-transmembrane G-protein coupled receptors (GPCRs) and comprise the largest gene family in this nematode. GPCRs are also involved in olfactory signal transduction across a broad spectrum of animals including insects, crustaceans, fish and mammals, but the *C. elegans* (and *Drosophila*) chemoreceptor genes have no sequence homology to vertebrate GPCR odour receptor genes and they also differ from vertebrate odour receptor genes in their genomic structure. In this presentation we will provide overview of the genomic structure and diversity of odorant and chemoreceptor gene families in vertebrates and invertebrates and we describe our attempts, using homology-based approaches, to isolate chemoreceptor genes in the entomopathogenic nematode *Heterorhabditis bacteriophora*.

093 Ecological aspects of small mammal nematode infections

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Nematodes have many adaptation and colonisation capacities. This explains how they were successful in colonising animal hosts from evolutionary lines of free-living nematode groups. Recent results on 18S rRNA gene sequencing and corresponding phylogenetic analyses show how the adaptation of a parasitic way of life was acquired by different free-living nematode groups independently. These analyses also suggest that phylogeny inferred from DNA sequences does not always agree with the present systematic groupings within the Secernentea. Moreover, studies carried out on nematode parasites of small mammals (rodents and insectivores) on islands in the last 20 years show that the resulting evidence does not support mathematical models which prove that nematode species following a one-host life cycle are more capable to colonise new environments and expand. Small mammal nematodes are excellent models for ecological and evolutionary studies because they include many nematode groups presenting very different types of life cycle patterns, from monoxenous to heteroxenous life cycles. Monoxenous nematode species infecting rodents and insectivores comprise geohelminths, pseudogeohelminths and geohelminths. Heteroxenous nematodes of small mammals include species transmitted by intermediate hosts pertaining to different invertebrate and vertebrate groups. This wide biological spectrum becomes a very useful tool for ecological and short-term evolutionary studies.

094 Studies on nematodes of wild carnivores

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The Iberian carnivores constitute a group of hosts where the diversity of its nematodes is not very high (45 species) compared with other mammal groups. From a faunistic point of view it is significant that: *i*) two endemic species are restricted to the Iberian Peninsula

(*Ancylostoma martinezi* in *Genetta genetta* (common genet); *Vigisospirura potekhina hugoti* in *Meles meles* (badger); *ii*) the high richness in nematodes species in *Vulpes vulpes* (fox) (16 species), *Martes foina* (stone marten) (14 species) and *Genetta genetta* (12 species), probably as a the result of a wide range of distribution of these hosts as habitat generalists. From an ecological point of view: *i*) there is an high ethologic incidence of the host to the nematode fauna; this is specially notable in the case of Mustelidae, which include hosts with aquatic, amphibian or terrestrial behaviours; the last group presents the highest number of nematode species; *ii*) the diet of the hosts is another important determining factor; moreover from indirect life cycle nematodes, it is possible to know with high precision which taxa are prey of these hosts. The specificity of reported nematodes in Iberian Carnivora also gives information about the phylogeny of the host.

095 Nematode parasitic fauna in Canary Island vertebrates

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In the Department of Parasitology in La Laguna University, many faunistic, ecological and biological studies on parasitic helminths in vertebrates have been undertaken over several years. Despite this, nematode studies have only been carried out on *Canis familiaris* and in the species which can cause zoonosis in man, *Toxocara canis* and *Dirofilaria immitis*. The influence of the Canary Island microclimate on their distribution and the insularity effect were also studied. Since 1999, parasitic helminths in Canary Island birds have been studied and the nematofauna is the best known group at the moment. The bird species (105) analysed include *Alectoris Barbara*, *Larus cachinnans*, *Columba livia*, *Fulica atra* and *Chalmydotis undulata*. The nematodes obtained were studied under optical microscopy by clearing with Amman lactophenol. In order to study the samples using scanning electronic microscopy (SEM), Mercer and Birbeck (1979), Ba and Marchand (1994) and Miquel and Marchand (1998) techniques were used. The helminth species identified in the different birds under study were as follows: in *Alectoris Barbara*: *Aonchotoca caudinflata*, *Ascaridia galli* (first report in this host), *Baruscaphillaria obsignata*, *Eucoleus annulatus*

and *Heterakis gallinarum*; in *Columba livia*: *Aonchoteca* sp., *Tetrameres fissipina*, *Synhimantus (Dispharynx) spiralis* and *Ascaridia columbae*, and in *Fulica atra*, *Capillaria* sp. were found and in *Larus cachinnans*, *Cosmocephalus* sp. The effect of insularity on the parasites is studied.

096 Some problems related to the control of nematode infections in domestic ruminants

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Gastrointestinal nematode infections in small ruminants are of great importance in animal husbandry worldwide. The control is based on the knowledge of the epidemiology, the detection of nematode eggs in faeces on a representative number of animals from the flock, the use of anthelmintic drugs and management practices. However, faecal egg counts are highly variable and must be done on a large number of animals in order to get accurate estimations of the parasite burdens within the flock. On the other hand, the routine and inadequate application of anthelmintics is another problem, due to the development of anthelmintic resistance (AR). In Spain, some recent reports on AR in small ruminants shows a flock prevalence of 21%. Although benzimidazoles (BZ) has been the most used anthelmintic during the last decade, BZ-resistance is still low. However, there is a high level of AR against imidazothiazoles. Two factors were significantly associated with resistance or suspicion of resistance: breed and anthelmintic efficacy according to the farmer. Foreign and crossbreed flocks seemed to be more prone to present AR. Higher probabilities of AR seemed to be related to flocks that used mostly private pastures or to those that bought replacement animals.

097 The cost of licensing and labeling a methyl bromide alternative

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There are many time consuming and costly processes involved with taking a new pesticide to market. While many researchers are searching for drop-in replacements for methyl bromide (MeBr), developing a new formulation or improving on an old one is just the beginning of the process. Efficacy data and field testing under one's own research programme might indicate promising results but the developer needs to be ready for a 3-4 year process which can cost in excess of US \$12 m. It is important that no steps or agencies are overlooked in the long road to a marketable product. There are many considerations, such as venture capital, the patenting process for both US and foreign proprietary rights, support for minor use pesticide field testing and addressing all steps required by the US Environmental Protection Agency. This presentation will cover what it takes to get a MeBr alternative to market in the US and beyond.

098 Soil fumigation: new uses for old chemicals and new compounds

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The imminent removal of methyl bromide (MBR) as a soil fumigant has stimulated a world-wide search for alternative treatments. This decade-long effort identified the old fumigants: 1,3-dichloropropene (1,3-D), chloropicrin, methyl isothiocyanate (MITC), and compounds that generate MITC, as the only materials available at present for development of preparations capable of substituting MBR in soil fumigation. New formulations and application technologies have been devised to increase the efficacy and practicality of these very old chemicals as MBR alternatives. Other compounds showing promise for future development as MBR substitutes are: inorganic azides, propylene oxide, methyl iodide and other iodinated hydrocarbons. Several naturally occurring 'biofumigant' compounds (e.g., citral, fufural, various mustard oils such as allyl isothiocyanate) are also being explored as MBR alternatives. The main difficulty in finding MBR substitutes for soil fumigation resides in the need to develop economical soil treatments that are safe, environmentally acceptable and effective, not only against plant-pathogenic nematodes but also weeds, and other soil-borne pathogens and pests.

099 New technologies for challenging situations – perennial and nursery crops in California

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Nursery crops that are grown in California for commercial plantings are required to be free of plant-parasitic nematodes. Nursery certification can be accomplished by extensive sampling or by use of an approved soil treatment. Approved treatments (materials and rates) vary with soil type, moisture, temperature, and cropping history of the field and show efficacy to a depth of 150 cm. Historically, the most commonly used material was methyl bromide. Prior to planting the certified trees and vines, growers often fumigated with methyl bromide to prevent replant disorder in their orchards and vineyards. Combinations of new and existing technologies, novel application methods, and registered and experimental materials are being tested for use in nursery and perennial replant situations. Global positioning satellite systems can be used to implement management strategies into areas selected for optimal performance based on the spatial variability of soil conditions. Drip irrigation technologies can be used to deliver emulsifiable formulations throughout the soil profile to the depth needed – especially for materials that do not move as readily as methyl bromide. Currently registered and experimental materials can be re-evaluated in light of these new technologies. Rescue strategies are necessary for situations where the management strategy has failed.

100 Basic strategies for defensive response of plants against nematodes

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In the last few years, our Ecological Chemistry research group has been engaged in molecular level studies on the host-plant interactions between banana cultivars and the pathogens: *Fusarium oxysporum*, *Mycosphaerella fijensis* and *Radopholus similis*. Several susceptible banana cultivars elicit a defence response to attack by the pathogens characterised by *de novo* production of a new

type of phytoalexins, hitherto undescribed. The pathogen resistant banana hybrid *SH-3481* (from FHIA), produces large amounts of these phytoalexins and phytoanticipins (constitutive natural antibiotics), suggesting that this new type of phytoalexins plays an important role in the resistance mechanisms of banana plants against fungal and nematode diseases. The significance of these findings will be discussed as they may open the way to development of new sustainable agricultural methodologies to manage plant diseases.

101 Quest for resistance to the peanut pod nematode

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Since its discovery in 1987, the peanut pod nematode *Ditylenchus africanus* is regarded as one of the economically most important pests of peanut in South Africa. The nematode is omnipresent in peanut production areas in this country and its most significant impact is on kernel and seed downgrading, which causes serious losses in income for the producers. This, in turn, has a detrimental effect on net national peanut production. Although a significant number of scientific publications have been published over the past few years, the occurrence of the peanut pod nematode seems to be restricted to South Africa. Screening of local peanut germplasm, selected elite breeding lines and hybrids provided at best a partial level of resistance to *D. africanus*. This partial resistance, however, is overcome at high nematode infestation levels, which is common in peanut fields, given the nematode high reproductive potential. The local genetic variation in peanut breeding material is small. Most popular hybrids are Spanish or Virginia ‘bunch’ types, with a few ‘runners’ still produced in remote areas. The uniqueness of this nematode problem and the narrow genetic background of available peanut germplasm present a challenge for the selection and application of resistance as a sustainable strategy to manage *D. africanus* in peanut.

102 Resistance to root-lesion nematodes in *Coffea canephora*

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Root-lesion nematodes (*Pratylenchus* spp.) are widely distributed in coffee plantations worldwide. In Central America, many of them are highly pathogenic on *Coffea arabica* causing important economic damage. The poor efficiency of nematicide treatments to control *Pratylenchus* spp. was demonstrated in Guatemala. In this country, *C. canephora* was used as a rootstock for 30 years empirically. Analysis pointed out the low level of root-lesion nematodes in the fields where this grafting technique was used. Comparative studies of penetration dynamics and reproductive fitness on coffee seedlings as well as field trials demonstrated the existence of pre- and post infection factors of partial resistance to *Pratylenchus* spp. in some *C. canephora* genotypes. Complementary histological studies of roots showed no noticeable structural differences between *C. arabica* and *C. canephora*. On the other hand, the presence of high amounts of polyphenols was observed in the roots of a *C. canephora* resistant genotype even before nematode penetration, suggesting the existence of constitutive factors of resistance. Sources and mechanisms of resistance must be investigated more exhaustively among *C. canephora* germplasm. Grafting onto *C. canephora* constitutes today the best alternative to nematicide treatments for controlling phytoparasitic nematode communities in *C. arabica* coffee crop, as some *C. canephora* genotypes also show resistance to highly pathogenic root-knot nematodes (*Meloidogyne* spp.).

103 *In vitro* screening as an early rapid and reliable tool to detect resistance against migratory endoparasitic nematodes

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Growing nematode-resistant crop varieties is considered as an effective and sustainable method for nematode management. Identification and selection of resistant varieties often occurs in the field, but field testing is time consuming, especially with large-sized plants.

Therefore, early and rapid screening of plant material for detection of nematode resistance is desirable. It allows a rapid selection at the very early plant stage of potentially interesting candidates. An *in vitro* screening method allows the elimination of susceptible germplasm, even earlier compared to early screening in climatic chambers or glass-/screenhouses. However, the final selection must still be performed in the field, but massive elimination of clearly non-resistant plant material would considerably reduce the size of such field screening. To date, the use of *in vitro* culturing techniques has been well demonstrated as a rapid and reliable tool for determining reproductive capabilities of plant-parasitic nematodes on known hosts, new hosts and resistant varieties. *In vitro* screening of varieties for resistance to plant-parasitic nematodes was first demonstrated for root-knot nematodes, *Meloidogyne* spp., using excised tomato roots. Nowadays, root-explant cultures of many different plants have been used for evaluating resistance to both sedentary and migratory endoparasitic nematodes. *In vitro* screening procedures were successfully developed for *Musa*, allowing selection of constitutive resistance against the root-lesion nematode, *Radopholus similis*.

104 Structure and function of food webs

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Several soil food webs have been thoroughly described and the importance of nematodes is evident from their representation at different trophic links – as plant-feeders, secondary detritivores, predators and omnivores. Carbon flow models have shown the importance of several pathways, including those through bacterial- and fungal-feeders to predaceous nematodes, and from plant roots through plant-feeders. The herbivory pathway has been observed experimentally by following a pulse of ¹³C, applied above-ground, through the soil community. Novel observations are also emerging on the effects of root-feeding fauna on above-ground food webs. Disturbance of food web groups causes a loss in stability, reduced biodiversity and changes in soil processes. Consideration of

biodiversity in food web models will allow exploration of biodiversity-productivity and biodiversity-stability relationships. Modelling interactions according to function rather than species reveal interesting interactions in above-ground food webs. Current soil food web models group organisms by function and taxa, but for improved understanding of the system information on broader functional groups may be more appropriate. Current initiatives and developments in food web research and theory highlight the importance of soil food webs in biosphere function and are providing insights into the preservation, conservation and management of soil systems.

105 Carbon and energy sources for nematodes

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Nematode species diversity, even at small spatial scales, provides an intriguing system for studies on resource partitioning and on links between biodiversity and ecosystem function. The ubiquity and abundance of nematodes in soils and aquatic sediments interest ecologists seeking models for fluxes of carbon, nitrogen, phosphorus *etc.* However, the complete spectrum of roles and the quantitative importance of nematodes in soils and aquatic sediments remain poorly documented. Food sources of nematodes are usually inferred from buccal morphology but there are many unresolved questions on food sources and feeding rates. Current functional guild classifications are useful but in some cases, including nematodes with minute buccal cavities and tylenchids with delicate spears, food sources are uncertain. We discuss whether, and how, a limited diversity of functional types can be reconciled with great species diversity and present a laboratory approach for studying

functional redundancy among bacterivorous nematode species. To relate laboratory-derived feeding rates to population growth rates under natural conditions, we address the effects of external influences on nematode activity in soils, including texture, aeration, temperature and moisture.

106 Decomposition pathways and successional changes

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In soils, energy and nutrient pathways are primarily mediated by bacteria or fungi. Bacteria-dominated systems rapidly transfer nutrients, directly and *via* consumers, to plants. In contrast, fungal-based decomposition channels are slower; they are driven by more complex organic resources. There are strong linkages between nematodes and their fungal or bacterial food sources. On one hand, consumer organisms affect rates of energy and nutrient release from their prey; on the other hand, they may regulate the prey biomass. The nature and abundance of available resources can be monitored by faunal analysis of fungal- and bacterial-feeding nematodes (f/b ratio, channel index). The resources change constitutively with time. Readily decomposable portions are rapidly consumed by bacteria and their predators so that the recalcitrant fraction becomes proportionally greater. That change is mirrored by corresponding increase in fungal decomposition and reflected in the nematode fauna. We discuss the relationship of nematode trophic structure with the nature of the incoming organic material and the prevailing state of the physical environment. For example, decomposition pathways of natural forests are predominantly fungal and those of agricultural systems are bacterial. We discuss the significance of the pathways in relation to the structure and functions of the entire soil food web.

107 Immuno-magnetic capture (IMC): a novel approach to nematode diagnostics

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Simple, rapid and reliable diagnostic techniques are important for identification and quantification of plant-parasitic nematodes. Current diagnostic methods have limitations for field sample detection. ELISA diagnosis is less sensitive than PCR and the sensitivity of PCR based methods is strongly affected by inhibitors from soil. IMC offers several advantages which overcome these drawbacks. In IMC an antibody which recognises the surface of target nematodes is incubated with a nematode suspension extracted from a field sample. Then, secondary-antibody coated magnetic beads are added and a magnet is used to capture target nematodes while other nematodes are discarded. We have used IMC to detect *Meloidogyne arenaria* and a virus-vector nematode *Xiphinema americanum*. Eighty and 60% of target nematodes, respectively, were recovered from mixed soil samples. These results show that IMC is an effective method of detecting specific nematodes in mixed soil samples. IMC converts field nematode samples to lab-friendly samples, containing highly concentrated target nematodes suitable for further serological or molecular techniques, such as ELISA, PCR or *Taqman*, which can be used to detect and quantify specific nematodes or viruses. We are currently examining the potential commercial applications of combining IMC with PCR-based diagnostics for routine detection of virus vector nematodes.

108 Detection of GFLV in *Xiphinema index* with DNA-based fluorescent probes

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A PCR based method was applied to the detection of grapevine fanleaf virus (GFLV). The virus was amplified from specimens of its vector *Xiphinema index*, collected in a grapevine orchard at Palagiano, Italy. The detection was carried out with a real-time fluorescent RT-PCR assay. A set of primers was designed for the GFLV RNA-2 amplification. A 2500 bp fragment was amplified by RT-PCR, from *X. index* adults and juveniles, cloned and sequenced. We compared the obtained GFLV sequences with those available in GenBank and two regions were selected for virus detection and/or strain identification. A fluorescent Scorpion probe was designed to amplify a 100 bp fragment within the conserved region of the cp gene. A 21 bp conserved motif at position 2855 was used as probe target. An additional region with strain-specific nucleotide variations at position 2502 was used to discriminate between the Palagiano and other GFLV strains. Specific oligos, constructed on the basis of GenBank sequences AF304015 and X16907, were used as controls. Furthermore, a set of three strain-specific molecular beacons was designed on this region and used with the amplified DNA or oligos. The successful detection of the probe targets was shown by fluorescent signals emitted during amplification or under UV excitation. The Scorpion probe proved to be useful in virus detection. Due to their single-base mismatch sensitivity, the molecular beacons appeared suitable for specific strain recognition. The potential of these technologies in the study of transmission and vector epidemiology are briefly discussed.

109 Advances in applied nematode research in South Africa after introduction of the SCAR-PCR technique for nematode identification

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The availability of well-defined nematode populations is essential for host plant resistance screening, breeding and crop rotation purposes. Identification of monoculture root-knot nematode species occurring in South African soils

and differentiation between species in mixed populations were done by means of the sequence characterised amplified region – polymerase chain reaction (SCAR-PCR) technique. *Meloidogyne fallax*, *M. chitwoodi*, *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* were identified from various crops. Multiplex internal transcribed spacer (ITS)-PCR amplified a fragment in an unknown root-knot nematode species for which no SCAR-marker is presently available. This technique enabled the identification of *M. fallax*, a quarantine organism in Europe, as a new record for South Africa where presence at two localities was established. Resulting from this study, the geographical distribution of *M. chitwoodi* was expanded from two to five known localities in this country. Root-knot nematode species composition of field and glasshouse isolates can be monitored frequently using the SCAR-PCR technique, which allows for routine analyses of root-knot nematode species used in research programmes.

110 Detection and eradication of plant-parasitic nematodes in imported germplasm in Brazil

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The Laboratory of Plant Quarantine of Embrapa Genetic Resources and Biotechnology makes phytosanitary analyses of most of the plant germplasm introduced in Brazil. During 2001, plant-parasitic nematodes were detected associated with 1738 germplasm accessions coming from several countries. The material infected with nematodes were corn, wheat, rice, soybean, oats, melon, sesame, cocoa, coffee, orchid, neen, clover and lespedeza. Those materials were introduced from the following countries: France, Mexico, USA, Colombia, Japan, Argentina, Venezuela, Trinidad-Tobago, Guatemala, Dominican Republic, Philippines, Spain and Holland. The techniques used for extraction and detection of the nematodes were Baermann funnel, tray technique, sieving and blending and cyst detection by Fenwick can. The plant-parasitic nematodes detected were *Ditylenchus dipsaci*, *Ditylenchus parvus*, *Ditylenchus* sp., *Aphelenchoides besseyi*, *Aphelenchoides* sp., *Aphelenchus avenae*, *Aphelenchus* sp., *Helicotylenchus* sp. and Hoplolaimidae. The accessions were submitted to dry and humid thermal treatments

and to chemical treatments for eradication of the nematodes, which was successful in all but a few accessions. With these phytosanitary methods the Laboratory of Plant Quarantine of Embrapa Genetic Resources and Biotechnology collaborates actively to reduce the risk of introduction of new plant-parasitic nematodes species in Brazil.

111 Professional automated extracting apparatus for free-living nematodes

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Nematode extraction by elutriation and subsequent separation on a cotton wool filter is labour-intensive, time consuming, and inefficient. Full automation is difficult. Extraction based on centrifugal flotation in swinging buckets is faster and more efficient but does not allow for extraction of large volumes and is still difficult to automate. A prototype of a fully automated extraction apparatus was built using a zonal centrifuge. With this prototype the sample volume was limited to 100 cm³ soil (10 cm³ extracted); subsequently, a version processing 200 cm³ (100 cm³ extracted) was constructed. Besides previous modifications, changes were made aiming at an easier and safer operation by using a conveyor-belt, a pneumatic drive, and a fool-proof design. The apparatus is commercial and can be used to extract other plant pathogens from soil.

112 Magnetic separation as a tool in a sample preparation procedure for direct detection and quantification of trichodorid nematodes

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The detection of trichodorid nematodes in soil samples is difficult as conventional methods are laborious, time-consuming and rely on specialist knowledge of morphological characteristics of the species. To overcome these difficulties the potential of magnetic separation for recovering target nematodes from soil populations

of nematodes was investigated. Lectins and polyclonal antiserum that bound specifically and reproducibly to the overall surface of *Paratrichodorus anemones* were identified and bound to monodisperse superparamagnetic particles (Dynabeads) to capture target nematodes from test suspensions. In recovery experiments, while both types of probe isolated nematodes from suspension, antibody-coated beads recovered them more efficiently than beads coated with lectins. The results obtained suggest that the immuno-magnetic separation (IMS) technique has potential to provide a serological-based target nematode enrichment process. Furthermore, it represents a significant advance over current methods for recovering nematodes as it facilitates high throughput to screen large soil populations quickly and economically and, being technically simple, requires minimal labour input.

113 Development and evaluation of a fluorogenic 5' nuclease PCR assay (TaqMan™) for the detection and quantification of virus-vector trichodorid species

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Molecular detection of pests and pathogens relies on rapid and dependable methods for the identification and quantification of these organisms. Here, the development of a qualitative and quantitative diagnostic method for trichodorid nematodes based on TaqMan™ chemistry is reported. Two independent primer/probe sets were designed targeting the 18S gene of the ribosomal cistron and producing an amplicon of 83bp for the virus-vector species, *Paratrichodorus pachydermus* and *Trichodorus similis*. The assay was applied to purified plasmid DNA containing clones of the 18S region from both nematode species, as well as to genomic DNA extracted from individual nematodes. Both primer/probe sets displayed high specificity as no cross-reaction was observed when tested with samples of two other trichodorid species. In experiments where dilutions of purified plasmid standards were used to test the analytic sensitivity, the TaqMan™ assay detected nematode DNA to the femtogram level. Quantification of the target present in unknown samples was performed by comparison of the fluorescence signals of the samples to those obtained from plasmid

standard dilutions. Currently, simultaneous detection and quantification of the above trichodorid species is being evaluated. Our data confirm this to be a rapid, accurate and sensitive molecular diagnostic.

114 A method for estimating the ratio of *Meloidogyne incognita* to *M. arenaria* in mixed populations using PCR-RFLP

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The most important root-knot nematodes in southwestern Japan are *Meloidogyne incognita* and *M. arenaria*. These two species are sometimes present in the same field. We devised a method to estimate the ratio of *M. incognita* to *M. arenaria* in mixed populations using PCR-RFLP and image analysis. Various ratios of the two nematodes were prepared, then PCR-RFLP was performed using each ratio. Brightness of species-specific RFLP bands for *M. incognita* and *M. arenaria* was quantified with image analysis using a computer program. The brightness of the *M. incognita*-specific band to that of the *M. arenaria* band for each RFLP pattern was used as an estimate for the ratio of the two nematodes. A regression line was calculated from these values to estimate actual ratios. We applied this method successfully to field samples. Attempts have also been made to apply this method to other nematode combinations, such as *M. incognita* and *M. hapla* or two different populations of *Pratylenchus coffeae*.

115 Detection of the reniform nematode in cotton using hyperspectral imagery

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The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira, 1940) is one of the two most prevalent nematodes on cotton (*Gossypium hirsutum* L.) and is quickly spreading throughout the southeastern United States. This nematode inhibits cotton plant development resulting in reduced plant growth and sometimes plant

death; cotton crop yield loss up to 40-60% has been due to reniform nematode infestations. A production field naturally infested with the reniform nematode and controlled field microplots are currently being used to study the correlation between reniform nematode population thresholds and reflectance properties exhibited by infected cotton plants. Reflectance properties, measured by a handheld hyperspectral spectroradiometer, will be used to develop hyperspectral images. These hyperspectral images along with nematode population threshold data will be used to determine relationships between cotton plant stress and nematode population thresholds. Hyperspectral imagery may be a useful remote sensing tool enabling cotton producers to test for nematode presence while avoiding the time consuming and sometimes costly soil sampling process presently used today.

116 Possibilities of autofluorescence in a nematological diagnostic laboratory

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Autofluorescence is a phenomenon that scientists often try to avoid because it interferes with the induced fluorescence they want to measure. We studied green autofluorescence in free-living and plant-parasitic nematodes at low magnification (12.5-100 \times). First observations indicate it is useful for the distinction between plant-parasitic and non-plant-parasitic nematodes, endo-parasitic nematode detection in roots, nematode viability tests such as live and dead counting of juveniles of potato cysts nematodes and for detection of other specific items like *Pasteuria* endospores. The Leica MZ FL III dissection microscope has been tested on a variety of nematological objects. The advantages and disadvantages will be highlighted.

117 Comparison of quantitative PCR and mister extraction for the assessment of *Pratylenchus neglectus* or *P. thornei*

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Seven wheat varieties and 13 doubled haploid wheat lines were inoculated with *P. neglectus* or *P. thornei* and grown in a glasshouse experiment for 8 weeks. Root systems were washed and nematodes were quantified using either a microscope or PCR. For assessment using the microscope, root systems were washed to remove soil, cut and misted for 4 days and the nematode suspension counted using a dissection microscope. For assessment using PCR, plant roots were washed and nematode DNA extracted. PCR (specific to either *P. neglectus* or *P. thornei*) was used to quantify DNA levels. There was higher variation between replicates with microscope (c.v. = 63%) compared with PCR assessment (c.v. = 22%). This was attributed both to errors associated with extraction using the misting chamber and in subsampling and counting the nematode suspension using the microscope. For both *P. neglectus* and *P. thornei*, significant positive linear correlations were obtained between the microscope and PCR methods (*P. neglectus* $r^2 = 0.80$; $P < 0.001$; *P. thornei* $r^2 = 0.512$; $P < 0.05$). The PCR technique for quantifying *P. neglectus* or *P. thornei* is currently being used for screening cereals in southern Australia.

118 Use of the mistifier for extraction of root lesion nematodes (*Pratylenchus* spp.) from soil

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Since 1995, we have used the mistifier for extraction of *Pratylenchus* from field samples of soil + roots. This required a filter that retained soil, allowed passage of nematodes, and remained intact through 96 h of misting. Coffee filters (Autocup[®], Thomas and Green Ltd, UK or Altra[®] Filters Inc., USA) were the most suitable material. Four filters are used per 50 g sample, providing clean extract without hindering passage of nematodes. Extraction and sample variation were compared with the tray method of Whitehead and Hemming using three naturally infested field soils and pasteurised soil inoculated with cultured *P. neglectus*. Eighty percent of nematodes from the field soils were *Pratylenchus*. Forty

eight percent more nematodes were extracted by mister than with the tray. Sample variation was similar for mister (16-23%) and tray (12-20%). Numbers recovered (64%) from inoculated soil did not differ between methods. Nematodes in natural soil could have been attached to soil particles or organic matter, contained within roots or anhydrobiotic. These nematodes were therefore extracted more efficiently by the mister than from the tray, probably due to the greater aeration and water flow on the mistifier. For field soils, extraction by mistifier resulted in higher *Pratylenchus* recovery.

119 PCR-based techniques for nematode diagnosis derived from the study of genetic diversity

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One of the main activities in the Department of Crop Protection (Agricultural Research Centre), is the development of identification and detection protocols. Identification of nematodes based on morphology and morphometrics is time-consuming and difficult due to overlapping of many characteristics. Recently molecular identification techniques based on PCR have been developed and successfully applied for nematode diagnostics. These techniques allow identifying genetic markers specific for certain nematode species or groups of populations. Genetic markers are identified by the analysis of PCR products using RFLP, direct sequencing and random PCR approaches like RAPD and AFLP. Markers are used to develop PCR with species-specific primers for rapid nematode detection and Real Time PCR for nematode quantification in samples. The poster presents several PCR-applications used for nematode identification in CLO-DGB.

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

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Xiphinema index (XI), *X. diversicaudatum* (XD), *X. vuittenezi* (XV) and *X. italiae* (XIT) are established or putative vectors of several nepoviruses of grapevine. All four species are closely related, which makes them difficult to identify reliably when only single or few individuals are available. With the aim of finding a solution, a simple diagnostic method was developed. The ITS1 region spanning the 18S and 5.8S ribosomal genes was sequenced in one population of each species, using two conserved primers from these respective genes. Sequence comparisons of ITS1, sized 1132 (XV), 1153 (XI), 1175 (XD) and 1190 (XIT) bp, suggested a genetic proximity between the two established vector species *X. index* and *X. diversicaudatum* on the one hand and *X. vuittenezi* and *X. italiae* on the other hand. The sequence variability of ITS1 allowed the design of internal sense primers, specific for each species, which amplified, in combination with the same antisense ITS1 primer, a single signature fragment (340 bp for XI, 414 bp for XIT, 591 bp for XV and 813 bp for XD). The primers were successfully used in a multiplex test for the reliable detection of two to four mixed species each represented by a single individual.

121 PCR based methods for the analysis of Dutch soil samples for the presence of root-knot nematodes

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The root-knot nematodes *Meloidogyne chitwoodi*, *M. fallax*, *M. hapla* and *M. naasi* are regularly found as plant-parasitic nematodes in Dutch agriculture, either present alone or as mixtures. The former two have acquired importance as quarantine pests while *M. hapla* has also increased importance. All three species cause considerable damage on most agricultural crops. *Meloidogyne naasi* is also found more frequently in agricultural plots, but its relevance is limited to grasses. Inspection services identify root-knot nematode species generally based on morphological characteristics. In a collaboration between PRI and the PD, analyses

of nematode samples have been compared. Root-knot nematode composition of samples of approximately 100 nematodes was first determined morphologically; subsequently, the samples were divided between PRI and PD and analysed using PCR assays for the detection of *M. chitwoodi*, *M. fallax*, *M. hapla* and *M. naasi*. For the former three species, species-specific PCR primers already existed; for *M. naasi* a new species-specific primer was developed. The results between the three analyses were comparable. With respect to routine diagnostic assays, PCR based assays seem to offer attractive alternatives: they are reliable, sensitive, relatively fast, easy to perform, and do not require nematological expertise.

122 Multiplex detection of nematodes and other soil related organisms using three dimensional micro-arrays

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Detecting the presence of nematodes and other soil related plant pathogens or beneficials in soil samples is helpful

to ensure safe and sustainable agriculture. However, the multiplicity of detection assays available for a specific pathogen leads to a lack of consistency among the various testing agencies and hampers standardisation. Micro-array technology, in which thousands of different oligos or proteins can be spotted on little more than 1 cm², enables the detection of many different target molecules in the same sample with increased specificity. Therefore, micro-arrays can meet the demands for fast, specific, efficient, cost-effective, user-friendly and reliable multiplex detection methods for different organisms. To develop the micro-array technology for diagnostic purposes, generic extraction methods for DNA and RNA have to be developed. Secondly, the sensitivity must be improved in order to detect low concentrations of extracted nucleic acids, with generic pre-amplification methods. Recently a revolutionary porous capillary solid phase micro-array has been developed. The capacity of this three-dimensional array to bind oligonucleotides is higher than that of a two-dimensional glass array resulting in a higher sensitivity. Moreover, the porous solid phase allows flow through measurements, resulting in fast hybridisation times of only 15 min instead of 18 h as on glass. Recent data to detect plant pathogens in this multiplex setting look promising.

123 Comments on intraspecific variability in the genus *Chiloplacus* Thorne, 1937

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The genus *Chiloplacus* Thorne, 1937 is one of the most widely distributed taxa among cephalobid nematodes, and some of its species are considered to be cosmopolitan. The genus currently includes 30 valid species, plus ten *incertae sedis*, which show a remarkable interspecific variability when they are studied under both optical microscopy and SEM. In Andalucía Oriental (SE Iberian Peninsula) the genus is represented by six species: *C. demani* (Thorne, 1925) Thorne, 1937; *C. magnus* Rashid & Heyns, 1990; *C. minimus* (Thorne, 1925) Andrassy, 1959; *C. tenuis* Rashid & Heyns, 1990; *C. trilineatus* Steiner, 1940 and *Chiloplacus* sp. In addition to the usual variability affecting the body length, number of lateral field incisures, postvulval sac length, tail morphology, etc., present study has revealed an interesting variability in several features of the lip region: separated or more or less amalgamated lips, labial probolae differing in the shape (biacute or bifurcated) and the length of their branches, cephalic probolae also differing in shape and size, more or less distinct primary and secondary axils, and cephalic margin smooth, serrated or bearing tines. The taxonomic interest of these features is discussed briefly.

124 Evidence for heteroplasmy and recombination in nematode mitochondrial DNA

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We previously reported the unusual multipartite arrangement of the mtDNA of the potato cyst nematode *Globodera pallida*. Its mtDNA consists of a population of small, genetically distinct mtDNAs which, presumably, exist in as yet unknown combinations in order to encode all the proteins necessary for mitochondrial function. Recently published biochemical evidence that the enzy-

matic machinery necessary for homologous recombination is present in animal mitochondria, along with phylogenetic evidence that recombination has been a feature of the evolution of primate mtDNA, has challenged the received orthodoxy that animal mtDNA is inherited clonally. The existence of a persistent heteroplasmic state as found in *G. pallida* would seem likely to provide ample opportunity for recombination. We now have evidence of mtDNA recombination both between distinct *G. pallida* mtDNA lineages, and between sub-types of single mtDNA lineages. Recombination is demonstrated using the latest methods from statistical phylogenetics, including hidden Markov models and Bayesian (Markov chain Monte Carlo) approaches.

125 Conversion of AFLP markers to PCR-based STS markers to identify *Heterodera glycines* populations

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Nine AFLP markers were identified that provide varying degrees of specificity to some of the 48 selected populations involved in a previous study that investigated the parasitic ability of the soybean cyst nematode, *H. glycines*. The 48 populations were obtained by subjecting field populations of races 1 through 6, 9, and 14 to several cycles of selection on each of six sources of resistance to *H. glycines*: Peking, Cloud, PI88788, PI89772, PI209332 and PI90763. The nine AFLP markers were cloned and their DNA nucleotide sequence determined before they were used to design oligonucleotide primers for PCR-amplification of genomic DNA from the original unselected field populations. Following this procedure, we have generated STS (sequence tag site) primers that are specific for races 1 through 6, 9, and 14 of *H. glycines*. These STS markers should be very useful as diagnostic probes for the identification of field populations of *H. glycines*.

126 Phylogenetic relationships between *Paratrichodorus* and *Trichodorus* species (Nematoda: Trichodoridae)

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Paratrichodorus and *Trichodorus* species (trichodorids) are soil-inhabiting ectoparasitic nematodes, several of which are natural vectors of Tobraviruses. Trichodorids used in this study were obtained from colleagues in Europe, North and South America, Asia, and from British soil samples. Phylogenetic analysis of the 18S gene from ribosomal DNA sequence data obtained from 17 different trichodorid species revealed a major differentiation corresponding to the two genera *Paratrichodorus* and *Trichodorus*, but *P. minor* and *P. porosus* were only distantly related to the other members of their genus. Analysis of the aligned sequences indicated the presence of 'mosaic' sequences in the 18S gene of *P. minor* and *P. porosus* populations and suggested recombination events. The cuticle is one of the main characters used to distinguish between the two genera and, recently, fine structure of the body cuticle in several trichodorids was found not to be genus specific. This observation, together with our results with *P. minor* and *P. porosus*, suggests that the current divisions in the Family Trichodoridae require a reassessment.

127 Molecular diagnosis of *Pratylenchus thornei*

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Root-lesion nematodes are economically important pathogens of a wide range of crops. This study describes the development of a species-specific primer pair designed from a RAPD fragment associated with *Pratylenchus thornei*, the cereal and legume root-lesion nematode. The RAPD fragment was cloned and sequenced and primers for specific PCR were developed from sequence data. Specific PCR analyses using the new primer pair and total genomic DNA extracted from a mixture containing all life stages of the nematode (eggs, juveniles and adult females) rendered a 1050 bp DNA fragment which proved specific

for *P. thornei*. This fragment was not amplified when total genomic DNA from other *Pratylenchus* spp., *Meloidogyne* spp., *Heterodera mediterranea*, *Zygotylenchus guevarai*, *Ditylenchus dipsaci* and *Radopholus similis* was used as template in PCR reactions using the newly designed primer pair.

128 Studies on the taxonomy of important genera and species of Criconematoidea from China

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More than 895 samples from 67 families, 189 genera, 246 species of plants in 73 cities and counties of 17 provinces and autonomous region were examined in China. The important genera, *Cricone-ma*, *Ogma*, *Discocricone-mella*, *Hemicycliophora*, *Hemicricone-moides*, *Cricone-mella*, *Paratylenchus* and *Gracilacus*, of Cricone-matoidea from China were systematically observed and identified. Forty species from eight genera of Cricone-matoidea were identified, including one species of *Cricone-ma*, one species of *Ogma*, one new species of *Discocricone-mella*, one species of *Hemicycliophora*, five species of *Hemicricone-moides*, ten species of *Cricone-mella*, 13 species of *Paratylenchus* and eight species of *Gracilacus*. Of the 40 species, there were five new ones: *Discocricone-mella sinensis* n. sp., *Hemicricone-moides parasinensis* n. sp., *Paratylenchus handansis* n. sp., *bayansis* n. sp., and *Gracilacus populus* n. sp., and there were 19 new recorded species in China.

129 The nematode collection at the Museum of Comparative Zoology, Harvard University

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The Museum of Comparative Zoology (MCZ) of Harvard University maintains several globally important research collections. The MCZ's Department of Invertebrate Zoology curates nearly one million specimens, 10 000 of which are type specimens; additionally, entomology and malacology collections are curated by separate

departments. The nematode collection is growing, and includes significant holdings obtained from several expeditions and studies conducted around the world. Most specimens are parasitic species from animal hosts, but some free-living and plant-parasitic species are included. Most were collected within the past 100 years, but some date closer to MCZ's founding in 1859. In 1980, MCZ transferred many nematode type specimens to the United States National Parasite Collection, but retained all other nematode specimens. Current collection activities focus primarily on voucher specimens for biotic surveys and ecological and experimental studies. Use of and contribution to the collections by nematologists outside Harvard for scientific and educational purposes is encouraged, subject to approval by MCZ on a case-by-case basis.

130 Protein variation in potato cyst nematodes revealed by capillary electrophoresis

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Capillary electrophoresis was used to characterise 49 Portuguese populations of potato cyst nematodes, 46 of *Globodera rostochiensis* and three of *G. pallida*, and eight representative populations of pathotypes defined for these two species (Ro1, Ro2, Ro3 and Ro4 for *G. rostochiensis* and Pa1, Pa2 and Pa3 for *G. pallida*). Protein extracts of 50 cysts per sample were separated using Beckman eCAP SDS 14-200 kit, in a capillary with 57 cm length and 100 μ m internal diameter and a run time of 40 min. Reproducible protein profiles were obtained and the populations were compared taking account the relative migration time and area of each peak recorded by the Gold Software Data System. Similarity indices (F) and genetic distances ($D = 1 - F$) between populations were calculated using peak area data and a dendrogram was constructed according to the UPGMA method. Protein profiles of each pathotype were obtained and some of the proteins were characteristic of each pathotype. However, Ro1 and Ro4 were not placed together, and Pa1 was not placed together with Pa2. Portuguese populations revealed a great variability and it seems that protein composition is not related to geographic origin of populations.

131 Testing a molecular barcode system against the biological species concept in culture of *Panagrolaimus* populations

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Previously we developed a molecular barcode system using the 18S of rDNA for defining a molecular operational taxonomic unit (MOTU) for the identification of nematodes from an experimental field site in the UK. Here we present the result of our attempt to identify five *Panagrolaimus* populations from this site, which we maintained in culture, morphologically, molecularly and biologically, *i.e.*, through breeding each population against the other. The results showed that biologically they belonged to two reproductively isolated species. The available morphological criteria, including SEM, were insufficient to differentiate between them; thus they probably belong to one species. The variation of the morphometrical data rendered its use subjective, more so it did not discern between the two biological species. The molecular operational taxonomic unit resulted from our barcode system, perfectly agreeing with the breeding results, clearly separated the populations into two groups. While understanding that the work was limited to only five populations of one genus, we maintain that this congruence of the MOTU concept with the biological species concept in the taxonomically difficult genus, *Panagrolaimus*, shows that the method could be promising as it is simple, comparable and transferable, thus universal.

132 Large-scale molecular barcode survey of soil nematodes

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We have developed a molecular barcoding system for identification of soil nematodes by DNA sequencing, and here report on the results of our first major survey. PCR was carried out on individual nematodes, and the 5' segment of the small subunit ribosomal RNA (SSU) gene amplified and sequenced. Resulting sequences, typically 450-500 bases, were aligned and

clustered using a neighbour-joining algorithm. Groups of similar or identical sequences were designated as molecular operational taxonomic units (MOTU). Two large-scale surveys were carried out (in July and October 2001) on a Scottish upland *Agrostis-Festuca* grassland soil (the Sourhope Soil Biodiversity Programme study site), using both molecular and morphological methods in parallel, in collaboration with Dr Eyuaem Abebe. Results indicate a general congruence between the two methods, affirming the reliability of the molecular barcode approach. Chemically treated plots (nitrogen, lime, nitrogen + lime and biocide, along with untreated controls) were included in this study, and the effect of these treatments on nematode diversity and distribution was analysed. This study establishes molecular barcoding as a rapid, time-efficient and robust method for large scale surveys of nematode diversity.

133 A phylogenetic view of longidorids

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The molecular phylogeny for 77 populations including 62 species of the Longidoridae (24 *Longidorus* spp., two *Paralongidorus* spp., 34 *Xiphinema* spp., and two *Xiphidorus* spp.) was constructed using Paup 4.0. Sequences obtained from the D2D3 region of the 26S subunit gene located in ribosomal RNA gene clusters were aligned and processed for constructing the phylogram using the maximum likelihood criterion. The species are clustered in two main groups: *Longidorus* and *Xiphinema*. *Paralongidorus* species are grouped with *Longidorus africanus*. *Xiphidorus* species are grouped with *Xiphinema krugi* and the subgroup *Xiphinema americanum*. Most of the branches obtained good support from non-parametric bootstrap analysis.

134 A proposed polytomous key for the genus *Aphelenchoides*

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The genus *Aphelenchoides* consists of over 140 nominal species, some of which are pathogenic to plants. In this study, a revised list of species was produced, deleting descriptions considered too poor for subsequent recognition. Key diagnostic characters were identified and promising features for future diagnostic use were investigated using 14 populations. The primary key characters were identified as the length of the post-vulval sac (pvs) as a percentage of the distance between the vulva and the anus, the shape of the tail terminus and tail, body length, and the ratios 'a' and 'c'. Promising key characters for the future are c', the distance from the vulva to the anus, vulval body width, pvs length as a measure of the vulval body width, head width and all measurements associated with the median bulb. A polytomous key was produced, and can be adapted for computer use. Diagnosticians and nominators of new *Aphelenchoides* species are invited to use the key and its associated recording form to collect more details of morphological and morphometric characters in this genus.

135 An interactive electronic key to nematode genera

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Using the DELTA[®] system, a database of the characteristics of genera of Australian freshwater aquatic nematodes and Dorylaimida has been constructed. This database lists over 700 characteristics for over 300 genera. The computer program INTKEY[®] can be used to interrogate this database to key out any genus easily. The database has been constructed so that the key will serve a range of users, from novice to expert, and a range of situations, from abundant good material to scarce damaged specimens. Characters from any particular part of the body may be omitted or used exclusively. Characters visible with high power microscopy may be used or those visible at relatively low magnifications only may be used (although these do not always lead to identification of a single taxon). All characters may be used, including those specific to local populations, or strictly taxonomic characters only may be used, leading to iden-

tifications which are by definition robust for areas outside the area specifically covered. Characters suitable for novice and experienced users can also be selected. The key is illustrated, and is available now online free at <http://www.ento.csiro.au/science/nematodes>.

136 A phylogeny of selected Longidoridae based on 18s rDNA gene sequences

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The 18s rDNA gene is highly conserved within taxa, which makes it particularly useful for phylogenetic studies. Phylogenies of several groups of plant-parasitic nematodes have been produced by various authors, but these refer only to endo- or semi-endoparasitic nematodes and have not involved 18s sequence data. The family Longidoridae is comprised of five genera, with two of these, *Longidorus* and *Xiphinema*, comprised of over 100 and 200 species, respectively. Individual specimens from representative populations of longidorids from around the world had their 18s gene fully sequenced. Analyses of the sequence data revealed a clear separation between *Xiphinema* and *Longidorus*; three major clusters of species were apparent in the genus *Xiphinema*: *X.*

americanum group of species, *X. pachtaicum*, and the other *Xiphinema* species with which the genus *Xiphidorus* clustered. The genus *Xiphinema* was most closely related to *L. camelliae*, followed by *L. litchi*, and then to a cluster that included several *Longidorus* spp., and two *Paralongidorus* spp. The remaining clusters of *Longidorus* spp. revealed strong evidence of geographical origin of the species.

137 Phylogenetic relationships based on 18S sequences among some *Xiphinema* species with three and four juvenile development stages

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Xiphinema americanum-group nematodes, comprising of approximately 50 putative species, have a cosmopolitan distribution and are known to vector four economically damaging viruses; consequently these nematodes are an important global agricultural pest. Previous studies have concluded that some species within the *X. americanum*-group have three juvenile stages with other species having four juvenile stages. Morphological characterisation of this group is problematical due to similarities of many of the taxonomic characters across the entire group. This lack of taxonomic resolution has crucial concomitant implications when trying to assess virus-vector relationships, *i.e.*, which species vector a particular virus. Analyses of 18S gene sequence have previously been reported to discriminate taxonomic units in many organisms including nematodes. 18S data showed that all the Asiatic (Chinese) populations of *X. americanum*, including those with three or four juvenile stages, were genetically identical inferring a single species. In contrast, speciation was evident in the studied populations from both North America and Europe. Furthermore, our data suggested that a greater genetic diversity existed in the studied European populations that originated from a smaller geographic area than the studied populations that originated in either North America or Asia.

138 Molecular diagnostics for virus-transmitting nematodes in German viticulture

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Virus-free grapevine plants required for viticulture have to be grown in soil free from virus-transmitting nematodes. Current soil testing methods are laborious and require highly experienced staff as they rely on morphological examination of the nematodes. Molecular diagnostics provide a simple and reliable alternative, but are not available for virus-transmitting nematodes. To develop this method for application with samples from German vineyards, we investigated species-specific primers for the most important nematodes in German viticulture *viz.*, *Xiphinema index*, *X. diversicaudatum* and *X. vuittenezi*. The 18S gene of rDNA, which is highly conserved in taxa, was sequenced using three primer pairs for ten, eight and four populations of these three species to confirm the homogeneity of each species. The genetic variation between the species was low. Consequently, in the first instance, species-specific primers identified from the genetically variable ITS-1 region of rDNA for each of these species, developed by INRA France, were used in interaction tests in PCR to confirm their specificity and robustness when used for detecting the species occurring in soil samples from German vineyards. Primer sets are also being developed for *Longidorus* and *Paralongidorus* virus-vector species. The preliminary results from these tests are presented and discussed.

139 Genetic variability of *Meloidogyne mayaguensis* isolates from the Caribbean

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Meloidogyne mayaguensis Rammah & Hirschmann, 1988 has been recently identified in the French West Indies from guava (*Psidium guayava*) orchards. This very aggressive root-knot nematode seems already widely distributed in the Caribbean (Cuba, Dominican Republic, Puerto Rico, Guadeloupe, Martinique). The objective of this study was to evaluate the genetic variability of *M. mayaguensis* isolates from several locations in the Caribbean, in comparison with African isolates from Burundi and Congo. For that purpose, nematodes were characterised using both isozyme electrophoresis and RAPD-PCR. At the biochemical level, for the two enzymatic systems used (EST and MDH), all the isolates shared the same electrophoretic profiles. At the molecular level, except one isolate from Martinique, RAPD analysis revealed a high level of genetic similarity (>78%) between all isolates. The UPGMA dendrogram deduced from the RAPD patterns showed that clustering of isolates neither reflected their geographic origin nor indicated how they might have been spread. Despite having been sampled from very distant locations, such a relative lack of genetic polymorphism suggests a common origin for all the *M. mayaguensis* isolates tested, and indicates that they may have been dispersed from a single source instead of representing local indigenous populations.

140 Four new species of soil nematodes (Nematoda: Dorylaimida) from Japan

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During the course of studies on biodiversity of soil nematodes in Japan, four new species of soil nematodes belonging to the order Dorylaimida were isolated from the soil samples collected from a non-tillage field of National Institute for Agro-Environmental Sciences, Tsukuba, Japan. Specimens killed in hot water were fixed in TAF, processed to anhydrous glycerin and mounted on slides. Specimens were observed under optical microscope and measured with the help of drawing tube attachment. Measurements and characteristics of these new species are as follows: *Egtitus* n. sp. is 1.7-2.0 mm long, a = 41-46, b = 3.9-4.3, c = 15-19, V = 50-53, odontostyle = 21-23 μ m, and is characterised by having sinuate odontostyle and smaller prerectum. *Axonchium* n. sp. having 1.5-1.6 mm long body, a = 39-43, b = 2.5-2.6, c = 48-54, V = 50-52, odontostyle = 9-11 μ m, and is distinguishable by its

larger body and absence of post-uterine sac. *Chitwoodius* n. sp. measuring 1.0-1.2 mm long, a = 26-31, b = 3.4-4.1, c = 47-55, V = 61-64, odontostyle = 30-33 μm , spicules = 48-55 μm and is characterised by having posteriorly located guiding ring and larger prerectum. *Labronema* n. sp. is 1.0-1.2 mm long, a = 22-25, b = 3.6-4.0, c = 47-50, V = 50-52, odontostyle = 20-21 μm , spicules = 55 μm and is characterised by smaller body and sub-digitate tail.

141 Four new species of soil nematodes (Nematoda: Dorylaimida) from Japan

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During the course of a study on biodiversity of soil nematodes in Japan, four new species of soil nematodes belonging to the order Dorylaimida were isolated from the soil samples collected from a non-tillage field of the National Institute for Agro-Environmental Sciences, Tsukuba. Specimens killed in hot water were fixed in TAF, processed to anhydrous glycerin and mounted on slides. Specimens were observed under optical microscope and measured with drawing tube. Measurements and characteristics of these new species are as follows: *Axonchium* n. sp. is 1.5-1.6 mm long, a = 39-43, b = 2.5-2.6, c = 48-54, V = 50-52, odontostyle = 9-11 μm , and is distinguishable by its larger body and absence of post-uterine sac. *Egtitus* n. sp. is 1.7-2.0 mm long, a = 41-46, b = 3.9-4.3, c = 15-19, V = 50-53, odontostyle = 21-23 μm , and is characterised by having sinuate odontostyle and smaller prerectum. *Labronema* n. sp. is 1.0-1.2 mm long, a = 22-25, b = 3.6-4.0, c = 47-50, V = 50-52, odontostyle = 20-21 μm , spicules = 55 μm and is characterised by smaller body and sub-digitate tail. *Prodorylaimus* n. sp. measuring 1.3-1.5 mm long, a = 37-40, b = 3.8-4.0, c = 5.1-6.0, V = 50-53, odontostyle = 21-24 μm , and is characterised by having rounded lip region and gradually tapering to long filiform tail.

142 Inter-population variability in *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida)

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Data corresponding to morphometrical characters of males and females belonging to populations of *Pratylenchus vulnus* from different geographic origin and under different developmental conditions were considered. An inter and intra-population variance was estimated for each character as well as the percentage of inter-population variance. Characters were grouped into three categories (low, medium and high variability). Considering those percentages, it was observed that the number of characters with low variability was higher in females than in males, being inverse in the case of characters with high variability. Depending on the percentage of inter-population variability for each character, it was inferred that those characters included in the low variability category would be barely influenced by environmental conditions, whereas those characters related to high values would be considerably influenced. Therefore, not all characters would have the same significance. Some of them appear to be more significant than others for the species identification (V ratio and spicule length for males and females, respectively, ratios O, MB, c', pharynx length) whereas other group of characters should be preferably considered when evaluating the existence of possible differences between populations.

143 Genetic variability in two populations of *Heterodera glycines* Ichinohe, 1952 from Argentina estimated with RAPD-PCR markers

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The genetic variability of two populations of *H. glycines* was analysed using Random Amplified Polymorphic DNA (RAPD-PCR) as markers. Thirty females of a population belonging to race 1 from Laguna Larga (Córdoba Province) and 31 females from a population of race 3 from Tortugas (Santa Fe Province) were studied individually. Twenty random primers from Biodynamics (Buenos Aires, Argentina) were tested. Five of them were selected because they originated reproducible polymorphic bands. Thirty nine consistent fragments were considered for the analysis. Allele frequencies were estimated assuming RAPD segregate as dominant and that the populations were in Hardy-Weinberg equilibrium. Proportion of polymorphic loci was 100% in both populations; mean expected heterozygosity (He) among individuals was 0.39 and 0.42 for females from Laguna Larga and Tortugas, respectively. F_{ST} values were significant ($P < 0.05$) for 67% of the loci. The AMOVA test showed a variation of 81.2% within specimens of a population, while the remaining 18.8% accounted for differences between populations. None of the populations presented exclusive bands, although many fragments showed significant differences in their frequencies. This study revealed a high intra-population variability and an important degree of genetic differentiation between populations of races 1 and 3.

144 Phylogeny of *Meloidogyne* spp. based on rDNA-ITS sequence and fluorescent AFLP

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A phylogenetic study of 12 populations of *M. incognita*, two populations of *M. arenaria*, two populations of *M. javanica* and newly-described *M. panyuensis* n. sp. was performed on rDNA-ITS sequences and fluorescent AFLP. For the rDNA-ITS sequence analysis, genomic sequences containing the partial 18S, complete ITS and partial 26S regions were PCR amplified, cloned and sequenced. Sequence analysis using the neighbour-joining cluster method showed that it is difficult to establish a reasonable phylogenetic relationship among the four species since the tree topology does not match with the established taxonomy. For AFLP analysis, 42 primer combinations were screened and AFLP with a total

number of amplified polymorphic fragments of 1267 from ten primer combinations were analysed with NTSYS-pc package. Populations from each species form a single clade and the phylogenetic relationship matches very well with previous phylogeny. *Meloidogyne arenaria* and *M. javanica* are closely related and form a branch that is monophyletic to *M. incognita*. *Meloidogyne panyuensis* is distantly related to the other three species. We concluded that AFLP is more reliable than rDNA-ITS sequences for nematode phylogenetic study since AFLP covers the whole genome whereas rDNA-ITS sequences come from only one locus. Furthermore, AFLP is much simpler than rDNA-ITS sequencing.

145 Enzymatic characterisation of *Meloidogyne* spp. associated with ornamentals and agronomic crops in Florida, USA

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The use of enzyme phenotypes has made the identification of species of *Meloidogyne* less subjective and more accurate. Biochemical analysis based on phenotypes of esterase (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD), and glutamate-oxaloacetate transaminase (GOT) was used to characterise 52 populations of *Meloidogyne* spp. collected from ornamentals and agronomic crops in Florida. *Meloidogyne javanica*, J3 phenotype, was the most common species (38.4%), followed by *M. arenaria* phenotype A2 (25.0%), *M. incognita* phenotypes I1 (15.4%) and I2 (7.6%), *M. graminis* (9.6%), and *Meloidogyne* sp. (3.8%). *Meloidogyne graminis* had only one slow moving activity EST band which was not present in any of the other phenotypes, three SOD bands (two intensely stained and a third less intensely stained but faster moving band), and one intensely stained MDH band identical to that of *M. mayaguensis*. The occurrence of *M. javanica* in peanut is being reported for the first time in Florida, USA.

146 Variability among isolates of *Pratylenchus penetrans* for an interaction with *Verticillium dahliae* and the potato early dying disease

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A synergistic interaction of *Pratylenchus penetrans* and *Verticillium dahliae* for the potato early dying disease has been demonstrated using a variety of potato cultivars and soil types. The purpose of our study was to determine if geographic isolates of *P. penetrans* vary for this interaction. We tested eight *P. penetrans* isolates from Wisconsin and one each from Washington, Minnesota and Ohio in a series of growth chamber experiments. Three-week-old potato cv. Russet Burbank grown from tissue culture was inoculated with nematodes and 1 week later with conidia of *V. dahliae*. Control plants were inoculated with only *V. dahliae* or mock inoculated with water and no pathogens. The isolates were ranked for the time required for three leaves to become symptomatic and area under the disease progress curve, as well as for population attributes such as female:male ratio and the rate of root egress. Three isolates from Wisconsin represented the range of variability for symptom expression; two ranked in the top third and one ranked in the bottom third consistently, but all isolates were able to cause disease in combination with *V. dahliae*.

147 Identification of some cyst-forming nematodes based on PCR-RFLP, sequence of rDNA and specific primers

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More than 50 populations of cyst-forming nematodes collected in Italy and Belgium were analysed using PCR-RFLP, ITS-rDNA sequences, and PCR with species-specific primers. Restriction of PCR products by eight restriction enzymes *AluI*, *AvaI*, *Bsh1236I*, *CfoI*, *HaeIII*, *MvaI*, *PstI* and *RsaI* allowed the identification of

several agriculturally important cyst nematode species and separation from their sibling species. RFLP profiles for some European populations of *Globodera pallida*, *G. rostochiensis*, *Heterodera carotae*, *H. fici*, *H. filipjevi*, *H. goettingiana*, *H. hordecalis*, *H. humuli*, *H. mediterranea*, *H. riparia* and *H. schachtii* are given. The species-specific primer developed by Bulman and Marshall clearly discriminated *G. pallida* from *G. rostochiensis*. *Bsh1236I* digestion of the PCR product separated both *Globodera* species from each other and from *G. tabacum*. Comparison of obtained sequences with those deposited in GenBank showed high similarities (99.8-100%). Relative high level of sequence divergence between populations of *H. hordecalis* (1.5%) suggests the presence of several biological species presently grouped under this taxon.

148 Intraspecies DNA polymorphism in the tobacco cyst nematode complex (*Globodera tabacum*) using AFLP

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AFLP was used to obtain information on the within-species genetic variability of the tobacco cyst nematodes (TCN) complex. AFLP was found to be well suited for this type of study. The current classification of TCN was confirmed. Results indicate that the *Globodera tabacum solanacearum* group, believed to be restricted to the USA, also occurs in Mexico. The within-species variability of the TCN is considerable. Populations from Mexico may form a new sub-group. AFLP group-specific markers were identified for two TCN subgroups: *Globodera tabacum tabacum* and *Globodera tabacum solanacearum*.

149 Reconstructing a phylogeny of Dorylaimida (Nematoda) with partial 18s sequence data using Bayesian inference

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Evolutionary relationships among members of Dorylaimida are investigated using approximately 600 bp of the 18s ribosomal DNA repeat. Sequence data obtained from selected taxa within Dorylaimida and from several related groups form the basis for the analyses. Results obtained under a range of optimality criteria and using several different methods, including parsimony, distance, and likelihood approaches, are presented and compared. The use of Bayesian inference in phylogeny reconstruction allows the combination of detailed likelihood models of DNA evolution with an assessment of the reliability of the results by summarising the posterior probabilities of an extensive sample of trees generated under a given set of model parameters. The resulting consensus tree, with summary branch lengths and clade credibility values, is analogous to a maximum likelihood bootstrap tree, but can be generated in a fraction of the time. We present a phylogenetic hypothesis for Dorylaimida and related taxa, along with discussion of several points of departure from recent classification schemes of this important group of terrestrial nematodes.

150 Genetic differentiation of individuals and populations of the endoparasitic nematode, *Globodera pallida*, using microsatellites

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The genetic differentiation of eight populations of the endoparasitic nematode *Globodera pallida* with known virulence characteristics was assessed at the individual nematode level using six microsatellite loci. This provided an opportunity to assess levels of genetic diversity between and within populations at a resolution that hitherto has not been possible. Intra-population genetic variability was evident, with four populations, Luffness, P5A, Gourdie and Halton being least genetically variable. Although *Globodera pallida* is a sedentary endoparasite and sexually reproducing species, F_{ST} values and heterozygosity data suggested that it had characteristics of an in-breeding species and supports published hypotheses postulating that many of the European *G. pallida* populations arose *via* multiple founder effects. Principal coordinate analyses derived from a) dissimilarity values

and b) mean F_{ST} values from an analysis of molecular variance, separated the eight *G. pallida* populations into four similar putative groupings. As with previous studies, P5A was distinct from all European populations and Luffness (and Lindley) were disparate from the other UK populations. The remaining populations formed two groupings suggesting further genetic differentiation between UK populations.

151 Variability of *Meloidogyne exigua* on coffee crops in the Zona da Mata of Minas Gerais State, Brazil

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Minas Gerais State is the most important producer of coffee in Brazil and 28% of its production occurs in the Zona da Mata region. Four major species of root-knot nematodes attacking coffee plants have been reported in Brazil, and some of them can cause plant death. The correct identification of species and/or race(s) of *Meloidogyne* present in roots of coffee is extremely important in deciding which measures are more appropriate for controlling the pathogens. In order to determine the occurrence and variability of *Meloidogyne* spp. in the region, 57 populations from 16 different locations were evaluated based on morphologic, enzymatic and physiologic traits. All of the 57 populations were identified as *Meloidogyne exigua* based on their perineal patterns. This characterisation was confirmed by phenotypes of esterase, malate dehydrogenase, sulphoxide dismutase and glutamate oxaloacetate transaminase. Thirteen populations presented the typical esterase phenotype showed by one band (VF1), while most of the populations (77.2%) exhibit a phenotype showed by two bands. No intraspecific variability was observed in the populations studied, and all of them were able to reproduce on tomato, pepper, cocoa, onion, bean and soybean.

152 Molecular characterisation of some species of the genus *Meloidogyne* from China

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rDNA-ITS RFLP and sequence analyses were used for identification and characterisation of ten *Meloidogyne* populations from China. The ITS regions were amplified using F195 and 5367 primers. PCR resulted in a single fragment of about 700 bp for all samples. Based on morphological and morphometrical characters, and ITS sequences, seven populations were identified as *M. hapla*, *M. javanica*, *M. arenaria*, *M. incognita* and *M. enterolobii*, while three populations belonged to two undescribed species. Twelve restriction enzymes were used for digestion of PCR products. The RFLP patterns generated by *HinfI* + *RsaI* separated all populations into three groups. None of the enzymes differentiated *M. incognita*, *M. arenaria* and *M. javanica* from each other. The RFLP and sequence analyses of the D2-D3 regions of the 28S gene permitted separation of several root-knot nematode species.

153 rDNA restriction fragment length polymorphism of *Heterodera avenae* in China

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The amplification of the rDNA-ITS region of *Heterodera avenae* (CCN) from China and Morocco with the current primers AB28 and TW81 yielded one fragment of approximately 1060 bp. A total of 27 scored fragments were obtained with 12 restriction enzymes. Intraspecific polymorphism was revealed within *H. avenae* by *AluI*, *RsaI* and *HinfI* digestion. *AluI* and *RsaI* digestion of ITS products of seven Chinese CCN populations yielded two fragments respectively (560 bp, 500 bp; 720 bp, 320 bp); but neither *AluI* nor *RsaI* could digest ITS product of Morocco. Those RFLP profiles revealed by *AluI* and *RsaI* classified the ITS of Chinese populations as 'Type B'

according to Subbotin *et al.* (2000). Digestion by *AluI* also showed heterogeneity in ITS regions of Morocco population, and two additional bands were obtained; the sum of the three fragments was approximately 2120 bp. *HinfI* digested PCR amplified ITS products of Chinese populations obtained two fragments (850 bp, 200 bp), but obtained three fragments (510 bp, 340 bp, 200 bp) from Morocco population. The results showed that Chinese populations may be distinctly different from Morocco population. Seven enzymes *CfoI*, *Bsh1236I*, *MsrFI*, *ScrFI*, *HaeIII*, *MvaI*, *MspI* produced restriction profiles identical for all CCN populations. *HindIII* and *AvaI* did not digest the ITS products of Chinese and Morocco CCN populations.

154 Genetic variability of *Heterodera schachtii* populations in the north of France inferred by microsatellite loci

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A better knowledge of the genetic structure of phytoparasitic nematode populations is essential to control these organisms, notably in the context of the use of resistant varieties leading to the selection of virulent individuals able to overcome resistance genes. With that aim, we first studied the genetic variability of nematode populations at various spatial scales using neutral genetic markers in fields where no resistant varieties have been grown until now. Our study focused on the sugar beet cyst nematode (*Heterodera schachtii*) in the north of France. Using five microsatellite loci on individuals sampled according to a hierarchical design, an Analysis of MOlecular VAriance (AMOVA) indicates that almost all the genetic variability is observed at the field scale (94% of the total genetic variability), with only 4% variability observed among fields of the same region and 2% among regions. This suggests that significant gene flows occur among fields and regions preventing genetic differentiation. In this diploid amphimictic species, Hardy-Weinberg equilibrium is observed only at the smaller spatial scale (*i.e.*, the individual plant), probably as a result of limited dispersal of individuals in the soil between hatching and mating.

155 The differential host test, mtDNA and rDNA PCR to distinguish *Meloidogyne arenaria*, *M. incognita* and *M. javanica* from vineyards

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The North Carolina (NC) differential host test and mtDNA were used to distinguish a collection of root-knot nematodes from South Australian vineyards. PCR amplifications of D3 expansion region of 28S rRNA gene and intergenic sequences of ribosomal DNA (IGS-rDNA) were also made to distinguish *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. The NC differential host test differentiated *Meloidogyne incognita* but not *M. arenaria* race 2 from *M. javanica*. The combination of the NC host test and mtDNA analysis differentiated among *M. arenaria*, *M. incognita* and *M. javanica*. The differentiation of these species with D3 expansion region of 28S rRNA gene was not possible. The sequences of this region are highly conserved among the species. The PCR amplification of IGS-rDNA from single females of each species produced distinct banding patterns that differentiated the species from each other. These species-specific banding patterns were reproducible across a range of individual nematodes of each species collected from different geographical locations of Australia. This method also produced DNA fingerprint variability within some individuals of each species. This variability could be applied to the examination of intraspecific variation and potentially development of race specific diagnostic marker(s).

156 A new species-specific satellite DNA family in the genome of the coffee root-knot nematode, *Meloidogyne exigua*

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A new satellite DNA family has been cloned in the coffee root-knot nematode, *Meloidogyne exigua*. It is represented as tandemly repeated sequences with a monomeric unit of 277 bp. The repeats are present at approximately 17 900 copies per haploid genome, and represent about

9.7% of the total genomic DNA. Twenty independent monomers have been cloned and sequenced. They are very homogeneous in sequence, with 2.4% average divergence from the deduced consensus. This result strongly suggests that this repeated family originated from a recent amplification event. Dot-blot experiments demonstrated that the satellite DNA distribution is limited to *M. exigua* isolates only. In squash-blot assays, it was possible to detect and positively identify single nematodes, independently of their developmental stage. Therefore, because of its high reiteration in the genome, and its species-specificity, this sequence could be used as a probe for the molecular diagnosis of the nematode.

157 Genetic diversity in field populations of tobacco cyst nematode in North Carolina

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Tobacco cyst nematode (TCN) is a fast spreading nematode problem in North Carolina requiring an efficient method of management. Resistant varieties are a good alternative to chemical nematicides. Quantification of genetic diversity is essential to decide the type of resistance to be incorporated. Cysts collected from infested fields of tobacco in Vance, Warren and Surry counties were used for RAPD-PCR analysis. Fourteen field samples for inter-population diversity and four populations, one from each county, for intra-population diversity were used. The size of the PCR amplified products varied from 220 to 2500 bp and the number of products varied from 11 to 22. UPGMA clustering grouped 13 populations into two major clusters at nodal interjunctions of 47 and 58%. One population was heterogeneous enough to fall out of these two clusters. For within population, percentage similarity ranged from 36 to 56 indicating considerable amount of genetic diversity. The heterogeneity observed in TCN suggests that breeding for resistance should be horizontal or broad enough to manage the damage at threshold levels.

158 The development of a query system for the Brazilian nematological bibliography references through the Internet

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Several bibliographical sources regarding nematode themes are organised in a bank and this will be put in a database which the Nematode Query System will access, for use *via* the Internet. When one wants to access nematode data, the search for such data becomes an arduous task for professionals. A standard form of systematic classification of such bibliographical sources is very important. In this context, the construction of a database in computational environment allows the data to be stored in a systematic and standard way, allowing efficient data recovery. Recently, EMBRAPA Genetic Resources and Biotechnology and Brasilia Catholic University have been constructing a database of these references which include several species of nematodes and plants belonging to different botanical families. This database allows a search of Brazilian publications including the distribution in Brazil and types of control. Currently, this database has 1850 bibliographical references recovered from 1981 to 2001. The nematode query system, which is a system of access to bibliographical references databases, is now under construction and will be accessible at EMBRAPA Center. A search of the nematode query system can be done by using the scientific name of either the nematode or the host plant.

159 A simple molecular diagnostic of marine Enoplida

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Marine Enoplida constitute a group of free-living marine nematodes, which do not share any known morphological synapomorphy. On SSU rRNA phylogenies the group also lacks solid statistical support. In recent studies it was found that an enoplid nematode *Trefusia zostericola*

possesses a rare nucleotide substitution within the region of hairpin 35 of the SSU rRNA secondary structure. The same substitution is common for all 17 Enoplida species studied to date, including *Trefusia* and *Xenella* (Trefusiidae), but is absent from other nematode lineages including Triplonchida and Dorylaimia. Thus, the substitution can be considered a molecular synapomorphy of Enoplida and Trefusiidae. Apart from this substitution, Enoplida are found to share a rare transversion in the loop of hairpin 48. This substitution is not strictly specific for Enoplida, as it is also known from Mesorhabditoidea and selected non-nematode taxa. These signatures may be used in designing specific primers and be considered as markers of the divergent sequences of marine Enoplida.

160 SSU rRNA gene of *Soboliphyme baturini* Petrow, 1930 (Nematoda: Dioctophymida) and a reappraisal of molecular bounds of Dorylaimia

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Molecular phylogenies based on SSU rDNA sequence data suggest that Dorylaimia, along with Enoplida and Chromadoria, constitutes the third mainstream in nematode evolution. Its contents are represented as follows: (Trichinellida (Dorylaimida (Mononchida: Mermithida))). However, high rates of sequence evolution inherent in the trichinellid lineage render this topology unreliable. Novel molecular data on the SSU rRNA gene structure of *Soboliphyme baturini* (Dioctophymida: Soboliphymidae) revealed the fifth lineage within Dorylaimia and provided new information for establishing its internal topology. Maximum parsimony, maximum likelihood and neighbour-joining algorithms with bootstrap replicates consistently inferred *S. baturini* as a sister taxon to Trichinellida. Inclusion of *S. baturini* had a stabilising affect on the integrity of the Dorylaimia clade through breaking the long branch leading to Trichinellida. Putative molecular synapomorphies reconstructed by parsimony for each node of the tree were analysed on a large dataset of metazoan SSU rDNA sequences to sort out

homoplasious characters that may potentially bias parsimony estimations. Strong synapomorphies located in highly conserved parts of the gene support monophyly of all Dorylaimia and suggest the following internal bifurcation pattern: ((Trichinellida: Dioctophymida): (Dorylaimida (Mononchida: Mermithida))). Molecular phylogeny of Dorylaimia juxtaposed with morphological evidence may alter our perception of morphological and ecological change in nematode evolution.

161 Phylogeny of Enoplia: parsimony estimations with SSU rDNA sequence data

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Molecular analyses suggest that Enoplia is the most divergent major clade within the phylum Nematoda. Disparities in rates of sequence evolution between lineages, lack of intermediate taxa to break possible long branches and the limited amount of phylogenetic information shared by sampled taxa make phylogenetic inference for Enoplia a difficult task. The dataset was carefully selected to maximally encompass enoplian biodiversity and utilise basal representatives of other clades, thus breaking long branches at all levels of the tree. It includes several genera for which molecular evidence has not been previously published: *Campydora*, *Leptosomatium*, *Thoracotomopsis*, *Bathylaimus*, *Tripylloides*, *Calyptonema*, *Viscosia*, *Halalaimus*, *Ironus*, *Alaimus*, *Xenella*, *Rhabdodomania*, *Tobrilus*, *Aporcelaimellus* and *Nygolaimus*. The data was examined for presence of phylogenetic signal using approaches based on sequential removal of categories of variable sites, with phylogeny reconstruction at each step. This case study proves that the NJ method performs

highly inconsistently with this problematic dataset and that bootstrap analyses (both NJ and MP) greatly underestimate repeatability of inferring even basal nodes of the nematode tree. Instead, comparing equally parsimonious topologies obtained in a series of MP runs with a number of statistical tests under the ML optimality criterion allowed the reconstruction of the major lineages within Enoplia.

162 Are data from the beta-tubulin gene family useful for cyst nematode phylogeny?

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Many eukaryotic genes have been found to be members of gene families. When similar, but different members of a gene family are recovered with the same PCR primers, it can be difficult to use such data for phylogenetic inference. To do so, we first inferred evolutionary relationships among 24 species of cyst nematodes from ribosomal DNA (rDNA) sequence data. Separately, we used beta-tubulin data, now known to be a gene family of six members in *Caenorhabditis elegans*. Topologies from the two trees were incongruent. Following restriction and hybridisation analysis, we concluded that beta-tubulin is a small gene family in cyst nematodes as well. We attributed six monophyletic groups on our beta-tubulin tree to putative gene family members. Some taxa appeared in more than one group, indicating the presence of multiple family members. The evolution of the gene family was assessed by tracing putative beta-tubulin genes on the rDNA tree, which we hypothesised to be a true species tree. A reasonable evolutionary scenario could be devised involving gene duplication, gene loss, and deep coalescence. Each of three different groups in Heteroderidae, monophyletic on our species tree, had different putative beta-tubulin gene members, and the data therefore corroborated the rDNA species tree.

163 RAPD of *Pratylenchus* populations from coffee, banana, ornamental plant and citrus in Brazil

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RAPD analyses of seven *Pratylenchus* populations from different hosts and regions of Brazil identified as *Pratylenchus coffeae* were studied. Three populations were extracted from roots of banana plant (Minas Gerais State), two from citrus (São Paulo State), one from coffee (São Paulo State) and one from *Aglaonema* (Rio de Janeiro State). *Pratylenchus penetrans* from oil palm was used as out group. They had been cultured on alfalfa callus tissues. Baermann extracted specimens of the populations from callus were smashed individually into a 200 μ l thermocycler tube with 5 μ l of lyses buffer and frozen at -20°C . After freezing for at least 1 week, the individuals of each population were pooled, and 5 μ l of the bulk of the population was used for PCR amplification. They were then run in 3% agarose gel for 3 h at 80 V. The gel was submitted in an ethidium bromide 0.25% and photographed. Polymorphic bands were analysed in Popgene software which grouped them in four clusters according to the host plants.

164 Utility of the heat shock protein gene, *hsp90*, for phylogenetic analysis and diagnostics of nematodes at several taxonomic levels

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Novel molecular traits are needed for nematode phylogeny and diagnostics, particularly when source specimens are limited or when currently used molecules do not provide enough molecular discrimination to distinguish genera, species, or isolates. We have examined the *hsp90* gene as a single-copy diagnostic molecule for nematodes at several taxonomic levels. We used degenerate primer PCR to amplify partial *hsp90* genomic sequences from a broad range of bacterial-feeding and plant-parasitic nematode genera. Genomic DNA alignments showed that *hsp90* introns had species-specific arrangements and genus-specific sizes. HSP90 amino acid phylogeny revealed several nematode-specific residues, and placed nematodes paraphyletic to insects. These results demonstrate that *hsp90* is informative and useful for evaluating the evolutionary relationships within and

between nematodes and other organisms. We performed another study to detect the presence of *hsp90* polymorphisms that may be useful for discrimination of nematodes at the sub-species level. Comparison of *hsp90* genomic sequences from several highly inbred strains of the soybean cyst nematode, *Heterodera glycines*, revealed unique combinations of intron polymorphisms in some strains, thus demonstrating the potential of this gene for discrimination of soybean cyst nematode genotypes.

165 Functional analysis of heat shock protein, HSP90, from the soybean cyst nematode, *Heterodera glycines*

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Developmentally arrested second-stage juveniles of many plant parasites are functionally similar to *Caenorhabditis elegans* dauers, and likely use molecules similar to those defined by the *C. elegans* dauer pathway to regulate developmental arrest, lifespan, and chemosensation. One *C. elegans* dauer pathway gene, *daf-21*, encodes an HSP90 molecular chaperone. The precise function of *daf-21* is unclear; however, HSP90 chaperones are known to refold denatured or misfolded proteins, especially under conditions of stress. In many systems, HSP90 guides the proper folding of specific target proteins, including nuclear hormone receptors and protein kinases. We are currently examining the effect of an HSP90 inhibitor, geldanamycin, on *C. elegans* development. Disruption of normal development by this compound would implicate a role for HSP90 in this process, and form the basis for similar studies in plant-parasitic nematodes. We have also initiated a yeast two hybrid screen using *Heterodera glycines* HSP90 as bait, to identify co-chaperones or target proteins, some of which may include dauer pathway homologs that are critical to plant-parasitic nematode development. In the absence of robust transformation or RNAi strategies for plant-parasitic nematodes, the yeast two hybrid approach should provide a tractable system for analysing gene function and identifying critical protein-protein interactions.

166 Phylogenetic and sequence analysis of ITS1, 5.8S gene, and ITS2 regions of the rDNA of *Steinernema*: comparison of populations and species

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Ninety new and 16 known complete ITS1+5.8S+ITS2 of rDNA sequences belonging to various populations of 22 nominal and several unidentified *Steinernema* species have been analysed using maximum parsimony and maximum likelihood methods. Analyses showed that more than 90% of the studied sequences formed four main moderately or highly supported clades ‘intermedium-affine’, ‘carpocapsae-tamsiamkayai-scapteriscī’, ‘feltiae-kraussei-oregonense’, ‘glaseri-cubanum-arenarium-karii-longicaudum’) and one weakly supported clade (‘bicornutum-ceratophorum-riobrave’). This grouping was obtained in most of the phylogenetic trees obtained from different ITS alignments and phylogenetic procedures. A short morphological characterisation of these clades based on infective juvenile (IJ) body length, amoeboid cell and lateral field structure is given. Groupings of species in the ITS based trees are largely congruent with previous D2-D3 based trees. Molecular analysis revealed at least ten new species among studied *Steinernema* isolates and proved the identity for two undescribed new species from Germany (types ‘B’ and ‘F’) with British undescribed cultures (types ‘B3’ and ‘D1’), respectively. Sequence differences between more than ten isolates of *S. feltiae* and *S. kraussei* were estimated. Several isolates from Switzerland, Sri-Lanka, Venezuela, Vietnam and USA with long and medium-sized IJ were found to be independent from related known species.

167 Molecular phylogenies of plant and entomoparasitic nematodes: congruence and incongruence with morphological and biological data

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One of the exciting developments in nematology during the last years has been the application of nucleic acid data analysis to problems of systematics of certain groups. In many cases, molecular data confirm the monophyly of species groups recognised by morphological studies. In other cases, the molecular data reveal taxa of which the phylogenetic relationship based on the morphology seems to be problematic. Molecular data put more light on problems of species boundaries and co-evolution of nematodes with their hosts. The early presumption that phylogenetic trees based on molecular data would more precisely reflect the true phylogeny than morphological data, seems to be wrong. Analyses of influence of alignment or tree building methods on phylogenies showed that molecular data could be subjected to many problems. Congruence and incongruence of results of analyses of molecular and morphological data sets and the possible reasons for conflicts are discussed with examples of plant-parasitic nematodes from the family Heteroderidae, Anguinidae, Longidoridae, and entomoparasitic nematodes of the Tylenchida and the families Steinernematidae and Heterorhabditidae.

168 Molecular phylogenetics of the cyst-forming nematodes (Tylenchida: Heteroderidae)

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Sequences of the complete ITS and the D2-D3 expansion segment of the 28S gene of rDNA obtained from 54 valid and seven undescribed species of cyst forming nematodes from the genera *Afenestrata*, *Betulodera*, *Cactodera*, *Dolichodera*, *Globodera*, *Heterodera* and *Punctodera* have been analysed using maximum parsimony. On the phylogenetic trees the species clustered in several major clades corresponding to the morphological grouping and present taxonomic division. The analy-

sis yielded six main morphological groups within the genus *Heterodera*: Avenae, Sacchari, Humuli, Schachtii, Cyperi and Goettingiana. Phylogenetic relationships between some of the *Heterodera* groups were not well resolved. The Goettingiana group occupied a basal position within Heteroderinae. Molecular data strongly supported monophyly of the Punctoderinae containing the genera *Cactodera*, *Globodera*, *Punctodera*, *Betulodera* and *Dolichodera*. *Punctodera* and *Dolichodera* have sister relationships. Analysis of the D2-D3 expansion segment of the 28S gene only partly resolved relationships within basal groups of Heteroderidae.

169 Phylogenetic relationships within the stem and gall forming plant-parasitic nematodes (Tylenchida: Anguinidae) as inferred from analyses of the ITS-rDNA sequences

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Internal transcribed spacer (ITS) sequences of rDNA from 53 populations and species of gall-forming nematodes of the subfamily Anguininae, along with five populations of the *Ditylenchus dipsaci* species complex were used for phylogenetic analyses. The molecular analyses support a concept of narrow specialisation for seed-gall nematodes and reveal distinction of at least nine undescribed species of *Anguina* inducing seed galls, previously identified as *A. agrostis*, and two species within the *D. dipsaci* species complex. Both the maximum parsimony and maximum likelihood analyses of the ITS data strongly support monophyly of the genus *Anguina*. Also, non-monophyly for *Subanguina* in the broad sense of Brzeski (1981) and of *Mesoanguina* and *Heteroanguina* according to Chizhov and Subbotin was indicated. Morphological and biological characters are congruent

with the anguinid groups supported by the ITS phylogeny. The test of topologies conducted by maximum likelihood analyses shows that the monophyletic origin of anguinids parasitising grasses and sedges could not be rejected. The main anguinid groups are generally associated with plant hosts belonging to the same or related systematic groups.

170 A new anguinid from dune grass in South Africa

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In October 2000, the senior author received dune grass (*Ehrharta villosa* var. *villosa*) with purple coloured galls on the stems and leaf sheaths. On opening, each gall contained four to six adult anguinid nematodes and numerous eggs and juveniles. Some galls were filled with a yellow substance containing bacteria. The bacteria were isolated and provisionally identified as *Clavibacter*. The galls and the nematodes were studied with the aid of the scanning electron microscope, the results of which are depicted on the poster. Molecular analysis of the anguinids showed that they are close to both *Anguina australis* and *A. microlaenia*. Both these species have been identified in Australia where they infect plants of the Poaceae, tribe Ehrharteae. According to molecular analysis, a host test and a light microscope study, the anguinid population from South Africa is new to science and in the process of being described.

171 Phylogenetic analysis of *Longidorus* and *Xiphinema* species (Nematoda: Dorylaimida) using ITS1 sequences of nuclear ribosomal DNA

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Some Longidoridae in the genera *Longidorus* and *Xiphinema* are important plant parasites as well as vectors of plant nepoviruses. Species discrimination based entirely on morphology is often questionable and controversial because of morphometric overlap and the lack of a phylogenetic framework for this group. Phylogenetic analysis using DNA sequencing of nuclear ribosomal DNA ITS1 was conducted to construct an evolutionary tree. Extensive interspecies sequence variation and minor intraspecies sequence variation were observed. The analyses suggest two groups within Arkansas Longidoridae species. The *Xiphinema* group includes *X. americanum*, *X. bakeri*, *X. chambersi*, and *Longidorus diadecturus*. *Longidorus diadecturus* shares some morphological characters of both *Xiphinema* and *Longidorus*. The *Longidorus* group includes *L. breviannulatus*, *L. crassus*, *L. fragilis*, five undescribed species, and two non-Arkansas species (*L. elongatus* from Europe and *L. africanus* from California). The phylogenetic analysis largely corresponded to the genera and species defined by morphology. Results demonstrated the potential for the use of rDNA ITS1 and 18S gene to infer phylogenetic relationships and PCR-RFLP based identification among longidorids.

172 Inter- and intra-specific variation in ribosomal DNA in some isolates of *Bursaphelenchus xylophilus* and *B. mucronatus*

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The genetic diversity of five isolates of *Bursaphelenchus xylophilus* and four of *B. mucronatus* from China or detected in package woods from Japan and Korea was studied. PCR-RFLP analysis of the ITS region of rDNA showed that *AluI*, *CfoI*, *HaeIII*, *HinfI* and *MspI* yield specific patterns. Using these patterns, both species were detected in a sample from Ningbo, Zhejiang, China.

The analysis of the ITS sequence alignment of the four populations of *B. mucronatus* and of three of the *B. xylophilus* populations revealed minimal (one nucleotide) intra-specific divergence. The inter-specific divergence was more than 100 bp. There were only 3 bp differences within populations of the same species, and 18 or 19 bp difference between species in sequences of the D2 and D3 expansion segments of the 28S gene.

173 Study of populations of *Ditylenchus dipsaci* from the Czech Republic by molecular methods

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Ditylenchus dipsaci is one of the most harmful parasitic nematodes in Central Europe. It is able to survive for a long time in soil without its host plants and that is why it belongs among organisms with quarantine importance. Nothing is known about *D. dipsaci* distribution in the Czech Republic. The aim of the study was to collect samples of *D. dipsaci* from the Czech Republic and to identify them by molecular methods. A region of rDNA including 3' end of S18 gene, ITS1, 5.8S gene, ITS2 and 5' end of S26 gene was amplified using general primers designed according to the DNA sequence of *Caenorhabditis elegans*. The amplicon (900 bp) was analysed by RFLP and SSCP. Restriction endonucleases Eco R1, Hinc II and Alu I can be used for differentiation of certain biotypes of *D. dipsaci*. At the same time, methods for DNA extraction from plant material and contaminated soil were optimised.

174 Study of populations of *Globodera rostochiensis* and *G. pallida* from the Czech Republic by molecular methods

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Potato cyst nematodes (PCN) are organisms of quarantine importance. That is why specific and sensitive methods for their determination are needed. According to the international classification, five pathotypes of *G. rostochiensis* and three pathotypes of *G. pallida* exist. They can be differentiated by biological tests using potato varieties resis-

tant to certain pathotype(s), but this method is time and labour consuming. For the application of molecular methods for species and pathotype differentiation, we used a relatively stable region in the genome of nematodes between genes S18 and S26 with the aim of finding polymorphisms enabling differentiation of PCN populations from the Czech Republic. Samples of PCN from different localities were tested by PCR, RAPD, RFLP and SSCP. Complete pathotype series from Scotland and Germany were used as standards. Six populations contained *G. pallida*. These results were identical to the results of biological tests and morphometrical observations of cysts.

175 An actual and modern identification key of the dorylaimid genera world-wide

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The poster introduces a new key of the dorylaimid genera of the world, updated to the latest taxonomic information available in spring 2002. The key comprises 234 genera, which are differentiated on the basis of nine characters and eight measures. The key is implemented as a graphics-based decision table in electronic form and is targetedly designed for rapid routine identification. Response time is as low as 100 ms, full identification (99% certainty) is achieved after eight mouse-clicks on average. Because the utilised inference mechanisms consistently rely on probabilistic calculations, the key offers innovative functionality: like other electronic keys it allows for *i*) a free sequence of identification decisions, *ii*) the omission of decisions, and *iii*) continuous feedback on identification progress. In addition, the key dynamically *iv*) indicates the best decision sequence onward, based on the previous selections made by the user, *v*) provides a quantitative certification of identification quality, and *vi*) enables nematode classification into feeding types and c-p classes prior to full identification, whenever possible. The poster explains and illustrates major characteristics of the key.

176 Molecular surveys of marine nematode diversity

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Using the molecular barcoding system developed for terrestrial nematodes, we performed a set of assays on marine samples to prove the utility of the method in additional habitats. The method relies on polymerase chain amplification and DNA sequencing of an informative segment of the small subunit ribosomal RNA (SSUrRNA) gene from individual nematodes. The sequence is then used to define operational taxonomic units (OTU) for the study population, and is compared to sequences from known taxa to attach taxonomic and ecological attributes. We surveyed nematodes from the low tide zone of three Scottish beaches (Loch Fyne (West coast, muds), Orkney (Northern, sandy) and Gullane (East coast, estuarine/sandy)). A second survey of a gas seep site in the Firth of Forth was carried out to assess the utility of the method for shorter range surveys. Over 250 sequences were determined and these define over 30 OTU. The molecular OTU identified were assigned to 'morphological' OTU by *i*) sequencing from specimens from the sites (identified in collaboration with Dr Tim Ferrero and Prof. J. Lambhead, NHM, London) and *ii*) by comparison to our growing database of nematode SSUrRNA sequences.

177 Free-living marine nematodes in two stations from Diego Perez Key, Cuba

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Free-living marine nematodes have been poorly studied in Cuba and, in general, they are named to class level as components of the meiobenthos. The sediment accumulated in the external slope of the reef (Station 1) and among the roots of the red mangrove in the interior lagoon of the key (Station 2) was collected. The temperature and salinity values were 29°C and 34‰, respectively. Granulometric composition of the stations was analysed. Diversity (H'), evenness (J') and richness were calculated. One species, 13 genera and

13 families belonging to six orders were identified. Station 2 registered not only the highest density of nematodes (658 ind./10 cm²) but also the highest number of families. Monhysteridae, Oncholaimidae and Desmodoridae predominated, and they represented 80.8% of all nematodes in this station. Station 1 was represented by only seven families with a density of 278 ind./10 cm², Desmodoridae being the dominant. The differences found in density, evenness and richness depend on the differential behaviour of the ecological factors. In this sense, stability and granulometric composition of sediments were important.

178 Trophic composition of free-living marine nematodes in two stations from Diego Perez Key, Cuba

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Free-living marine nematodes are ecologically very heterogeneous and occupy different trophic positions in the benthic food web, but in Cuba they are poorly studied. Sediment was collected at the external side of the reef (Station 1) and from among the roots of red mangrove in the interior lagoon of the key (Station 2). Diversity (H'), evenness (J') and richness were calculated. Nematodes were divided into four trophic guilds: deposit feeders (DF), epistrate feeders (EF), scavengers (S) and predators (P). Trophic behaviour at guild level reflected high differences among stations. The diversity in the guilds was higher in Station 2: this behaviour is due to the distribution of individuals by family, more than the amount of represented families in the case of EF, S and P guilds. The component that produces a higher diversity in DF guild is the amount of families. In DF guild the abundance and richness presented the highest differences between habitats. In Station 2, the two main families are included in two guilds associated with the detritus chain, but in Station 1 the two dominant families are in the same guild related to the grazing chain.

179 Biology and taxonomic notes on sibling species of *Oncholaimium* (Nematoda: Enoplida) from Russian Far Eastern part of the Sea of Japan

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Species of the marine nematode genus *Oncholaimium* are widely distributed in the benthos of the Sea of Japan. We determined that the *Oncholaimium* complex from Russian Far Eastern part of the Sea of Japan was composed of at least three sibling species: *O. olium*, *O. paraolium* and *O. ramosum*. Populations from habitats in three localities have been compared. These three species are very similar in morphology. Multivariate analyses were done for 14 morphometric characteristics in males, and 13 in females. In addition, a series of minor variations in morphology were found (structure of terminal parts of the tubular organ, form of supplementary organ). The sympatric sibling species *Oncholaimium* complex form a series on the successional scale between the most perturbed conditions occupied by nematode *O. ramosum*, and less impacted, slightly more predictable and stable conditions, characterised by sub-littoral nematode *O. paraolium* and by littoral nematode *O. paraolium*. Detailed field studies of *O. ramosum* have found that it is a dominant member of the epifauna in areas subjected to severe organic disturbance and heavy metal and petroleum hydrocarbon contamination, and has the ability to withstand high levels of H₂S, a combination facilitating the primary opportunist role of this species of the complex.

180 Free-living marine nematode communities in the northern part of Tatarskyi Strait in the Sea of Japan

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The northwestern part of the Sea of Japan, especially in the region of Amurskyi Liman, is the most productive and sensitive to modern negative anthropogenic activity. Meiofauna were sampled in shallow (3-20 m) continental shelf at 15 stations on June to August 2001. Total meiofauna abundances ranged from three to 1730 individuals per 10 cm² with a mean of 810 individuals per 10 cm². In meiobenthos we see reinforced nematodes dominance comparative with other groups. Nematofauna abundances ranged from two to 1350 individuals per 10 cm² with a mean of 700 individuals per 10 cm². A total of

46 nematode species were recorded; the most numerous species is widely-distributed *Sabatiena pulchra*, followed by *Anoplostoma cuticularia*, *Halanonchus arenarius* and *Tershellingia* sp. Nematode communities were identified using classification and ordination techniques. The identified communities were than characterised with respect to species composition, density, diversity, size structure and feeding type. Deposit feeders were most dominant in all communities, followed by omnivore/predators, explained by the muddy nature of the sediment. The structure of the nematode communities from the Tatarskyi Strait is compared with: *i*) the communities from adjacent oxic deep sea sediments; *ii*) another area of continental shelf water off the coast of Sakhalin; and *iii*) shallow reduced environments such as sediments of eutrophic bottoms in Peter the Great Bay.

181 The embryonic cell lineage of the nematode *Pellioiditis marina* shows evolutionary adaptations towards faster development in the Rhabditidae

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Until now only the embryonic cell lineage of the model organism *Caenorhabditis elegans* has been described. The complete embryonic cell lineage (until muscle contraction) of a *Pellioiditis marina* embryo was established by means of 4D-microscopy. *Pellioiditis marina* is a close relative of *C. elegans*, but has adapted to a marine, brackish environment. The overall cell lineage resembles strongly that of *C. elegans*, with a few small differences. At the stage of muscle contraction (when most cells are established), *P. marina* has as many cells as *C. elegans* (571 cells) but less cell deaths (60 and 106, respectively). In more derived forms of development, like in *P. marina* and *C. elegans*, developmental rate is hastened by adapting their lineage, so that cells originate close to their final position. This could explain the 'chaotic' cell lineages of *P. marina* and *C. elegans* where tissues are derived from different founder cells. In the slower developing *Hali-cephalobus* sp., sublineages form identical cells, which migrate to their exact location. *Pellioiditis marina* and *C. elegans* have adjusted these lineages to avoid these migrations and hasten their development.

182 **Thalassinidean burrow walls: a cradle for nematodes**

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As a part of a study of the biology of the intertidal-burrowing shrimp *Callianassa tyrrhena* (Thalassinidea) the meiofauna from burrow walls and chambers was compared with that of surface sediment and embedded ambient debris. Abundance and size spectrum of nematodes from all samples were determined. Nematodes from burrow wall and surface sediment samples were identified to genus level. The highest number of nematodes especially juveniles was found in the burrow wall samples. *Halaphanolaimus* was the most dominant of the 19 different genera found in this sample category. In surface sediment samples 12 different genera were found, with *Chromadorina* being the most dominant. Seven genera are common to both sample categories. The densely packed sediment of the burrow walls apparently has enough interstitial space for a large meiofauna community, and also represents a retreat for juvenile nematodes.

183 **Phylogenetics of marine nematodes – a molecular approach**

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Historically, the classification of nematodes has been based solely on the interpretation of the evolutionary history of morphological characters, resulting in a set of mutually incompatible systems. Molecular phylogenetics

offer the promise of being able to resolve conflicts in this field by measuring relatedness using a single metric and verifiable, model-driven analysis. Phylogenetic relationships of marine nematodes from UK waters were inferred from partial small subunit ribosomal RNA gene sequences. As part of the experimental design a new sequence of techniques was set up to ensure species identification prior to DNA extraction of the whole animal. Phylogenetic trees derived from these sequences showed that, within 'Adenophorea', only the Monhysterida form a monophyletic group, and that the Chromadorida and Enoplia are both polyphyletic. Even at higher taxonomic levels, different clusterings of species are evident in molecular compared to morphological phylogenies. The data support earlier studies using molecular techniques in that classical views of nematode systematics have to be revised.

184 **Nematodes of the order *Dorylaimida* (Pearse, 1942) in different high-mountain lakes of the Sierra Nevada (Granada, Spain)**

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A total of 20 lakes was sampled in the Sierra Nevada mountains (Granada, Spain) in the summers of 1990 and 1991. At the beginning of this period in 1991, the lakes were still covered with ice. Despite their altitudes of 2820-3050 m and being oligotrophic lakes, the nematofauna proved abundant. The orders with the greatest total biomass were: *Dorylaimus asymphidorus* Andrassy, 1969 (45.2% of the total of the dorylaimids in 1990 and 62% in 1991); *Mesodorylaimus* cf. *litoralis* Loof, 1969 (35% in 1990 and 7.7% in 1991) and *Paractinolaimus macrolaimus* Meyl, 1957 (4.5% in 1990 and 14.2% in 1991). No correlation was found between the dorylaimids and the physico-chemical parameters analysed; however, this nematofauna correlated positively with surface area, depth and catchment area of the lakes.

185 **Marine nematodes as hosts for chemoautotrophic symbionts**

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Marine free-living nematodes (Stilbonematinae, Desmodoridae, Adenophorea) have ectosymbiotic sulphur-oxidising chemoautotrophic bacteria attached to their cuticle in a genus or species specific pattern. In the genus *Eubostriechus* the bacteria are up to 100 μm long non-septate filaments containing many nucleoids. The worms supply the bacteria alternately with oxygen and sulphide by migrating through the chemocline in sheltered sandy sediments. In turn, the bacteria are eaten by the worms and probably constitute their only food. The nematodes possess conspicuous epidermal glandular sense organs which are unique to the Stilbonematinae. The mucus produced by these glands is most probably connected to the recognition mechanism between the worms and the bacteria. The latter are shed with each moult and have to be recruited from the environment. Preliminary data suggest a lectine/sugar bond. According to the sequence of the 16S rRNA gene the primary symbionts all belong to the γ -proteobacteria. In addition, the complex bacterial coat of some genera harbours a variety of other bacteria, including α - and ε -proteobacteria and cytophaga. The stilbonematid/bacteria symbiosis is found in tropical to cool temperate shallow waters. Highest diversity and abundance, however, is found in tropical calcareous sediments.

186 *Draconema hoonsooi* n. sp. and *Draconema youngeouni* n. sp. (Adenophorea: Chromadorida: Draconematidae). The first record of free-living marine nematodes from Korea

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Two free-living marine nematodes, *Draconema hoonsooi* n. sp. and *Draconema youngeouni* n. sp. of the family Draconematidae, are described on the basis of the specimens sampled at depth 30-60 m of the Sea of Japan and Jeju Island of Korea. *Draconema hoonsooi* n. sp. is recognised by the possession of the following characteristics: having a cup-shaped amphideal fovea in both male and female, five pairs of evenly tapered anal setae (male), 15 pairs (male) and 18 pairs (female) of posterior sublateral adhesion tube (SIAT), and five pairs of setae on non-annulated tail region in both sexes. *Draconema youngeouni* n. sp. can also be easily distinguished from all known congeneric species in having a combination of the following features: the greater body length and slender body shape, the bowl-shaped amphideal fovea in both male and female, 13 pairs (male) and 17 pairs (female) of posterior sublateral adhesion tube, five pairs of evenly tapered anal setae (male), and the absence of long setae on non-annulated tail region in both sexes. The photomicrographs of the new species by scanning electron microscopy (SEM) and differential interference contrast (DIC) microscopy are presented with a detailed morphological description.

187 Molecular evolution among geographic isolates of *Steinernema carpocapsae*

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Ribosomal DNA (ITS regions 1 and 2) from ten disparate geographic isolates of *Steinernema carpocapsae* was sequenced and analysed in order to better understand intraspecific variation, speciation, and the molecular evolution of this locus. Taxa assumed to represent panmictic populations were sampled from the USA, Poland, Russia, France, Sweden, UK and Mexico. No phylogeographic patterns emerged from the data set. Among the populations, transitions were as common as transversions, and a single insertion event was observed. Intra-individual polymorphic characters with states fixed among other taxa indicate an intermediate phase in concerted evolution and tokogenetic relationships among the sampled taxa. These data are used to explore the mode and tempo of evolution at this locus, and as an operational model for comparing applications of species concepts.

188 Efficacy of steinernematid nematodes against three insect pests of crucifers in Quebec

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Steinernema feltiae, *S. carpocapsae* and *S. riobrave* were evaluated against imported cabbageworm *Artogeia rapae*, diamondback moth *Plutella xylostella*, and cabbage looper *Trichoplusia ni* under laboratory and field conditions. LD₅₀ values ranged from 3.6-18.2 for *A. rapae* L2, from 2.3-24.5 for *P. xylostella* L3, and from 4.7-10.1 for *T. ni* L2. *S. feltiae* UK was the most pathogenic strain on all three pests. Maximum larval mortality rates recorded on *A. rapae* were influenced by temperature: 100% with *S. riobrave* 335 at 30°C, 95.8% with *S. feltiae* UK at 25°C, 91.7% with *S. feltiae* 27 at 25°C, and 75.7% with *S. carpocapsae* All at 30°C. Mortality of *A. rapae* L2 increased with contact time to *S. carpocapsae* All and *S. feltiae* UK with a 76 and 78% maximum, respectively, reached after 12 h contact. At 20°C and 70% RH, survival rates of *S. carpocapsae* All, *S. feltiae*

UK, and *S. riobrave* 335 on cabbage leaves were 43, 2, and 0%, respectively, 4 h following application. Under field conditions, foliar applications of *S. carpocapsae* All provided 24.9, 19.4 and 14.9% control of *A. rapae* on Brussels sprout, broccoli, and cauliflower, respectively.

189 Discrimination between isolates of *Heterorhabditis bacteriophora* Poinar, 1976 (Nematoda: Heterorhabditidae) from the province of Córdoba, Argentina

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Up to the present, eight isolates of *Heterorhabditis bacteriophora* from the province of Córdoba have been described, and significant differences have been found for all their morphometrical characters. The aims of this work were: to discriminate these isolates based on morphometrical characters, to identify the principal characters that permit their separation and to evaluate the possible relationship between isolates. Isolates from six departments corresponding to two different phytogeographical regions of the province were considered. Hermaphroditic females, amphimictic females, males and infective juveniles (IJ) were analysed. An evident separation of one isolate with respect to the others was observed in adults. In IJ the isolates were separated in three groups. The most relevant morphometrical characters for discrimination were: distance between anterior end and nerve ring, body diameter at anus, distance between anterior end and excretory pore, ratios a, c, D in adults; distance between anterior end and pharyngeal basal bulb, distance between anterior end and excretory pore, ratios a, D, E in IJ. Similarity relationship among isolates was different depending on the stage considered.

190 The use of the glycosylase-mediated polymorphism detection for the study of gene flow in entomopathogenic nematodes

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Entomopathogenic nematodes are traded for control of difficult pests. The greatest limitation on understanding how indigenous populations are affected by new strains or escaped genes is the problem of recognising the genetic structure of wild populations and the levels of gene flow between them. We are aiming at studying gene flow in Irish entomopathogenic nematode populations by detecting DNA polymorphisms in the intron regions of highly conserved genes. We have used EPIC-PCR to amplify part of the major sperm protein gene from *Steinernema feltiae* and *Heterorhabditis megidis* containing a putative intron. DNA sequence analysis will follow to confirm the presence of the intron. Amplifications were achieved using degenerate primers designed after aligning the major sperm protein sequences from seven nematode species taken from GenBank. Our research is still ongoing for additional suitable genes. Genetic variation in the introns will be studied by the use of the glycosylase-mediated polymorphism detection (GMPD). Introns will be reamplified using the four dNTPs including uracil and having adjusted the dTTP to dUTP ratio. Products will then be subjected to the action of uracil DNA glycosylase, which results in DNA cleavage at specific sites thus generating an allelic profile for each population studied.

191 Life cycle of *Gastromermis massei* Doucet & Cagnolo, 1997 (Nematoda: Mermithidae)

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The life cycle of *Gastromermis massei* was studied for 3 years. Samples were taken from the bed of Cosquín River, Córdoba, Argentina and individuals in their free-living stage were collected with Surber trap. Samples were processed through suspension and 40 µm pore sieving. Larvae, pupae and adults of *Simulium (Ectemnaspis) wolfhuegeli* (Enderlein) were collected for the study of the parasitic stage. Parasites and immature stages of hosts were kept under laboratory conditions. Cycle was completed in 30-40 days. It includes adults, eggs, and four larval stages. Eggs are deposited freely on the substrate, without showing segmentation. The embryonic

development is completed 12-15 days after oviposition at 26°C. The pre-parasitic juvenile emerges from the egg, living only 48 h, when it must find and penetrate the host. The parasitic juvenile develops in the host, being the only feeding stage. The post-parasitic juvenile abandons the host through cuticle perforation. It moults into adult, male or female, in the environment. Moulting, copulation and oviposition were observed only when numerous individuals were interlaced. The biological observations performed agree with what was indicated for other species of the genus *Gastromermis*.

192 Phoretic association of *Steinernema feltiae* (Nematoda) with *Eisenia fetida* (Annelida)

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Earthworms improve soil characteristics and have a notable influence on edaphic organisms; however, interactions between them have not been studied in detail. After protozoa, nematodes are the most abundant and diverse group of soil invertebrates and an important part of food webs. Entomopathogenic nematodes are an environmentally-friendly alternative for insect pest control. However, their dispersal capability is limited and it is often difficult to reach the expected level of control. Since earthworms are able to change soil structure and move large amounts of soil, some authors think that earthworms could be used as vectors to introduce and disperse beneficial organisms. As part of the study about interactions between earthworms and nematodes, we show the infectivity results of *Steinernema feltiae* after passage through the *Eisenia fetida* gut. Moreover, entomopathogenic nematodes have no deleterious effect on earthworms.

193 Combination of *Steinernema carpocapsae* (Weiser) and pheromone lure: a promising strategy for biological control of the banana black weevil *Cosmopolites sordidus* (Germar) on bananas in Martinique

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Cosmopolites sordidus (Germar, 1824) is one of the major pest of bananas in the Caribbean. Several techniques can be applied to avoid use of insecticides. On one hand, laboratory studies have shown that some specific strains of *Steinernema* spp. could contribute efficiently to the control of this pest. Unfortunately, field applications of formulations of *S. carpocapsae* often give inconsistent results due to formulation and conservation constraints. On the other hand, mass-trapping using pheromone lure has given good field results, excepted when the *C. sordidus* population levels were very high. In this study, we used pheromone traps to contaminate weevils with entomopathogenic nematodes instead of killing them. During a 10 month study, we evaluated the interest of pheromone traps using pheromone lure on which we replaced each week new *Galleria mellonella* larvae previously inoculated with *S. carpocapsae* (Weiser). In this experiment, promising results were obtained with a reduction of attacked plants from 98.5 to 40% and a reduction of the attack severity (according to Vilardebo's coefficient) from 42 to 23.

194 Effects of storage temperature on survival, infectivity and lipid content of the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae)

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The Belgian Lxm 31 population of *Steinernema feltiae* was cultured *in vitro* or *in vivo* and stored in water at 5, 10, 15 or 20°C. Its survival, infectivity and lipid content were evaluated after 0, 0.5, 1, 2, 3, 4, 5, 6, 12 or 18 months. Temperature, storage time and culture method significantly influenced the survival of the infective juveniles (IJ). Survival decreased with increasing storage temperature and was negatively correlated to storage time. After a long-term storage, the percentage of surviving IJ was higher when cultured *in vivo* than when produced *in vitro*. Storage temperature also influenced infectivity. When kept at low temperatures (5-10°C), IJ infectivity decreased during the first half month and then increased. When IJ were stored at 20°C, their infectivity increased

during the first half month after which it decreased. The lipid content of IJ was negatively correlated to the storage temperature and time. Lipid content and IJ survival were positively correlated; lipid content and IJ infectivity were not correlated.

195 The potential of *Heterorhabditis marelatus* for control of Colorado potato beetle

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Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) is an important pest of potato and has considerable resistance to many insecticides. With current and future restrictions on pesticides, ecologically safe alternatives are needed. The goal of this project is to develop *Heterorhabditis marelatus* (*Hm*) as a biological control alternative for CPB through understanding its pathogenicity and adaptation to field edaphic factors. *Hm* had best survival and pathogenicity in sand and sandy loam soil and at 60-100% moisture content under controlled conditions. In naturally CPB infested field plots (322 m²), treatments of 0, 333 million, 667 million or 1 billion *Hm*/m² of soil in 2000 and 2001 showed higher numbers of beetles in control plots compared with nematode-treated plots. Field cages (2 m³) artificially infested with five CPB adults per plant were treated with either 0 or 1 billion *Hm*/m² at the emergence and peak of 4th instar, or at peak pupation. Time of application was not significant, but more adults emerged in controls than in *Hm* treated plots. Overall, the results suggest that *Hm* has a potential for managing CPB under field conditions.

196 Abundance of endemic entomopathogenic nematodes following application of *Steinernema riobrave*

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Caged insect larvae were buried at monthly intervals in a Florida citrus orchard during 2000-2001 to monitor nat-

ural control of *Diaprepes abbreviatus* by entomopathogenic nematodes (EPN). The survey occurred in plots that were treated or not treated with *Steinernema riobrave* during 1998-2001. Mean monthly insect mortality in control plots ranged from 13-74% and endemic EPN (*Steinernema* sp., *Heterorhabditis zealandica*, *H. indica*, and *H. bacteriophora*) were recovered from 22-81% of the cadavers each month. Endemic EPN in each plot were inversely related to *S. riobrave* and directly related to the numbers of adult weevils (*D. abbreviatus* and *Pachnaeus litus*) captured in modified Tedders traps. Insect mortality was higher and cadavers containing endemic EPN were more numerous in control plots than in *S. riobrave*-treated plots, except during months in which *S. riobrave* was applied. In treated plots, recovery of endemic EPN exceeded that of *S. riobrave* in all but the treatment months. The net efficacy of *S. riobrave* treatments against *D. abbreviatus* attenuated over time, possibly due to competitive displacement of EPN that are better adapted than *S. riobrave* to persist in Florida citrus orchards.

197 Profitability of applications of *Steinernema riobrave*, metalaxyl and supplemental fertilisation for management of *Diaprepes abbreviatus* and *Phytophthora nicotianae* in a Florida citrus orchard

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An experiment was conducted (1998-2001) in a central Florida citrus orchard infested by *Diaprepes abbreviatus* and *Pachnaeus litus* to evaluate the profitability of recommended IPM tactics for insect control. Main plots were *i*) not treated for insect control, *ii*) treated twice annually since 1998 with commercially formulated *Steinernema riobrave*, or *iii*) treated twice annually with *S. riobrave* and 15 times annually with liquid fertiliser in place of dry fertiliser (three times/year) used in the other treatments. Split plots were untreated or treated twice annually with metalaxyl. Carbaryl was applied once (May 1998) to treatments 2 and 3. During 4 years, numbers of *D. abbreviatus* in plots were directly related to abundance of *Phytophthora nicotianae* and inversely related to fruit yield. Fertiliser treatment did not affect yield or abundance of any pest. Treatment with *S. riobrave* reduced numbers of both insects and abundance of *P. nicotianae* by an average of 45-60% annually except in 2001. Metalaxyl did not affect abundance of the fungus.

A single treatment of carbaryl followed by 4 years of treatment with *S. riobrave* increased the cumulative yield (1999-2001) of citrus by 16-23%, a 4-11-fold return on investment, depending on fruit variety and market destination.

198 Liquid culture production of biocontrol nematodes

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For mass production nematodes are produced in liquid media pre-incubated with the symbiotic bacterium for 1 day before the dauer juveniles are inoculated. Nematode yields and the process duration can vary significantly. Several different species have nowadays been cultured successfully in liquid media. For example, *Heterorhabditis megidis* dauer juvenile yields can reach 100 000/ml; however, the mean of several processes is usually not higher than 40 000/ml. The process time is either 15 days (one generation) or 24 days (two generation process). Yields depend on the nematode species cultured. Species with smaller dauer juveniles can reach a dauer juvenile density of >500 000/ml. A major reason for process instability is a variation of the nematode population dynamics. Another influencing factor on yields is the bioreactor design. Internal loop bioreactors equipped with a marine propeller can provide the necessary oxygen at low shear forces. The system has been proven to be superior to airlift and conventionally stirred bioreactors and has been successfully scaled up to 3000 l.

199 Factors influencing dauer juvenile recovery of *Heterorhabditis bacteriophora* in liquid culture

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Recovery is the step of the developmentally arrested infective third stage juvenile (dauer) to feeding third stage juveniles, which continue growth to reproductive adults. It is a response to yet unidentified food signals, which reflect the nutritional conditions in the environment. Unlike dauer ju-

veniles (DJ) of *Caenorhabditis elegans*, which recover in the presence of yeast extract, entomopathogenic nematode DJ need more specific signals. In liquid culture a defined number of hermaphrodites (approximately 5000/ml in the tested medium) results in maximum yields. This density, however, cannot easily be obtained by just defining the DJ inoculation density. In liquid culture DJ respond to food signals produced by their symbiotic bacteria, which cause between 18 and 90% of the DJ to recover within a period of several days. This unsynchronised and low recovery in *in vitro* cultures is the major reason for process instability and variable yields. In order to increase recovery and thus improve the management of nematode population density, several process parameters were investigated for their influence of DJ recovery in liquid culture.

200 Effect of the *Steinernema feltiae*-*Xenorhabdus bovienii* complex on *Meloidogyne javanica*

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Metabolites from the *Steinernema feltiae* – *Xenorhabdus bovienii* complex were tested for their efficacy against *Meloidogyne javanica* penetration and egg production in soybean. *In vitro* metabolites of *X. bovienii* were collected from 4-day-old YS-broth cultures by ethyl acetate extraction. *In vivo* metabolites of the *S. feltiae* – *X. bovienii* complex were collected from 4-day-old *S. feltiae*-infected *Galleria mellonella*. Daily applications of *in vitro* exudates at 1 mg/plant/day, or *S. feltiae* juveniles at 1000 or 10 000 infective juveniles/plant/day, for up to 3 days, were ineffective at reducing *M. javanica* penetration or egg production, compared to water treatment. A single application of *in vivo* metabolites applied at 250 mg-insect-cadaver/plant reduced *M. javanica* penetration by 42% compared to water treatment, but the effect was lost using multiple applications. Daily applications of *in vivo* metabolites did not affect *M. javanica* egg production. Multiple treatment applications increased *M. javanica* establishment in soybeans. *Steinernema feltiae* – *X. bovienii* complex effects on *M. javanica* were highly variable.

201 Thermal adaptation of entomopathogenic nematodes: acclimation, trehalose accumulation, and survival at environmental extremes

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Cold acclimation leads to trehalose accumulation in entomopathogenic nematodes. We hypothesised that trehalose accumulation in nematodes is a general strategy to prepare for survival at environmental extremes. Therefore, we tested whether the nematodes will accumulate trehalose during acclimation at sub-lethal warm and cold temperatures and whether the accumulated trehalose correlates with enhanced desiccation, heat, and freezing tolerance. Three species, *Steinernema carpocapsae*, *S. feltiae* and *S. riobrave*, were acclimated at 35 and 5°C for 1 and 4 days, respectively, and their trehalose contents were measured. Survival of acclimated- and non-acclimated nematodes at –20 and 40°C and in 25% glycerol was compared. *Steinernema riobrave* and *S. carpocapsae* accumulated high trehalose at 35°C, and *S. feltiae* at 5°C. Heat tolerance in acclimated *S. carpocapsae* and *S. feltiae* was high, but unaffected in acclimated *S. riobrave*. Freezing tolerance in acclimated *S. carpocapsae* and *S. riobrave* was increased, whereas in acclimated *S. feltiae* it was unaffected. Heat acclimated *S. carpocapsae* and cold acclimated *S. riobrave* showed the highest desiccation survival at 5°C. Overall, the accumulated trehalose levels appear to enhance desiccation, freezing and heat tolerance of entomopathogenic nematodes.

202 Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from diverse geographic localities

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We compared the susceptibility of the introduced *Popillia japonica* and the native *Cyclocephala borealis* to 16 species and strains of entomopathogenic nematodes isolated from within or outside the geographic ranges of the two scarabs. We found large variation in the virulence of the species/strains of nematodes with over

50% mortality of *P. japonica* produced by *Heterorhabditis zealandica* strain X1 and *H. bacteriophora* strain GPS11 and of *C. borealis* by *H. zealandica* and *H. bacteriophora* strains KMD10 and NC1. *Heterorhabditis indica* and *H. marelatus* caused less than 20% mortality of both scarab species. When considered as a group, the nematode species and strains from within and outside the geographic ranges of either *P. japonica* or *C. borealis* did not differ in virulence towards either scarab species. Results suggest that the introduction of the exotic *H. zealandica* into the front-line states with respect to the movement of *P. japonica* and *A. orientalis* should be explored as a tactic to delay their establishment and spread. The results also suggest that the manipulation of the indigenous *H. bacteriophora* populations may help in delaying spread and mitigating losses caused by the invasive grub species.

203 Control of overwintering black vine weevil *Otiorhynchus sulcatus* by a cold-active entomopathogenic nematode *Steinernema kraussei*

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The susceptibility of black vine weevil larvae *Otiorhynchus sulcatus* to a cold-active entomopathogenic nematode, *Steinernema kraussei* (isolate L137) and a commercially available nematode, *Steinernema carpocapsae* (Exhibit) was compared in winter field trials. Nematodes were applied to outdoor potted strawberry plants at a range of doses in early winter. Parasitism and mortality of vine weevils were assessed at the end of the winter experiment. Up to 83% of vine weevils were infected and killed by *S. kraussei* at the high dose of 60 000 nematodes per pot, whereas treatments with *S. carpocapsae* caused no significant mortality. *S. kraussei* (L137) was able to survive winter field conditions including prolonged exposure to low temperatures in contrast to *S. carpocapsae* which showed poor survival. These results suggest *S. kraussei* (L137) has great potential as a commercial biocontrol agent against overwintering *O. sulcatus*.

204 Effect of soil texture on *Steinernema feltiae* (Filipjev) virulence against *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Spodoptera littoralis, a serious pest in African and Mediterranean countries, has highly polyphagous larvae, which feed mainly at night and hide below ground by day. Pupation takes place just below the surface of the soil, and there are at least seven generations a year in warm climates. Although chemical pesticides and *Bt* are used for control, resistances have recently been reported in field and laboratory. Entomopathogenic nematodes are virulent against many species of insects. Their ability to actively locate and kill an insect host in a few hours as well as their high reproductive potential and capacity for mass production make these organisms a key to establishing environmentally-friendly alternatives for controlling insect pests. However, their dispersal capability in soil can be affected by the texture and biological factors of the isolation strain. Thus, soil texture/infectivity bioassays can improve on selection of a more accurate field dose application. The virulence of *Steinernema feltiae* (Rioja strain) against *S. littoralis* in three soils with different clay contents was studied under laboratory conditions. Replicate samples of various doses of *S. feltiae* juveniles were applied to the soil surface. Nematode virulence against *S. littoralis* was reduced in soil with 24% clay content.

205 The genetic selection for improved desiccation tolerance of *Heterorhabditis megidis* 01 PL

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The process of selection was induced in a Polish isolate of *Heterorhabditis megidis* (01 PL). The objective of the selection procedure was to improve the nematode desiccation tolerance in extreme environmental conditions. A series of base populations, 10 000 infective juveniles (IJ) each, was exposed to environments with reduced relative humidity (r.h.) levels in order to establish the threshold of 30% survival after 24 h. The experiments were conducted in desiccators with various saturated salt solutions at 20±1°C. The initial r.h. level for the selection was set at 60%. After each round the selected nematodes were reproduced in last instar larvae of *Galleria mellonella*. Evalua-

tion of the selection efficacy was based on the nematode survival after each round. Although the selection is still in progress, overall changes in the population survival at low humidity levels are observed. This trial was to assess the possibility of permanent enhancement of the nematode desiccation tolerance although the genetic determination of this characteristic still remains unknown. It is planned to continue the research in the future. The survival and pest control efficacy of the selected nematode population when exposed to open-field conditions will be tested.

206 Identifying nematode-inducible genes in *Xenorhabdus nematophila*

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The bacterium *Xenorhabdus nematophila* is a mutualist of the entomopathogenic nematode, *Steinernema carpocapsae*. Most of the life cycle of these two organisms takes place inside larval-stage insects where the nematode and bacterium reproduce individually. However, a non-feeding form of the nematode, the infective juvenile (IJ), resides in the soil and is the vector for the nematode germline and for bacteria between insect hosts. *Xenorhabdus nematophila* colonises a specialised intestinal organ of the IJ and can survive in this location for longer than 5 months despite a lack of nutritional uptake by the nematode/bacteria complex. Factors mediating colonisation of the nematode and the metabolic state of the bacterium during existence inside nematodes have not been characterised. We have developed a strategy to identify and characterise bacterial genes that are specifically expressed during colonisation and growth inside nematodes. The results of this analysis will yield insight into those bacterial functions that are important for establishing and maintaining a mutualistic interaction with the nematode. We are currently examining several nematode-inducible bacterial loci to identify the specific genes that are subjected to nematode-specific expression. Future analysis will include targeted mutation of nematode-inducible genes and determination of the resulting mutant phenotypes with regard to nematode-colonisation.

207 The bioassay of entomopathogenic nematodes on agricultural insect pests in laboratory conditions

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Entomopathogenic nematode/bacterium (EPN/EPB) symbiotic complexes have been providing a rather efficient and environmentally friendly way of controlling agricultural insect pests. Since the bacterial partner plays the important role of pathogenicity, we have developed several new EPN/EPB combinations between *Heterorhabditis* spp. and *Photorhabdus* spp. strains at the Genetic Department of the Eötvös Loránd University, and tested them on several agricultural insect pests at the Plant Protection Institute of the VE Georgikon Faculty of Agriculture. The results of the previous gnotobiological analyses have been published elsewhere. In 1998 we tested some EPN/EPB symbiotic complexes that were applied in three doses (1/1, 1/10, 1/100 infective juveniles (IJ)/caterpillar) on last instar wax moth larvae of *Galleria mellonella* as well as in two doses (3000 and 5000 IJ/white insect) on second stage white grubs of maybeetle (*Melolontha melolontha*) in laboratory conditions. It was concluded that the effectiveness of the new symbiotic complexes could be compared to that of natural EPN strains. In 1999 we tested the effectiveness of various *Steinernema* species (*S. anomali*, *S. serratum*, *S. riobrave*, *S. glaserii*, *S. carpocapsae* Mex., T1, T2, T4, *S. feltiae* Nyíregyháza., IS6, (Israel), on German cockroaches (*Blattella germanica*) and on *Periplaneta americana*. Two tests were applied: i) filter paper method: 5000 IJ/5 cockroach/plate; ii) feeding experiment: wax moth killed by nematodes were given to cockroaches. Wax moth larvae killed by *H. bacteriophora* Brecon and wax moth larvae killed by freezing were used as controls. In conclusion, *S. carpocapsae* gave positive results against cockroaches and the cockroaches ate the wax moth larvae killed by *Steinernema* but avoided those killed by *Heterorhabditis*.

208 Identification of *Steinernema* spp. and *Heterorhabditis* spp. using scanning electron microscopy of the labial region, lateral fields, vulval area, spicules and gubernacula

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Scanning electron microscopy techniques were used to identify morphological characters that are readily observed under light microscopy for the identification of species of *Steinernema*. Head shape and structures in the labial region of infective juveniles (IJ) can be used to divide steinernematids into four groups: *i*) head without labial papillae, *ii*) with six labial papillae, *iii*) with two labial horns, and *iv*) with swollen head. Formulae based on numbers of ridges in the lateral field from anterior end to tail tip are also informative. Scanning electron microscopy of *Heterorhabditis* reveals that the *H. megidis* IJ has a membranous tube associated with its dorsal tooth. The spicules, gubernacula and vulval areas provide diagnostic characters among species of both genera.

209 Non-target mortality by and host searching behaviour of *Heterorhabditis bacteriophora* in the presence of an aphid predator, *Aphidoletes aphidimyza*

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Aphidoletes aphidimyza occurs naturally in several North American, European, and Asian field crops and is released in glasshouses for aphid biocontrol. Experiments were conducted to determine how it might interact with an entomopathogenic nematode, *Heterorhabditis bacteriophora*. Fewer *A. aphidimyza* adults emerged from soil in 60 mm diameter Petri dishes inoculated with 1000 and 10 000 *H. bacteriophora* infective juveniles (IJ) than from uninoculated soil. No difference in percent adult emergence of *A. aphidimyza* was observed when IJ were applied 1, 6 or 9 days prior to emergence. *Aphidoletes aphidimyza* did not interfere with *H. bacteriophora* host searching behaviour: *e.g.*, no difference was observed in the number of IJ infecting *Galleria mellonella* larvae at the bottom of 276 ml styrofoam cups in the presence or absence of 100 *A. aphidimyza* near the soil surface. *Heterorhabditis bacteriophora* was not preferentially attracted to *A. aphidimyza* in a soil-filled 150 mm diameter Petri dish with one side containing one or 30 *A. aphidimyza* and the other containing none. This study suggests that *H. bacteriophora* applications may reduce

A. aphidimyza populations, but that the presence of *A. aphidimyza* does not interfere with *H. bacteriophora* host searching behaviour.

210 The first report of genetic diversity among Indian populations of entomopathogenic nematodes, *Steinernema* spp. and *Heterorhabditis* spp.

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Insect pests are one of the major constraints of agricultural productivity in India. To utilise entomopathogenic nematodes for the management of regional insects, *Steinernema* spp. and *Heterorhabditis* spp. from different agro-climatic regions of India are being isolated, cultured and conserved in our laboratory. Nineteen EPN populations were assayed for their insect host range and analysed for genetic diversity using RAPD and PCR-RFLP of ribosomal DNA. *Steinernema carpocapsae*, *S. glaseri* and *S. thermophilum* – a new species recently reported from India – were included for comparison of *Steinernema* populations. Both and PCR-RFLP of rDNA could not detect any polymorphism among populations of *Heterorhabditis* spp. Both the profiling systems revealed a high degree of polymorphism among *Steinernema* populations. RAPD UPGMA analysis clustered the populations into two main clusters. PCR-RFLP of rDNA of 13 *Steinernema* populations revealed similarity of only one population to *S. glaseri*. The internal transcriber spacers 1 and 2 of rDNA has been sequenced and aligned. Differences in the sequence alignment and the correlation between the observed genetic diversity revealed by RAPD and PCR – RFLP analysis will be discussed.

211 Entomopathogenic nematode occurrence in an Irish dune system

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Entomopathogenic nematodes are obligate parasites of insects. The two genera *Steinernema* and *Heterorhabditis* are ubiquitous soil organisms with world-wide distribu-

tions. Insect-killing nematodes are traded globally as biological control agents. Infective juveniles (IJ) emerge from the insect cadaver to search out a new host. The IJ invade the host *via* the mouth, anus or spiracles and release a symbiotic bacteria which kills the insect by septicaemia. Development into either males or females (*Steinernema* spp.), or a hermaphrodite (*Heterorhabditis* spp.) eventually leads to the production of further infective juveniles. These then emerge from the cadaver into the soil after 10-25 days depending on the species. Intensive sampling of the dune system on the geologically new Bull Island (Dublin Bay, Republic of Ireland) revealed the co-occurrence of the two genera. The genera were intimately associated, but *Heterorhabditis* occurred at lower frequencies. Prevalence was low with only 4.4% of samples revealing nematode presence (*Steinernema* = 2.5%; *Heterorhabditis* = 1.9%). Populations were patchy and the distribution of each genus seems to be related to stable and unstable habitats.

212 Entomopathogenic nematodes in Norwegian forest soils

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A project on the use of entomopathogenic nematodes (EPN) for control of the pine weevil (*Hylobius abietis*) has been initiated. In the first phase we are investigating the natural occurrence of EPN in forest soils. Soil samples were collected from several clear cut forest sites in autumn 2001. Samples were taken around the roots of tree stumps at each site. EPN were isolated using the *Galleria* technique. Preliminary results indicate that several species in the genus *Steinernema* are present. So far *Steinernema krauseii* has been identified which is a new record for Norway. Identification of the nematodes isolated is being conducted using morphometric and molecular techniques.

213 Survey of entomopathogenic nematodes endemic to pecan orchards of the southeastern US and their virulence to the pecan weevil (Coleoptera: Curculionidae)

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The pecan weevil, *Curculio caryae* (Horn), is a major pest of pecans in the southeastern US. Entomopathogenic nematodes and fungi are potential alternatives to chemical insecticides for *C. caryae* control. Our objective was to survey pecan orchards in the southeastern US for entomopathogenic nematodes and fungi determine the virulence of the new isolates to *C. caryae*. Soil was collected from 105 sites in 21 orchards in Arkansas, Georgia, Louisiana, and Mississippi. Entomopathogens were isolated by exposing soil to *C. caryae* and greater waxmoth larvae, *Galleria mellonella* L. We isolated entomopathogenic nematodes from six of the 21 orchards surveyed, respectively. Nematodes included *Heterorhabditis bacteriophora* Poinar, *Steinernema carpocapsae* (Weiser), *S. glaseri* (Steiner), and *S. rarum* (Doucet). This is the first report of *S. rarum* in the US. In laboratory assays, virulence of 15 nematode isolates to *C. caryae* larvae was tested in small plastic cups containing soil. Results indicated poor susceptibility of the *C. caryae* larvae to entomopathogenic nematodes. However, high levels of nematode virulence were observed toward adult weevils, and several entomopathogenic fungal isolates caused significantly higher mortality in *C. caryae* larvae than other strains (including a commercial strain of *Beauveria bassiana*).

214 Integration of *Azadirachta indica* with entomopathogenic nematodes for control of root grubs (*Holotricha serrata*)

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Studies were conducted to explore the integration between neem seed kernel extract (NSKE), neem oil (NO) and *Steinernema carpocapsae* (SC) on root grub *Holotricha serrata*. Field collected third instar grubs were kept individually in microcosms filled with soil. Soil was exposed to various concentrations of NSKE, NO and *Steinernema carpocapsae*. Infective juveniles were added 0 to 16 days after application of NSKE and NO root grub mortality was noted every day. After combining and immediately applying the *Steinernema carpocapsae*, higher mortality was obtained against root grub. The survivability and virulence of SC in neem were maintained for 20 days with or without aeration. SC survived well in the neem concentrations and also the virulence was not affected by the neem concentrations with aeration and without aeration. Neem enhances the activity of nematodes. Entomophagous nematodes and neem can represent an eco-friendly strategy to control root grub in agriculture and forest ecosystems.

215 Ecological consequences of inundative biological control: impact of entomopathogenic nematodes on the nematode community in turfgrass

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Biological control is often touted as an ecologically safe pest management approach. This notion is based on its relative safety to humans and above ground flora and fauna. However, there is a paucity of data to support that large-scale releases of biocontrol agents do not have an impact on below ground food webs. We addressed this issue using entomopathogenic nematodes and native nematode community in a turfgrass ecosystem as a model. Application of *Heterorhabditis bacteriophora* strain GPS11, *H. bacteriophora* strain HP88, and *H. indica* significantly reduced the abundance, species richness, diversity, and maturity of the nematode community by reducing the number of genera and abundance of plant-parasitic but not free-living nematodes. In contrast to the entomopathogenic nematode treatments, trichlorfon, a commonly used insecticide in turfgrass, reduced the number of genera, abundance and diversity of nematode community by adversely affecting both plant-parasitic and free-living nematodes. The reduction in abundance and diversity of plant-parasitic nematodes without any

adverse effect on free-living nematodes that play a role in nutrient cycling may be a beneficial non-target effect of entomopathogenic nematodes.

216 Detection of entomopathogenic nematodes and their possible use under IPM in Pakistan

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In Pakistan the studies on biology, taxonomy and efficacy of entomopathogenic nematodes (EPNs) were initiated in 1996. Since then during extensive surveys, over 1000 soil samples were collected from diverse habitats of Pakistan with emphasis on Sindh Province. Nematodes were collected using *Galleria mellonella* soil trap method. Three isolates of *Steinernema* and 59 of *Heterorhabditis* were detected; of which, 22 isolates were identified as *H. indica*. Out of the identified EPNs, two are new species; one has been described as *Steinernema pakistanense* Shahina et al. 2001, while the other, *Steinernema* species is in the process of description. Pathogenicity of new indigenous EPN, *S. pakistanensis* was tested against seven insect pests of common occurrence and agricultural importance under laboratory conditions and confirmed on *Helicoverpa armigera*, *Earias insulana*, *E. vittella*, *Papillio demoleus*, *Leucinodes orbonalis*, *Etiella zinkenella*, *Holotrichia consanguinea*. Mortality was higher (up to 100%) on early stages of insect larvae (*H. armigera*) as compared to fourth and fifth stage larvae.

Search for more indigenous EPNs is continuing and under the National IPM Programme, field-testing of promising species will be undertaken during the year 2002. The farmers would be trained for conservation of natural enemies including EPNs through the farmer field school (FFS) approach particularly in cotton crop, which receives the largest amount (60%) of pesticides in Pakistan.

217 Heritability of the liquid culture potential of the entomopathogenic nematodes *Heterorhabditis bacteriophora*

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The infective stage of *Heterorhabditis bacteriophora* is the mobile, but developmentally arrested dauer juvenile (DJ). For commercial application, nematodes are produced in liquid culture. Prior to the inoculation of the DJ, their symbiotic bacterium *Photorhabdus luminescens* is cultured. The DJ exit from the arrested stage (recovery) and develop to reproductive adults. Recovery is a response to bacterial food signals. In liquid culture the percentage of DJ recovering from the DJ stage is highly variable, which significantly influences the number of reproducing hermaphrodites and the final DJ yields. The heritability of the disposition to recover from the DJ stage and of the final DJ yield in liquid culture has been evaluated. From a hybrid strain 30 homozygous inbred lines were established by inbreeding over seven generations. These inbred lines were propagated in liquid culture several times; the percentage of recovered DJ and the final DJ yields were recorded. The calculated heritability for the DJ recovery was low ($h^2 = 0.38$). No significant genetic variability could be detected for this trait. In contrast, a high heritability ($h^2 = 0.90$) was found for the total number of DJ produced in the liquid medium.

218 Potential new targets for entomopathogenic nematodes in biological control of urban tree pests

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Many leaf feeding pests of urban trees pupate and/or overwinter in the soil, the most suitable environment for entomopathogenic nematodes. During this period the insects can be challenged with *Heterorhabditis*- or *Steinernema*-based bioinsecticides. Susceptibility of the most important urban tree leaf beetles and sawflies to *S. feltiae* and *H. megidis* was examined in laboratory and field conditions. Last instar larvae and pupae of all tested leaf beetles (*Altica quercetorum*, *Agelastica alni*, *Phytodecta quinquepunctata* and *Phyllodecta laticolis*) and some sawflies (*Acantholyda erythrocephala*, *Arge berberidis*, *A. pagana*) proved to be highly susceptible to nematode infection. A tight pupal cocoon of the remaining sawfly species (*Pristiphora abietina*, *Caliroa*

annulipes), however, made nematode penetration difficult or impossible. For the susceptible hosts nematodes significantly reduced the number of adult insects emerging from the soil after pupation. They could also develop and recycle in the insect cadaver. Significant differences were found between the efficacy of *H. megidis* and *S. feltiae* strains and isolates tested against different insect species. The results suggest that entomopathogenic nematodes should be considered as potential effective control agents which could be safely used against many tree leaf beetles and sawflies damaging urban parks and forests.

219 Potential of entomopathogenic nematodes for the control of cabbage root fly

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The cabbage root fly (*Delia radicum*) is the major pest of brassica vegetable crops in the northern temperate region; larvae cause extensive damage by feeding on plant roots. Chemical insecticides have traditionally been used to control this pest. However, with the imminent withdrawal of organophosphorus insecticides, alternative management strategies need to be developed which may include the use of entomopathogenic nematodes as biopesticides. Under glasshouse conditions, the susceptibility of cabbage root fly larvae to ten isolates of entomopathogenic nematodes was examined, using cauliflower (*Brassica oleracea* var. *botrytis*) as the host plant. *Steinernema affine* (isolate 179) gave the highest level of control, killing approximately 66% of larvae within 28 days. The least significant control was given by *Steinernema* E1 and F1 (194 and GWE63) and two *Heterorhabditis* isolates (K122 and UK211) which killed approximately 30% of larvae in 28 days. A second experiment compared the performance of *S. affine* and Nemasys® (Microbio Ltd), a commercial formulation of *S. feltiae*, over a range of doses (from 8000 to 64 000 nematodes per pot). *Steinernema affine* controlled significantly more of cabbage root fly (54%) than Nemasys® (36%) and may have potential for commercial development.

220 On the entomopathogenic nematodes belonging to genera *Steinernema* and *Heterorhabditis* from the Russian Far East and comparison with entomopathogenic nematode fauna in Japan

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Entomopathogenic nematodes were collected in southern Primorye and southern Sakhalin, grant-aided by Gene Bank Project of Ministry of Agriculture, Forestry and Fisheries and supported by the Institute of Marine Biology and the Institute of Parasitology of the Russian Academy of Sciences. A total of 197 soil samples from 34 sites were tested for the presence of the nematodes using the *Galleria* baiting technique. Forty-two nematode isolates were detected from 14 sites. The nematodes were divided into six RFLP types. Three of them were identified as *S. kraussei*, *S. feltiae* and *Heterorhabditis megidis* and another three were tentatively designated as *S. spp. primorye1*, *sakhalin1*, *sakhalin2*. *Steinernema kraussei* was the dominant species (nine/14 sites) in this survey and was isolated from coasts to mountains. However, it has been detected only from two coastal sites in Hokkaido, the closely related region with the Russian Far East. *Steinernema feltiae* was detected only from a coastal grassland in Sakhalin and also mainly from coastal sites in Hokkaido. *Heterorhabditis megidis* was isolated from both Vladivostok and Sakhalin, but has not been isolated from Hokkaido. *Steinernema sp. sakhalin2* corresponded to *S. sp. MY7* from Japan and *S. sp. primorye1* was recently recognised among the isolates detected from Hokkaido.

221 Growth and reproduction of *Heterorhabditis bacteriophora* on *Xenorhabdus poinarii* RS92

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Heterorhabditis bacteriophora is an entomopathogenic nematode which has a specific symbiotic relation to the bacterium *Photorhabdus luminescens*. To understand the symbiotic effect of *Photorhabdus* and *Xenorhabdus* on *H. bacteriophora*, the bacteria were examined for their potential to support nematode growth. Axenic 1st stage larvae of *H. bacteriophora* obtained by surface sterilisation of eggs that were inoculated on lipid agar plates along with 24 h bacterial cultures. *Heterorhabditis bacteriophora* grew and reproduced well not only on *Photorhabdus* bacteria isolated from *H. bacteriophora* and *H. megidis* but also on *X. poinarii* RS92 from *Steinernema glaseri*. Nematode growth was not supported by the symbiont of *H. indica* and by other *Xenorhabdus* spp. tested. The hermaphrodites of *H. bacteriophora* grown on *X. poinarii* RS92 were significantly smaller than those grown on their own symbiont. Dauer juveniles obtained from the *X. poinarii* RS92 culture did not retain the bacteria in their intestine nor recovered from the dauer stage in a fresh *X. poinarii* RS92 culture. Also, *H. megidis* and *H. indica* as well as *S. carpocapsae* grew and reproduced on *X. poinarii* RS92. These results indicate that *X. poinarii* RS92 is a *Xenorhabdus* bacterium that supports the growth of both heterorhabditid and steinernematid nematodes.

222 Different developmental responses of virulent and avirulent isolates of *Bursaphelenchus xylophilus* to *Monochamus alternatus*

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It is known that there is a great variation in virulence of *Bursaphelenchus xylophilus*, which is the causative agent of pine wilt disease and is transmitted from wilt-killed to healthy pine trees by the insect vector, *Monochamus alternatus*. To compare the developmental responses to *M. alternatus* between the two nematode isolates of different virulence, the production of dispersal forms and the number of nematodes carried by newly-emerged *M. alternatus* adult (initial nematode load) were investigated using pine bolts. The numbers of total nematodes, dispersal juveniles (third-stage dispersal juvenile and fourth-stage dispersal juvenile) and fourth-stage dispersal juveniles were much smaller in the avirulent isolate than in the virulent one around pupal chambers. The proportions of dispersal juveniles produced in a population and the proportion of third-stage dispersal juveniles developing to fourth-stage dispersal juveniles were also smaller in the avirulent isolate. Although the proportion of fourth-stage dispersal juveniles boarding beetles was equal between the two isolates, the number of them was much smaller in the avirulent isolate. A small initial nematode load of the avirulent isolate was ascribed to a smaller rate of reproduction and lower productive rates of third- and fourth-stage dispersal juveniles for the avirulent isolate.

223 Effects of phytohormones and root diffusates on the surfaces of plant-parasitic nematodes

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The direct effects of root diffusates and phytohormones (auxin and kinetin) on the surface lipophilicity of the plant-parasitic nematodes *Globodera rostochiensis* and *Meloidogyne incognita* were investigated. The fluorescent lipid probe AF18 (5-N-(octodecanoyl) aminofluorescein) was used to detect surface changes. Root diffusates increased AF18 uptake by *G. rostochiensis* while it had no effect on *M. incognita*. Kinetin and auxin decreased AF18 uptake in *G. rostochiensis*, while they had an opposite effect on *M. incognita*. Auxin/kinetin ratio was also found to be important in triggering the surface changes, especially at high concentrations. Whether plant nematodes have auxin and/or kinetin binding proteins is discussed as well as the mechanism behind the surface lipophilicity changes. The implication of these changes in the establishment of infection is also discussed.

224 Effect of population densities of *Heterodera glycines* race 3 on leaf area, photosynthesis and yield of soybean

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The effect of *Heterodera glycines* race 3 on photosynthesis, leaf area, and yield of susceptible soybean 'Embrapa 133' was studied in two experiments carried out under glasshouse conditions. Soybean seeds were sown in 1.5 l (Experiment 1) or 5.0 l (Experiment 2) clay pots filled with a sterilised mixture of field soil + sand (1:1). Eight days after sowing, seedlings were thinned to one per pot, and 1 day later inoculated with 0, 1200, 3600, 10800, 32400, or 97200 second stage juveniles (= *Pi*). Experiment 1 was continued until 45 days after inoculation whereas experiment 2 extended until harvest time. Data on plant physiological processes were taken at 10-day intervals during the experiments. Data on fresh root weight, top dry weight, grain yield, and nematode reproduction factor were obtained at the end of the trials. For both experiments, a statistical design of randomised blocks with six treatments (*Pi* values) was established and each experimental unit (one plant growing per pot) was replicated ten times. There was a marked reduction in both photo-

synthetic rate and chlorophyll content, as well as evident yellowing of the leaves of the infected plants. Even at the lowest Pi , the effect of *H. glycines* on top dry weight or grain yield were quite severe. Also, soybean yield was highly correlated with the integrated leaf area.

225 Leaf area, photosynthesis and yield of soybean as affected by initial population densities of *Meloidogyne javanica*

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The effect of *Meloidogyne javanica* parasitism on the leaf area, photosynthesis, and yield of two Brazilian soybean varieties, Embrapa 133 (susceptible) and Coodetec 201 (tolerant), were studied under glasshouse conditions. Two similar experiments were set up, one continued until 48 days after plant inoculation, and the other until plant harvest. The plantlets were individually inoculated with 0, 1200, 3600, 10 800, 32 400 or 97 200 second stage juveniles (= Pi). Data on physiological processes of the plants were taken at 10-day intervals throughout the experiments, whereas data on fresh root weight, top dry weight, grain yield and nematode reproduction factor were obtained at the completion of the trials. A statistical design of randomised blocks with treatments arranged according to a 2×6 factorial model (varieties $\times Pi$) was established and each experimental unit (one plant growing per pot) was replicated ten times. Values of photosynthesis rate, stomatic conductance, chlorophyll fluorescence, leaf colour and chlorophyll content were slightly affected by *M. javanica* for both tested varieties. Conversely, soybean leaf area, fresh root weight, top dry weight, and grain yield were markedly reduced by the nematode at the highest Pi values. Also, soybean yield was highly correlated with the integrated leaf area.

226 Characterisation of a member of the LEA gene family from the anhydrobiotic nematode *Aphelenchus avenae*

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When subjected to desiccation some nematodes can enter a state of suspended animation known as anhydrobiosis. Anhydrobiotic nematodes synthesise high concentrations of the non-reducing sugar trehalose during the induction of anhydrobiosis. Trehalose is believed to protect membranes and proteins from desiccation damage by replacing structural water, and to contribute to the formation of an intracellular organic glass, which is thought to stabilise the cell contents. In anhydrobiotic plants, sucrose plays a similar role. However, several lines of evidence indicate that non-reducing sugars alone are not sufficient to confer anhydrobiosis, and that additional adaptations are required. To identify these additional adaptations, we have investigated gene expression in *Aphelenchus avenae* undergoing anhydrobiosis. A number of genes were found to be upregulated in nematodes exposed to 90% relative humidity for 24 h. Of particular interest was a strongly induced transcript whose sequence suggests that it encodes a member of the group 3 subclass of LEA (Late Embryogenesis Abundant) proteins. LEA proteins accumulate in response to water deficit in many plants but are especially abundant in anhydrobiotic plants and in maturing seeds and pollen. We will present our data on the molecular phylogeny of the *A. avenae* LEA3 gene and on its induction in *A. avenae* by various environmental stresses.

227 Dual modes of reproduction in *Caenorhabditis elegans*

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Caenorhabditis elegans is a model system for studying development and reproduction. The relative investment of energy in growth, maintenance, or reproduction is a classic question in the evolution of reproductive strategies. Here we report that when *C. elegans* is starved, it alters its mode of reproduction from ovipary to vivipary wherein larvae develop within the parent body before live birth. Facultative vivipary was observed in worms placed in water, on culture plates and in soil microcosms, was induced by starving late L4s, young adults, and gravid adults, and was reversible by restoring food. Without

food, vivipary resulted in the death of parent worms because their progeny appeared to consume the parent body contents. The larvae that resulted from vivipary developed to reach a normal lifespan and had normal egg production, as compared to larvae arising from ovipary. Larvae produced by vivipary were able to enter the dauer stage under crowding and limited food but, in contrast to larvae arising from ovipary, some larvae produced by vivipary were able to enter the dauer stage under complete starvation. We infer that the combination of vivipary, androdioecy, and the dauer stage represent life-history adaptations that ensure survival of dispersal propagules under extreme food limitation.

228 Inhibition of sterol metabolism in *Caenorhabditis elegans* by AY-9944

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Caenorhabditis elegans and some other nematodes are capable of attaching a methyl group to the nucleus of sterols at the C-4 position. In *C. elegans*, 4-methylcholest-8(14)-enol is the most abundant 4-methylsterol produced, and smaller quantities of 4-methylcholest-7-enol also occur. The purpose of this study was to treat *C. elegans* with a known inhibitor of steroid C-7 double bond isomerisation in order to more fully understand the pathway of 4-methylsterol formation. Thus, *C. elegans* was propagated in sterile semi-defined medium with or without 50 mg/l AY-9944. The media were supplemented with sitosterol as a dietary sterol. Lipids were extracted from the nematodes and saponified; sterols were isolated with column chromatography and identified with gas chromatography. The sterols of inhibitor-untreated nematodes contained 91.4% 4-desmethylsterol plus 8.6% 4-methylsterol; the inhibitor-treated nematode sterol contained only 4.6% 4-methylsterol. The 4-methylcholest-8(14)-enol:lophenol ratio in control nematodes was 9.75 but only 0.71 in treated nematodes. The results are consistent with a pathway involving the methylation of cholest-7-enol to form lophenol as an intermediate in the production of the final product of the methylation pathway, 4-methylcholest-8(14)-enol.

229 The kinase cascade possibly involved in the odorant signaling of *Meloidogyne artiellia*

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The plant parasitic nematode *Meloidogyne artiellia* causes significant economic losses worldwide by attacking the roots of several cultivated crops. Although very little is known about the host-parasite interactions, chemosensation processes might play a key role in the recognition of the plant host thus involving specialised signal transduction pathways. We are investigating some aspects of the mechanism of signal transduction using molecular and biochemical approaches in order to elucidate roles of specific proteins in the signal cascade. In particular, we are interested in RAS-Mapk pathway that was demonstrated to be involved in olfaction in *C. elegans*. We have isolated and characterised in *M. artiellia* the corresponding genes of *let60*, *lin45*, *mek2* and *mpk1* that constitute this pathway in *C. elegans*. The comparison of the sequences of these four genes from *C. elegans* and *M. artiellia* and kinase assays with GST-recombinant proteins *mek2* and *mpk1* of the parasite confirm the role of the isolated genes in the RAS-Mapk pathways. The approach of a phosphorylation screening of a cDNA library of *M. artiellia* will allow the isolation of all the possible substrate proteins of the last member of this cascade *mpk1*.

230 Suitability of a mathematical model to describe the effect of temperatures and exposure times on the survival of the sugar-beet nematode *Heterodera schachtii*

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The effect of combinations of temperatures and exposure times on the survival of *Heterodera schachtii* was assessed in experiments under laboratory conditions. In the first experiment cysts in water were exposed at 25, 35, 37.5,

40, 42.5, 45, 47.5, 50 or 52.5°C for a maximum of 2 h. In the second experiment cysts in naturally infested soil were exposed at 32.5, 35, 37.5, 40, 42.5, or 45°C for a minimum of 2 to a maximum of 2048 h. Viability of eggs in cysts was assessed by hatching tests in a 3 mM zinc chloride solution. Viability in water was suppressed after 2 h exposure at 50°C and inhibited after 1-2 h at 52.5°C. Emergence of juveniles from cysts in soil increased after treatments at rather low temperature x exposure time combinations and was suppressed at high combinations. Egg mortality started after exposures of 256 h at 40°C, 32 h at 42.5 and 16 h at 45°C. Eighty one, 31 and 7 h of exposure were necessary to kill 50% of the nematode egg population at 40, 42.5 and 45°C, respectively. Data fitted the models $P_t = P_0 10^{-t/(q+mT)}$ and $P_{T1} = P_{T0} 10^{-\Delta T/(z-pt)}$ for $m = -0.0111$, $q = 0.8238$, $z = 2.444$ and $p = -0.236$.

231 Interaction of root-knot nematode infection and drought stress in cotton

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Drip irrigation was used in a field with a loamy sand naturally infested with *Meloidogyne incognita* to create low, medium and high levels of drought stress, though rainfall minimised drought stress early in the season. Fumigation with 1,3-dichloropropene (0 or 56 l/ha) was used to create a range of nematode densities. Three metres of each plot were hand harvested and ginned to provide lint and seed yield. A subsample of lint was analysed for fibre quality, including fibre length and micronaire. Covariance analysis was used to determine within each level of drought stress the relationship between nematode densities at mid-season and seed weight, lint weight, fibre length, and micronaire at harvest. For all levels of drought stress, seed weight ($P = 0.03$), lint weight ($P = 0.05$), and fibre length ($P = 0.07$) decreased and micronaire ($P = 0.02$) increased as nematode populations increased. For seed weight and micronaire, the relationships with nematode densities were similar ($\alpha = 0.10$) among the three levels of drought stress (similar slopes and intercepts), but only slope was similar for length. The decrease in lint weight with increasing nematode densities was greater ($P = 0.07$) at the high level of drought stress than at the lower levels of stress.

232 Detection of changes occurring during recovery from the dauer stage in *Heterorhabditis bacteriophora*

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When conditions are unfavourable for reproduction in *Heterorhabditis bacteriophora*, a long lived, non-feeding, survival and dispersal stage, the dauer juvenile (DJ), is formed. This DJ stage is also adapted for host finding and infection. When it infects a suitable host, the DJ recovers and resumes growth and development. We describe a series of methods for improved detection of recovery in *H. bacteriophora*. We also describe some of the physiological changes that occur immediately after the onset of recovery in these nematodes as revealed using fluorescent nucleic acid binding SYTO dyes. Although recovery could be monitored using morphological changes, we found that observation of the uptake of fluorescent latex microspheres by recovering nematodes was a far more sensitive and efficient means of detecting recovery. SYTO dyes were also found to be useful indicators of recovery, binding to the pharyngeal glands and genital primordia as little as 3 h after the onset of recovery. The use of SYTO dyes also indicates that the pharyngeal glands produce large quantities of RNA following the onset of recovery, implying that these structures may produce proteins important in the infection and/or feeding process of *H. bacteriophora*.

233 Isolation and characterisation of genes induced by desiccation stress in the insect-killing nematode *Steinernema feltiae*

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The insect-killing nematodes of the Steinernematidae infect and kill a wide range of insect species and are considered among the most promising alternatives to chemical control of insect pests. *Steinernema feltiae* can partially tolerate desiccation by entering a shallow

dormancy and thus may serve as a model to study desiccation stress tolerance for steinernematid nematodes. Using cDNA subtractive hybridisation we identified IS6 genes that are differentially expressed during exposure to desiccation stress. One hundred and ten genes were identified, among them Late-Embryogenic-Abundant gene (*Sf-LEA*) and aldehyde dehydrogenase (*Sf-ALDH*), both known to be involved in response to water stress in other organisms. Furthermore, using real-time PCR, we detected a significant increment in the steady state level of the gene transcription products upon 8 h of nematode exposure to desiccation, and further increase upon 24 h of desiccation. Future studies of desiccation tolerance, including identification of additional desiccation-related genes and study of their biological roles and regulation, will shed light on the genetic and biochemical alterations evolved in desiccation tolerant organisms.

234 Are there hatching inhibitor factors of *Globodera pallida* in potato tubers?

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Co-evolution of potato plant *Solanum tuberosum* and potato cyst nematode (PCN) *Globodera pallida* for millions of years has resulted in a closed interrelationship in which the nematode biological cycle depends on root diffusates that stimulate the hatching process of second stage juveniles (J2). Hatching factors (HF) are present not only in roots but also in leaves and other parts of the potato plant, except for tubers. However, the existence of chemicals which inhibit the HF is well-known, and these have been found in several non-host species for PCN, such as white mustard, asparagus or *Blupearum salicifolium*. Recently hatching inhibitors (HI) have been identified in potato root leachates, though information does not exist about their presence in tubers. The potato industry produces thousands of tons of potato tuber wastes every year and this material could be used as a potential source of HI. Bioassays with cysts of *G. pallida* have been carried out using different fractions of peel tuber extracts in n-hexane, dichloromethane and Ethyl acetate. After

18 days the cysts were subjected to the action of potato root leachates. In those cysts with an n-hexane fraction pre-treatment no hatching of J2 was observed. This could indicate the presence of HI in potato peel.

235 Effect of heavy metals (zinc and lead) on soil nematode fauna

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Changes of nematode fauna in soil polluted with zinc ($ZnSO_4$ – 2500 mg/kg) and lead ($PbNO_3$ – 500 mg/kg of soil) were investigated. Faunistic diversity, nematode population density, ecotrophic structure and maturity index of nematode communities were analysed. Soil samples were collected monthly in 2000 (April – September) and twice in 2001 (May and September). Simultaneously, an experiment on the growth of nematodes of genus *Cephalobus* on medium with addition of Zn and Pb in increasing concentrations under laboratory conditions was carried out. Both salts were toxic for nematodes. They caused a decrease in population density and diversity. Trophic structure of the nematode communities was changed, immediately after heavy metal application. The number of representatives of Rhabditidae sharply increased (up to 78%, especially in the variant with Zn); later the number of polyphagous and predaceous nematodes decreased. Under Zn application these groups disappeared totally from the nematode fauna. Maturity index of the nematode communities (ΣMI) was lower in variants with heavy metals (2.1-2.6) compared to the control (2.7-2.8). In the laboratory experiment, the toxic effect of Zn and Pb was expressed in a decrease in fecundity of nematodes and size of individuals in the population. Negative effects were intensified with increased heavy metal concentration.

236 Presence of *Meloidogyne* sp. males in thickenings of the end of soybean lateral roots

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Soybean plants (*Glycine max* L. Merr. A 5435 variety) from Berrotarán, province of Córdoba, Argentina, showed highly galled radical systems. Females of *Meloidogyne* sp. were extracted from inside prominent galls located on principal and lateral roots. Several lateral roots showed particularly thickened ends. Dissection of the lateral roots revealed the presence of several males inside them. The analysis of histological sections showed giant cells around them. Thickenings at the end of soybean lateral roots represent another element of analysis to detect nematodes of the genera *Meloidogyne*.

237 Effect of plant defence elicitors and cations on reproduction of RKN on tomato

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A number of plant defence elicitors, such as salicylic acid (SA), acetylsalicylic acid (AcSA), jasmonic acid (JA) and isonicotinic acid (INA), has been used to test their effect on root-knot nematode reproduction on tomato. Furthermore, tomato response to RKN was assayed after incubation of roots in concentrated solutions of K^+ , Na^+ , Fe^{3+} and the Ca^{2+} chelator EDTA. In particular, roots of young seedlings of susceptible tomato were immersed overnight in appropriate solutions of SA, AcSA, JA, INA, KH_2PO_4 , Na_2HPO_4 , EDTA, $FeCl_3$ and water as a control, washed and immediately inoculated with over 100 *Meloidogyne incognita* active J2 per seedlings. Inoculated tomato plants were grown in a glasshouse at 25-27°C for 6 weeks. Reproduction index was determined as number of egg masses per root system and compared to untreated control. Previous treatment of tomato seedlings with INA, Na^+ and EDTA markedly reduced RKN reproduction on tomato. The effect of different concentrations of chemicals has been also evaluated. Generally, the effect on RKN reproduction of the single chemical does not exceed a 50% decrease compared with untreated controls. The maximum inhibitory effect is associated with a threshold

concentration; higher concentrations, if not phytotoxic, are ineffective in controlling the parasites.

238 Olfaction and odour discrimination in the insect parasitic nematode *Heterorhabditis bacteriophora*

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The survival of any organism depends greatly on its ability to monitor the external environment. Organisms have developed a wide range of sensory systems to detect, transduce and evaluate relevant environmental signals. Olfaction is a primary neurosensory function and it remains one of the major senses by which organisms assess their environs. *Heterorhabditis bacteriophora* lives at a soil-water interface and so encounters many types of volatile and water-soluble molecules in its environment. We show that its complex chemical vocabulary enables *H. bacteriophora* to bind and identify many classes of odorant molecules. These include many host-related chemicals, including carbon dioxide, as well as more complex organic cues such as long chain alcohols. We show that *H. bacteriophora* detects numerous volatile and water-soluble chemicals, which can be either attractant or repellent depending on their concentration. Using behavioural assays, we have identified several classes of receptors and have investigated their affinity for host-related cues.

239 Video-assisted analysis of anhydrobiotic process in *Aphelenchus avenae* on sucrose-amended agar substrates

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The fungivorous nematode, *Aphelenchus avenae*, comes into anhydrobiosis under slowly drying conditions, however, the process leading to the anhydrobiotic state has not yet been fully understood. The present study individually examined the process leading to coiling of the nematodes on agar plates with added sucrose and their survival after desiccation treatment. Fourth stage juveniles or young adults of *A. avenae* individually

placed on 5% agar substrate containing 0.8 M sucrose showed the highest proportion of nematodes in the coiling posture. Video-assisted image analysis indicated that the nematodes became quiescent within 2-3 h after being placed on the agar plate, then gradually resumed mobility and became coiled. Coiled or uncoiled nematodes were individually transferred, without destroying their shape, to a desiccator (<20% r.h.) and exposed for 24 h. Desiccation survival was positively related to the pre-exposure treatment of nematodes on agar. Survival rates were 0, 3, 70, and 90% of nematodes pre-exposed for 0, 2, 5, and 12 h on the agar plate, respectively. All nematodes pre-exposed for 24 h or more revived immediately after rehydration following the desiccation treatment, irrespective of whether their postures either remained coiled or were un-coiled artificially.

240 Characterisation of feeding and digestion in *Meloidogyne incognita*

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The digestive proteinases of plant-parasitic nematodes have been the target of a successful proteinase inhibitor-based transgenic defence. Characterisation of the target enzymes of cyst nematodes led to the isolation of genes encoding cysteine, serine and aspartic proteinases from female cDNA libraries. To extend the work to the root-knot nematode *Meloidogyne incognita*, a cDNA library was constructed from feeding female nematodes. cDNA fragments corresponding to proteinases were amplified by PCR using degenerate primers and were subsequently used to screen the library to obtain full-length clones. cDNA clones encoding three cysteine, two serine and one aspartic proteinase have been identified, some of which are predicted to be extracellular. The expression patterns of the genes have been characterised by northern analysis and *in situ* localisation, showing varying expression patterns at different stages of development. A cysteine proteinase inhibitor expressed transgenically in *Arabidopsis* and potato has been shown to have a detrimental effect on *M. incognita*. The effect of serine proteinase inhibitors and inhibitor combinations is now being tested.

241 Variation in the assimilation and transpiration rate in nine accessions of *Passiflora* inoculated with *Rotylenchulus reniformis*

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Seeking resistance to *Rotylenchulus reniformis*, nine accessions (236-2, 221, 214-1, 247-2, 241, 232-1, 219-1, 219-2) of *Passiflora edulis* f. *flavicarpa* and 231 of *P. quadrangularis* from the germplasm collection of CENIAP were evaluated. Only accession 219-1 showed a susceptibility reaction. An experiment to study the variation in the assimilation and transpiration rates between control and *R. reniformis* inoculated plants was carried out. In order to estimate the assimilation and transpiration rate, four measurements were made on each material with an infrared gas analyser (IRGA). Results demonstrated that all accessions showed a different behaviour in the assimilation and transpiration rate between control and inoculated plants, except accessions 247-2 and 231 where plants in both treatments exhibited similar responses. Preliminary results indicate that even though there was a resistance reaction in eight of the materials, they behave physiologically differently in the presence of the nematode. It is necessary to continue this type of study for a longer period of time and with different levels of inoculum.

242 Effects of DL-methionine on hatching and survival of *Meloidogyne incognita*

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Several concentrations of DL-methionine *in vitro* and applied to soil were tested in the laboratory for their effects on *Meloidogyne incognita* egg hatching and juvenile survival. Solutions of 2.5×10^2 mg per ml reduced egg hatching *in vitro* after 2 days of treatment. After 7

days in methionine solutions, proportion of hatched eggs were reduced by 23.3% in 2.5×10^{-1} mg/ml and by 76.4% in 2.5×10^4 mg/ml methionine concentrations, compared to controls in distilled water. An effect of methionine solutions on juvenile activity was also shown after 24 h when percentage of active juveniles was reduced from 99.3% in controls to 83.0% in 2.5×10^4 mg per ml treatment. Further reductions were observed at lower methionine concentrations as time of exposure increased. When methionine was applied to soil infested by *M. incognita*, reductions in egg hatching and juvenile activity were observed at 0.1 and 1 mg of methionine per g of soil, either in sand or clay-loam soils. The percentage of hatched eggs 1 week after methionine application was lower in sandy than in clay-loam soils. Active juveniles were not found after 1 week in soil containing 1 mg of methionine per g of soil.

243 Effects of plant secondary metabolites on plant parasitic nematodes

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Plant secondary metabolism is responsible for the production of three general groups of metabolites, flavonoids, terpenoids and alkaloids, with diverse functions in plants, including defence against pathogens. The aim of the study was to test members of the three groups of metabolites for their effects on plant parasitic nematodes. The nematode species studied included the migratory endoparasites *Pratylenchus penetrans* and *Radopholus similis* and the sedentary endoparasites *Meloidogyne incognita* and *Globodera rostochiensis*. The nematodes were subjected to *in vitro* toxicity tests, chemotaxis tests and tests for the inhibition of egg hatch. Results indicate that the flavonoids juglone, caffeic acid and umbelliferone are toxic to *P. penetrans* with LD₅₀ values of 90, 374 and 418 ppm respectively after 72 h exposure. The monoterpenoid thymol and the sesquiterpenoid rishitin have LD₅₀ values of 395 and 387 ppm after 24 h exposure. Both caffeic acid and rishitin repel *P. penetrans* and *R. similis* at concentrations as low as 50 and 20 ppm in the chemotaxis tests. Other flavonoids with repelling effects include quercetin (20 ppm), resveratrol (100 ppm), kaempferol (100 ppm) and genistein (100 ppm).

244 The susceptibility of cucurbitaceous plants to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949

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Meloidogyne incognita is an important pest of forced cucumber. As there are no cucumber varieties resistant to *Meloidogyne incognita*, the use of cucumber plants grafted onto tolerant cucurbit species offers a highly effective, as well as an environmentally friendly means of control. Two cucurbit species, *Sycios angulatus* Harry and *Cucurbita ficifolia*, have been used by Hungarian growers as rootstock for cucumber. However, the tolerance level of these two species against *M. incognita* has not been fully elucidated. These two species, along with eight other previously untested species belonging to the *Cucurbitaceae*, were evaluated for their resistance to *M. incognita*. Tests were carried out under glasshouse conditions. The damage caused on the roots was assessed by using two six-graded scales (measuring size of galls and number of galls). Stochastic homogeneity test was used for statistical analysis. We found that the galls formed on the roots of *S. angulatus* Harry were small but abundant. Galls occurring on the roots of *C. ficifolia* were not only abundant but also large. Three cucurbit plants, *Cucurbita pepo* Alba, *Cucurbita pepo* Sárga óriás and *Cucurbita moschata* Orange, were identified as potential rootstocks for glasshouse cucumber.

245 Nematode losses in cotton in the USA

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Cotton is grown on more than 15 million acres in 17 states in the United States. Plant parasitic nematodes have been found in every state where cotton is grown. Each year losses to diseases and nematodes are reported to the National Cotton Council by plant pathologist and nematologist in each state where cotton is grown. Compared to other plant diseases, nematodes are the

largest cause of yield loss. In 2001 plant-parasitic nematodes accounted for an estimated loss of 979 357 bales valued at 381.9 million dollars. Root-knot was responsible for a loss of US\$200.5 million, reniform for US\$154.5 million, and other species for US\$25.5 million loss. Beltwide losses due to nematodes have increased more than 50% in the past 10 years. Most experts say that economic losses have probably been underestimated in the past when losses due to nematodes were erroneously attributed to poor soil fertility, pH, or 'weak fields'. Improvements in properly identifying nematode damage have been made, thus impacting the perceived trend of increased losses due to these pests.

246 *Meloidogyne incognita*, a new threat to soybean production in Illinois

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Meloidogyne incognita is an emerging threat to crop production in southern Illinois. This pathogen has been identified in six soybean fields, eight vegetable fields and in five peach orchards. The potential impact of *M. incognita* to soybean germplasm in northern latitudes is unknown. In 2001, four soybean varieties (Pioneer 9481, Pioneer 9492, Gateway 493 and LS 94-3207) were selected and planted in infested fields. Nematode population densities were recorded at planting and every 6 weeks until harvest. At planting, the population density of *M. incognita* averaged eight juveniles/100 cm³ soil. Reproduction by *M. incognita* was higher in the plots planted to P 9481. At harvest, the population densities (juveniles/100 cm³ soil) were 508, 41, 37 and six for P 9481, P 9492, GW 493 and LS 94-3207, respectively. Across the four varieties, the increase in the population density of *M. incognita* was concomitant with a linear decrease in soybean yield.

247 Host suitability and damage thresholds of arable crops to the root lesion nematode *Pratylenchus penetrans*

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Root lesion nematodes (*Pratylenchus penetrans*) are of growing concern in Dutch arable farming. Especially on the light sandy soils damage occurs in potatoes, black root and carrots. Dutch farmers try to prevent the problems by choosing a non-host as preceding crop to vulnerable crops. Most data on host status and damage thresholds were determined in the 1950s and 1960s. PPO started in 1998 to reconsider the multiplication of *P. penetrans* on new crops and varieties within modern growing practices. The poster presents the host status of important arable- and green manure crops determined in field- and in pot experiments. In addition, field results of initial populations (P_i) and crop yield are shown for some arable crops.

248 Effect of the root-knot nematode *Meloidogyne incognita* on the yield of parsley (*Petroselinum crispum*)

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A greenhouse experiment was conducted in 1000 cm³ plastic pots to evaluate the effect of increasing population densities of *M. incognita* on yield of parsley. Initial nematode densities were: 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs + second stage juveniles (J2)/cm³ soil. The Seinhorst model, $y = m + (1 - m)z^{Pi-T}$, was fitted to average plant top and total fresh weight and top dry weight. Tolerance limits (T) to the nematode for top fresh weight, total fresh weight and top dry weight were 0.17, 0.02 and 0.05 eggs + J2/cm³ soil, respectively. The minimum relative yields (m) were 0.5 at $P_i \geq 16$ eggs + J2/cm³ soil, 0.55 at $P_i \geq 2$ eggs + J2/cm³ soil and 0.4 at $P_i \geq 4$ eggs + J2/cm³ soil for top fresh weight, total fresh weight and top dry weight, respectively. The maximum nematode reproduction rate was 37-fold at $P_i = 0.25$ eggs + J2/cm³ soil and the equilibrium density was 5 eggs + J2/cm³ soil.

249 Reniform nematodes – a threat to cotton production in Alabama (USA)

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Reniform (*Rotylenchulus reniformis*) and root-knot (*Meloidogyne incognita*) are the two major nematode species that attack cotton in Alabama. Nematode samples from Auburn's nematode analytical laboratory and two statewide nematode surveys conducted in 1990/1991 and again in 1999/2000 revealed that reniform is the most widespread and damaging nematode species in Alabama. This species is responsible for over 90% of the nematode damage on cotton in the state. Twenty-one percent of the cotton fields sampled in north Alabama and 26% of fields in central Alabama have reniform populations greater than 500 juveniles/100 cm³ soil. Yield losses in a cotton field with high reniform populations can be as little as 10% or as high as 75%, depending on the presence of favourable or adverse growing conditions in any given year. When growing conditions for cotton in Alabama are normal, yield losses usually average around 30-40%. Statewide yield losses during the years 1999, 2000 and 2001 were estimated to be US\$14 million annually. Most cotton producers use nematicides to successfully manage reniform in cotton. A 1-year rotation with a non-host crop in Alabama has been shown to be more effective than a nematicide. However, most Alabama growers, who prefer to grow cotton continuously in the same fields, have not adopted this practice. Reniform resistant or tolerant commercial cultivars are not currently available. Research continues for more effective crop rotation schemes, improved nematicide application techniques and the search for reniform resistance in commercial cotton cultivars.

250 Investigations on *Ditylenchus dipsaci* damaging carrot in Italy

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Investigations were undertaken in southern Italy on the epidemiology of an Apulian and a Sicilian population of *Ditylenchus dipsaci*, on carrot under field conditions, from September to the following March. The host status of some plants to the Sicilian population of the nematode

was also assessed. In Apulia, on carrot sown in early September, the nematode infection started at the end of October on leaves and in early December on tap roots. Death of leaves and tap root rot were observed the following January on mature plants. On carrot sown 1 month later nematode infection occurred by the beginning of November and greatly suppressed plant growth. In Sicily, on carrot sown in early October the infection was obvious on aerial plant parts at the end of November and on tap roots in mid December. Leaf death and tap root rot occurred at end of January. Early symptoms of nematode attacks were straddled leaves, multi-bud plant crowns and light discolorations of tap root tops. The portion of the plant most affected by *D. dipsaci* was that 2-4 cm below and above ground. The Sicilian population of the nematode reproduced on carrot, broad bean, oats, rye, Italian ryegrass, celery, *Amny majus* and *Galium aparine*.

251 Effect of *Globodera rostochiensis* on the yield of potato cv. Kennebec in Venezuela

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The relationship between initial densities (P_i) of *Globodera rostochiensis* pathotype Ro2 and yield of the susceptible potato cultivar Kennebec was investigated in 30 dm³ microplots at Cubiro, Lara State, during the January-April growing season. P_i used were 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 eggs/cm³ soil. Tuber yield response to P_i fitted the model $y = m + (1 - m)z^{P_i - T}$ and a tolerance limit (T) of potato to *G. rostochiensis* of one egg/cm³ soil was derived. Yield losses of 20 and 50% occurred at P_i of eight and 32 eggs/cm³ soil, respectively. The minimum relative yield (m) was 0.35 at $P_i \geq 128$ eggs/cm³ soil. The maximum nematode reproduction rate was 25-fold at $P_i = 0.125$ eggs/cm³ soil and the equilibrium density 64 eggs/cm³ soil. Symptoms of the nematode attack became obvious on aerial plant parts at $P_i = 64$ eggs/cm³ soil.

252 Parasite-host relationship and virulence of the most damaging nematodes in fields in Armenia

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We have studied the roots and the top-soil under tomatoes, cucumbers, pepper, cabbage, carrot and watermelon as well as wheat, sweetcorn and potato. Thirty-nine species of nematodes recorded in ten crops belonged to 17 genera. The most virulent included *Macropostonia xenoplax*, *Criconemoides mongolensis*, *Heterodera crucifera*, *Globodera rostochiensis*, *Meloidogyne hapla*, *M. arenaria*, *M. incognita*, *Tylenchorhynchus brassica*, *T. davaini*, *T. parvus*, *Quiniculcius acti*, *Helicotylenchus pseudorobustus*, *H. eritrinae*, *H. conicephalus*, *H. californicus*, *H. digonicus*, *Ditylenchus destructor*, *D. dipsaci*, *Aphelenchoides subparientinus*, *A. ritzemabosi*, *Longidorus elongatus* and others. The nematode fauna of crops studied was diverse and abundant, and was distinguished by a large number of dominant species. The species diversity of nematodes and their population density in the top soil was related to the agrotechnology measures which lowered the number of species.

253 Pathogenicity of *Pratylenchus coffeae* on seedlings of coffee cv. Mundo novo

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The pathogenicity of two Brazilian *Pratylenchus coffeae* isolates was evaluated in two glasshouse experiments on coffee seedlings (*Coffea arabica*) cv. Mundo Novo. In the first, the effect of initial population densities ($P_i = 0, 333, 1000, 3000$ and 9000 nematodes/plant) was studied using *P. coffeae* isolate from Marília, SP. The data were fitted to the Seinhorst model. At 270 days after inoculation, all plants inoculated with 9000 nematodes and the majority inoculated with 3000 nematodes were dead. Plant growth and photosynthesis were reduced with inocula of 333 and 1000 nematodes, respectively. In the second experiment, pathogenicity of two *P. coffeae* isolates (from Marília and Rio de Janeiro) was compared using coffee seedlings. Although photosynthesis was similarly reduced for both isolates with an inoculum of 8000 nematodes/plant, the Marília isolate caused intense darkening of the roots, leaf chlorosis and a greater reduction of root and shoot growth.

In both experiments, reproduction (*Pf/Pi*) of the isolates was low, suggesting that coffee is a poor host for both *P. coffeae* isolates. The differential pathogenicity observed in the second experiment supports previously published data that reported differences in morphology and host ranges between these isolates.

254 Virulent populations of *Meloidogyne* in vegetable crops from Uruguay

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Some tomato varieties that are carriers of the *Mi* gene, which confers resistance on them to the root-knot nematodes *Meloidogyne arenaria* and *M. incognita*, appeared severely affected by these pathogens, making the crop non-viable in many cases. This happens frequently in the departments of northern Uruguay, especially Salto and Artigas, where soil temperatures often rise above 27°C, which is one of the main causes for the break in resistance. Various *M. incognita* populations from northern Uruguay have been characterised, proving to be virulent to resistant tomato varieties. Several pepper cultivars with the *N* gene have also been selected that demonstrate resistance to these nematodes. It can be affirmed that conventional crop methodologies are contributing to the selection of virulent populations, which could make the use of resistant varieties impossible in the future. Biofumigation and other crop techniques proved effective in the regulation of virulent nematode populations of the *Meloidogyne* genus, without the use of nematicides.

255 Virulent populations of *Meloidogyne* in vegetable crops in Spain

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It has been observed that tomato varieties having the *Mi* gene and pepper varieties with the *N* gene, which

confers resistance on them to the root-knot nematodes *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, are seriously affected by these pathogens, even leaving the crop non-viable in some cases. It has been observed that plants resistant to these nematodes, either because they are carriers of the above-mentioned gene or because they are grafted onto a resistant stock, are susceptible principally to attacks by *M. incognita* and *M. javanica*. In most cases, this is due to an incorrect application of these alternatives, sometimes because the plant loses its resistance due to exposure to soil temperatures over 27°C or by selection of virulent populations. The use of crop management techniques that decrease nematode populations, such as biofumigation, has been regarded as efficient in the regulation of virulent populations of nematodes belonging to the genus *Meloidogyne*.

256 Pest risk analysis of *Meloidogyne chitwoodi* in Norway

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Meloidogyne chitwoodi may be introduced into Norway through plant material and soil. The nematode may easily establish on common crop plants. During the growing season two generations are expected to develop in southern Norway, while conditions in the central parts would allow for the development of one generation. *M. chitwoodi* starts to develop at 5°C. Planting and sowing in Norway starts when soil temperatures reach around 4°C. The use of plastic tunnels to increase soil temperature is a common practice in early potatoes and vegetables, and would enhance nematode development. *M. chitwoodi* requires 600-800 degree days (DD) to complete one generation from the time of planting, and 500-600 DD for subsequent generations. These temperature sums are commonly recorded in potato growing areas of Norway. Qualitative damage on potato tubers would require the re-infection and development of the second generation, and is expected in the southern areas. Qualitative damage on carrots does not require re-infection, and is expected also in central parts. The possibility that the nematode could cause damage to other vegetables and to cereals is an additional concern that supports its status as a quarantine pest organism.

257 Progress of two kinds of symptom appearing in the body of recovered *Pinus thumbergii* from pine wilt disease

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Pine wood nematode can cause fatal damage to Japanese black pine, *Pinus thumbergii* and red pine, *Pinus densiflora*. However, not enough is known about the mechanisms leading to the death of pines killed by this organism. Hence, study of the various aspects of pine trees which are purported to represent the processes of the disease from its early stages through to its lethal stages has helped to clarify both disease development and causal mechanism. Thionazin or mesulfenfos was injected into 11-year-old *P. thumbergii* infected with pine wilt disease following inoculation of pine wood nematodes 2, 5 and 6 weeks prior to treatment with nematicides. At the time of injection with nematicides, all the trees tested were judged as having pine wilt disease because they had ceased to exude pine resin. Recovery, expressed as resumption of resin exudation by surviving trees, was observed in pines injected with thionazin 2 weeks after inoculation. By examining cross-sections of recovered pine trees felled 2 years after this experiment, two apparently distinct symptoms were observed, one an early symptom associated with the formation of small non-water-conducting patches, and the other associated with cambium death. Both symptoms seemed to begin in and progress from the pith.

258 Impact of *Meloidogyne graminicola* on yield of lowland rainfed rice in Bangladesh

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Replicated plots with and without the application of Furadan 5G (40 kg/ha) were established at an agricultural research station and on farmer fields in northwestern Bangladesh to assess the impact of *Meloidogyne graminicola* on lowland rainfed rice yield in the rice-wheat production system. At the research station, rice yields in-

creased by 0.2, 0.8 and 1.1 t/ha where Furadan was applied to the seedbed only, to the field only, and to both the seedbed and field, respectively, compared to a non-treated control. Averaged across three farmer fields, rice yield increased by 1.1 t/ha where Furadan was applied to both the seedbed and field compared to the control. At harvest, soil levels of *M. graminicola* in the non-treated plots were more than three times greater than those in the treated plots at both locations. Prior to transplanting, seedling shoot height and dry weight were significantly greater, and soil levels of *M. graminicola* significantly less, in the treated seedbed plots compared to the non-treated plots. This is the first on-farm study to demonstrate a negative impact of *M. graminicola* on rice growth and yield during the monsoon season in Bangladesh.

259 Reaction of olive to *Pratylenchus vulnus* infections in Italy

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The reaction of seven olive cultivars, Cellina di Nardò, Cima di Bitonto, Coratina, FS 17, Frantoio, Leccino and Yusti and of the rootstock DA 12 I to *Pratylenchus vulnus* infections was evaluated in a glasshouse experiment. Plants were artificially inoculated with 200 nematodes per pot. Plant growth and nematode reproduction parameters were recorded. Host reaction was rated according to the reproduction factor $r = Pf/Pi$. All the tested cultivars and rootstock were good hosts for *P. vulnus*, although the highest susceptibility was shown by FS 17. No statistical difference was found among the *P. vulnus* reproduction parameters on the other cultivars.

260 Impact of *Pratylenchus neglectus* infestation on wheat in Western Australia

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Pratylenchus neglectus is very widespread in cereal-producing regions of Western Australia. A recent study revealed that the average nematode population in 64% of the surveyed locations exceeded the lowest level that causes significant yield loss. Experiments were

established between 1999 and 2001 in nematode-infested fields at five locations in central and southern wheat-belts to study impact of the nematode on performance of wheat. Large plots (1.8×40 m or 1.44×20 m) were sown to susceptible wheat (cvs Machete or Cunderdin or Brookton). In half of the 64 plots (paired plots), aldicarb (Temik 150G) was applied (20-30 kg/ha) at seeding. Ten weeks after sowing nematode densities in roots were assessed. Nematode density and wheat yield in the nematicide-treated and check plots were compared using analysis of variance. Aldicarb partially controlled the nematode populations. Yield enhancement with nematicide application was 6-14%. At one location, yield differences following use of aldicarb were not significant, partly due to inadequate nematode control. The study established economic damage potential of *P. neglectus* in Western Australia; however, this loss assessment could be an under-estimation of actual loss as nematicide provided only partial protection to plants from nematode infection.

261 Population dynamics of, and yield loss caused by, *Pratylenchus neglectus* and *P. thornei* in cereal crops in Australia

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A range of initial soil densities of either *P. neglectus* or *P. thornei* was established in field sites in South Australia. This was achieved by growing replicated plots of susceptible wheat (cvs Machete and Spear), moderately resistant wheat (cv. Excalibur), moderately resistant barley (cv. Chebec) and resistant triticale (cv. Tahara) in the 1st year. In the 2nd year, these cultivars resulted in initial *P. neglectus* densities for Machete, Spear, Excalibur, Chebec and Tahara of 18, 8, 4, 2 and 3/g soil, respectively. For *P. thornei*, these cultivars resulted in initial densities of 14, 10, 3, 1 and 1/g soil. Plots were oversown in the 2nd year with an intolerant oat cultivar (Echidna). Initial (P_i) and final numbers (P_f) of nematodes were assessed from soil of all plots in each year. For both *P. neglectus* and *P. thornei*, a significant negative linear relationship was observed between yield of the intolerant oat and initial nematode density in the 2nd year. Yield losses of 27% for *P. neglectus* and 22%

for *P. thornei* were estimated. In addition, an exponential relationship was observed between initial density and nematode multiplication rate (P_f/P_i).

262 Infection of *Arachis hypogaea* by *Meloidogyne arenaria* may increase aflatoxin contamination of kernels

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A field-microplot study was conducted to determine whether aflatoxin levels in peanut, *Arachis hypogaea*, were correlated with pod and root galling caused by *Meloidogyne arenaria* (*Ma*). The experiment was a completely randomised 2×2 factorial with six replicates per treatment. Factors were nematodes, plus and minus *Ma*, and fungus, plus and minus *Aspergillus flavus* (*Af*). Peanut seedlings, either infected with *Ma* or uninfected, were transplanted into 2.3 m^2 plots previously fumigated with methyl bromide. Inoculum of *Af* was sprinkled over the plant canopy at mid bloom. Drought was induced after pod set by covering plots during rain with a fibreglass shelter. Pod- and root-gall indices (1-5 scale) were determined for all plants in the plot and averaged. Pods from each plot were bulked, shelled, and a subsample of kernels was used to determine aflatoxin concentration and percentage colonised by *Af*. Neither factor, nematodes or fungus, affected aflatoxin concentration; however, there was an interaction between these factors ($P = 0.003$). In plots without added fungus, there was a correlation between aflatoxin concentration vs pod-gall index ($P = 0.001$, $r = 0.82$) and vs root-gall index ($P = 0.006$, $r = 0.74$). Colonisation of kernels by *Af* increased with increasing pod galling ($P = 0.04$, $r = 0.42$).

263 Yield losses of barley, oat and wheat due to root lesion nematode in South Australia

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Wheat (*Triticum aestivum*) is the principal host of *Pratylenchus neglectus* in southern Australia. By comparison, barley (*Hordeum vulgare*) and oat (*Avena sativa*) are moderate hosts. Mean yield loss for intolerant wheat in 1995-1998 was 15-20%. Trials at six sites were assessed in 2000 to compare losses for barley, oat and wheat. Natural variation in nematode density between and within trial sites allowed comparison of plot yields over a range of nematode densities. Final nematode density (*Pf*) was determined during grain development in mid-spring (October). Negative correlations between yield and *Pf* were significant for barley ($r = 0.789-0.875$), oat ($r = 0.654-0.892$) and wheat ($r = 0.524-0.828$). Yield loss for each genotype was calculated from the maximum genotype yield predicted by the linear relationship between yield and *Pf*. Mean yield loss for intolerant barley and oat was 8.4%, and for wheat 7.3%. Barley and oat are more resistant than wheat (*Pf*, respectively, 1.0, 1.6 and 2.5 *P. neglectus*/g dry soil), but suffer comparable yield loss and are therefore as intolerant as wheat. *Pratylenchus neglectus* is a constraint to cereal production in southern Australia, and these yield penalties represent significant financial loss to the grower.

264 Response of four potato (*Solanum tuberosum*) cultivars to varying levels of root-knot nematode (*Meloidogyne hapla*) inoculum

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The effect of varying levels of *Meloidogyne hapla* inoculum on the growth and yield of four potato cultivars was studied under glasshouse conditions. Plant height, fresh root and top weights, and tuber yield were significantly affected by the nematode. Plant height was reduced by 28-45% when *Pi* was 1000 juveniles per pot, and 30-57% when the number of infective juveniles present around young seedlings was 10000. Fresh root weight was decreased by 35-44, 39-51, 41-50 and 37-46% in cvs Berolina, Igorota, Solibao and Granola, respectively, when *Pi* was 1000-10000. Significant yield reduction was also noted in the four cultivars used when *Pi* was 1000-10000 juveniles per pot: 44-56% in Berolina; 75-88% in Igorota and Solibao and 85-100% in Granola. Based on the gall index rating used, cvs Berolina, Igorota, Solibao and Granola were rated resistant, moderately resistant, moderately susceptible and susceptible, respectively, to *M. hapla* infection.

265 Novel gene discovery in plant-parasitic nematodes

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The potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) are economically important pests both in the UK and worldwide, and cause significant losses to commercial potato production. As a means of investigating the complex host-pathogen interactions involved in *G. pallida* and *G. rostochiensis* infections, cDNA AFLP and suppression subtractive hybridisations (SSH) using resistant and susceptible potato cultivars were carried out. Novel genes expressed during invasion of the host plant and subsequent nematode development were identified by harvesting plants at several time points ranging from 24 h to 14 days post-infection. A significant proportion of candidate genes from the SSH matched nematode EST such as FABP-1, haemolysin, calponin, beta-1,4-endoglucanase, cytochrome p450 monooxygenase and ribosomal proteins. Other sequences gave no reliable database matches. Localisation studies are being carried out on these genes by *in situ* hybridisation to obtain functional information and levels of gene expression during nematode invasion and development are being investigated using *rt*-PCR and southern blot analysis. To further quantify levels of expression during the host-pathogen interaction an analysis using real time PCR is currently underway.

266 Cloning of the chorismate mutase homolog of the soybean cyst nematode *Heterodera glycines*

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The chorismate mutase is a nematode gland secreted protein that was first cloned from root-knot nematode *Meloidogyne javanica*. This enzyme is extensively studied in root-knot nematode and it was recently reported as an EST from *Globodera rostochiensis*. This enzyme is believed to be involved in nematode parasitism. Using degenerate primers to the conserved regions of both known nematode chorismate mutase proteins,

we successfully amplified the chorismate mutase of *Heterodera glycines*, the soybean cyst nematode (SCN), from cDNA extracted from 4-day-old parasitic juveniles. The SCN homolog gene has been cloned and sequenced. The derived protein sequence of the SCN chorismate mutase showed about 25% identity to that of *M. javanica* and about 55% identity to that of *G. rostochiensis*. The SCN chorismate mutase is being characterised by southern blot, enzymatic analysis and *in situ* hybridisation analysis.

267 Establishing a *Lotus japonicus*-nematode pathosystem to search for variations in their interactions

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In order to complete their life cycles, both root-knot and cyst nematodes must interact with their host plants. Much attention has been devoted to the nematode factors involved in root penetration, migration and feeding site formation. However, very little is known about plant host factors that are needed for the susceptible interaction to occur because, in general, natural host plants are not easy to manipulate genetically. *Lotus japonicus* has emerged in the past few years as a suitable model plant to study interactions with nitrogen fixation symbionts. A set of genetic resources and tools are rapidly becoming available for *L. japonicus*, including ecotypes, mutants, transformation procedures and a sequencing project. We have established a pathosystem using *Lotus japonicus* as a host plant for both a plant cyst nematode and a root-knot nematode. The current search for variation in the interactions between either of these nematodes and a battery of *L. japonicus* ecotypes and mutants will eventually lead to the isolation and cloning of plant factors responsible for such interactions.

268 Isolation of putative parasitism genes from *Globodera pallida*

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Potato cyst nematodes (PCN) are devastating pests in the United Kingdom and other European countries. While *Globodera rostochiensis* can be controlled in the UK by using resistant potato cultivars, no effective resistant genes are available to control *G. pallida* populations. Research targets for the control of *G. pallida* include parasitism genes needed to complete its life cycle. To isolate these targets, two *G. pallida* cDNA libraries were built from either preparasitic juveniles (J2) and adult (gravid) females or J2 individuals. A collection of more than 1000 EST from the former library has been released into the public domain. Data mining in this collection reveals EST encoding for putative secreted proteins with similarities to Tylenchida sequences, but not to free-living *C. elegans* sequences, which suggests that they may have a role in plant nematode parasitism. A number of PCN specific sequences have no homologies to other sequences in databanks. These novel sequences encode for proteins containing features of secreted proteins, which make them candidates for further research into their putative role in PCN-specific parasitism and evolution.

269 Using monoclonal antibodies to discover parasitism proteins from the virus-vector nematode *Xiphinema index*

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Xiphinema index is a plant ectoparasitic nematode that transmits plant viruses. *Xiphinema index* induces changes in plant cells similar to those induced by endoparasites such as *Meloidogyne* spp. *Xiphinema index* is large and can be cultured, making it an attractive model system for some aspects of plant nematology. Although

much work has been performed on the interaction between the nematode and the virus, most research on the nematode itself has been limited to taxonomic studies. Antibody methodology has been successfully used to discover parasitism genes from endoparasites. We therefore produced a panel of MAbs against *X. index*. A series of secreted proteins present in different dorsal ducts, which related to particular periods of the nematode feeding cycle, were recognised by one monoclonal antibody. Other MAbs were obtained which recognise the cuticle surface, muscle fibres, nerve cords and oocytes. Some of these MAbs have the potential to be developed for use as nematode diagnostics. An antibody that recognises the virus-retention site within the nematode odontophore may be useful for further virus-nematode interaction studies. In future work we will use western blots of 2D electrophoresis gels coupled to MALDI-TOF analysis to identify the secretory proteins in gland cells recognised by the MAbs.

270 Peptides that bind to and inhibit cellulase of *Heterodera glycines* identified by combinatorial library screening

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The feasibility of identifying inhibitors of the products of nematode parasitism genes is being investigated using the secreted cellulases of *Heterodera glycines* as a model target. The HG-eng-2 endoglucanase gene of *H. glycines* was overexpressed in a *Pichia pastoris* host, and active recombinant HG-ENG-2 was affinity-purified. Purified rHG-ENG-2 was used as a target in biopanning experiments with a commercial phage-display combinatorial 7-mer peptide library. Two stringent rounds of biopanning produced about 1000 plaques with peptides that bound to rHG-ENG-2. Individual plaques are now being investigated to determine the number of unique peptides that bind to rHG-ENG-2 and to identify those that inhibit the ability of rHG-ENG-2 to degrade carboxymethylcellulose. One promising 7-mer peptide

that binds to and inhibits the activity of rHG-ENG-2 has been identified to date.

271 Proteins and expressed genes of the ectoparasite *Xiphinema index*

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Xiphinema index, the natural vector of Grapevine fan leaf virus, is a plant ectoparasite that induces changes in host cells similar to those induced by biotrophic endoparasites such as *Meloidogyne* and *Heterodera*. *Xiphinema index* is large, readily cultured and all life stages occur outside the plant making it an attractive model system for some aspects of plant nematology. We have begun a programme aimed at identifying genes and proteins important in the host parasite interaction of this species. Expressed sequence tags are being obtained from a mixed stage cDNA library of this nematode. Over 500 EST have been obtained and a variety of genes identified including proteases and collagens. To date no EST from genes acquired from bacteria by horizontal gene transfer have been found. Two-dimensional electrophoresis has been used to identify proteins specific to nematode portions containing the pharyngeal gland cells. In future work we will use MALDI-TOF analysis coupled to the data from our EST programme to identify the genes encoding such proteins.

272 Defining a plant-parasitic nematode: a profile of putative parasitism genes expressed in the pharyngeal gland cells of *Heterodera glycines*

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The most evolutionary advanced adaptations for plant parasitism by nematodes are the products of parasitism genes expressed in the pharyngeal gland cells and secreted through the stylet into plant tissue. A direct method

of microaspiration of cytoplasm from the pharyngeal gland cells of ten parasitic stages of *Heterodera glycines* from different time points in the parasitic cycle provided expressed mRNA to construct a gland cell-specific cDNA library by long-distance-PCR. Of 2345 cDNA clones sequenced, deduced protein sequences of 231 cDNAs had a N-terminal signal peptide for secretion and, thus, could have roles in *H. glycines* parasitism of soybean. High-throughput *in situ* hybridisation with probes of 112 cDNA clones encoding signal peptides resulted in 40 unique clones specifically hybridising to transcripts within the subventral (11 clones) or dorsal (29 clones) gland cells. PSORT II predicted 30 of the proteins to be extracellular and ten proteins as nuclear localised. In BLASTp analyses, 25 of the predicted proteins were novel. Those proteins with similarities to known proteins included venom allergen like proteins, β -1,4-endoglucanases, a pectate lyase, a chitinase, RanBPMs, and a cellulose-binding protein. Only two of the 40 gland-expressed genes had homologues in *Caenorhabditis elegans*.

273 Activity of plant endoglucanase gene promoters in nematode feeding cells

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Five endoglucanase (*Ntcel*) genes were demonstrated to be upregulated in syncytia and giant-cells formed in *Nicotiana tabacum* by *Globodera tabacum* and *Meloidogyne incognita*, respectively. 1.5 kb of the 5'-flanking genomic DNA region of the most strongly upregulated tobacco endoglucanase gene, *Ntcel7*, was fused to the GUS reporter gene in a binary vector and used to produce hairy roots of tobacco and tomato, *Lycopersicon esculentum*, via *Agrobacterium rhizogenes*-mediated transformation. The 1.5 kb *Ntcel7* promoter/GUS construct was expressed specifically within plant root tips, lateral root initials, and appeared to be expressed within the feeding cells of root-knot and cyst nematodes in both tobacco and tomato hosts. These findings agree with earlier evidence that an *Arabidopsis*

thaliana endoglucanase (*Atcel1*) promoter was active in the feeding cells of root-knot nematodes in transgenic tobacco. The apparent expression of the *Ntcel7* promoter in feeding cells of cyst and root-knot nematodes in two plant species suggests that these promoters may have similar activity in other plant systems.

274 Identification and localisation of tomato expansin gene expression in nematode-induced syncytia

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In the model system *Arabidopsis thaliana*/*Heterodera schachtii* (*Hs*) it was shown that specific plant expansins are activated in nematode-induced syncytia. To prove whether the situation in the model system is transferable to susceptible crop plants, tomato roots were infected with *Hs* and *Globodera rostochiensis* (*Gr*), respectively. RT-PCR experiments with primers discriminating seven tomato α -expansins showed that six expansins (*Le-Exp1*, -3, -4, -5, -9 and -18) were expressed in or close to the syncytium, 10 and 14 days after inoculation. *Le-Exp5* was further analysed by *in situ* RT-PCR. Amplified tomato expansin 5 cDNA could only be localised in the cytoplasm of syncytial cells but not in the surrounding root tissue. This indicates that tomato expansin 5 is specifically expressed in syncytial cells and presumably plays an important role in the development of syncytia induced by cyst nematodes.

275 *Arabidopsis thaliana* expansin genes are expressed in syncytia induced by *Heterodera schachtii*

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The differentiation of syncytia of *Heterodera schachtii* (*Hs*) in roots of *Arabidopsis thaliana* is accompanied by cell wall loosening, extension and local degradation. In order to study whether specific expansins are involved in this process, RT-PCR experiments with a syncytium-specific cDNA library and primers differentiating between 17 α - and 3 β -expansin cDNAs of *Arabidopsis* were performed. Specific *Arabidopsis* mRNAs of 8 α - and 1 β -expansins were present in 5-7 day-old syncytia induced by *Hs*. *In situ*-RT-PCR experiments indicate that amplified cDNA of *At-Exp6* is localised in the cytoplasm of syncytial cells and not in the surrounding root. These results could be confirmed with transgenic *Arabidopsis* promoter:GUS lines. GUS activity was specifically observed in syncytia and primordia of lateral roots.

276 Identification of gene expression differences between *Globodera pallida* and *G. mexicana* by suppressive subtractive hybridisation

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Globodera pallida and *G. mexicana* are two closely related species that are able to mate but usually develop on different solanaceous plants. Identification of nematode genes involved in parasitism is one of the most critical steps leading to the elucidation of disease resistance mechanisms in plants. In this study, we have used the Suppression Subtractive Hybridisation (SSH) technique to investigate differences between the transcriptomes of *G. pallida* and *G. mexicana* J2. None of the cDNA fragments isolated in the SSH experiments appeared to be completely absent from the other transcriptome, but differences in expression levels of some cDNAs between the two species were confirmed in reverse northern experiments. Sequence analysis revealed that a high proportion of the cloned sequences were pioneer genes for which no putative homologues were present in the databases. However, homologues of a cellulase and a putative pathogenicity factor previously described in *G. rostochiensis* were isolated. cDNAs corresponding

to these sequences were therefore compared between *G. pallida* and *G. mexicana*.

277 Identification of putative parasitism genes expressed in the pharyngeal gland cells of *Meloidogyne incognita*

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Cloning parasitism genes coding proteins secreted from the pharyngeal gland cells and injected through the stylet into plant tissue is the key to understanding the molecular basis of nematode parasitism of plants. Microaspiration of the cytoplasm from the pharyngeal gland cells of 43 parasitic stages of *M. incognita* representing different time points in the parasitic cycle provided expressed mRNA to construct a gland cell-specific cDNA library by long-distance-PCR. Of 1200 cDNA clones sequenced, deduced protein sequences of 141 cDNAs had an N-terminal signal peptide for secretion and, therefore, could have roles in root-knot nematode parasitism of plants. Of 101 cDNA clones encoding signal peptides tested by high-throughput *in situ* hybridisation, probes of 21 unique clones specifically hybridised to transcripts within the subventral (seven clones) or dorsal (14 clones) gland cells. PSORT II predicted 18 of the deduced proteins to be extracellular and three as nuclear localised. In BLASTp analyses, 19 of the predicted proteins were novel proteins. Those with similarities to known proteins included a transcription factor and an avirulence protein. None of the 21 gland-expressed genes had homologues in *Caenorhabditis elegans*.

278 Expression of *Arabidopsis thaliana* gene PROLIFERA in nematode-induced feeding sites

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The initiation and formation of feeding sites in plant roots, induced by root-knot and cyst nematodes, involve the re-activation of cell cycle genes, resulting in multinucleated giant cells and syncytia. The feeding site serves as the nutrient source for the development and reproduction of the nematode. In order to extend our understanding of the interaction between nematodes and plants during the infection process, we have examined expression of the *Arabidopsis* PROLIFERA (PRL) gene during the development of nematode feeding sites (NFS). PRL is expressed in dividing cells throughout plant development and encodes an MCM protein thought to function in the initiation of DNA replication during S phase. We will present the expression pattern of PRL during formation of both giant cells and syncytia in the root of *A. thaliana* seedlings.

279 Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach

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The stylet secretions of sedentary plant-parasitic nematodes are thought to be major pathogenicity factors involved in host invasion and feeding site induction and maintenance. A procedure was developed that allowed the direct qualitative analysis of proteins secreted by *Meloidogyne incognita* infective juveniles. After stimulation in semi-sterile conditions, the secreted proteins whose pI ranged from 5.0 to 7.5 were separated by 2D electrophoresis and the seven most abundant proteins were identified by micro-sequencing. A protein highly homologous to calreticulins was identified. The peptide sequences were used to design degenerate oligonucleotides that allowed the cloning of the corresponding *Mi-crt* cDNA. Transcription of *Mi-crt* in infective juveniles and adults was demonstrated. Expression of the calreticulin in the subventral pharyngeal glands of infective juveniles was evidenced,

suggesting that calreticulin is a component of naturally produced stylet secretions and supporting the validity of the purification procedure. Calreticulin is a calcium binding protein involved in multiple cellular functions. It is secreted by several animal-parasitic nematodes and trematodes during parasitism.

280 Identification and expression analysis of pectin degrading enzymes from the root-knot nematode *Meloidogyne incognita*

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The establishment of sedentary parasitic root-knot nematodes in a host root is dependent on their efficiency in invading the root tissue and in inducing the formation of a permanent feeding site. Several works have brought evidence that stylet secretions injected in the root tissue during parasitism are involved in those two phases of parasitism. Secreted enzymes involved in the invasion phase can be identified by a candidate gene approach. This strategy has previously allowed the identification of cellulolytic endoglucanases secreted by *Meloidogyne incognita*. In this study, pectin degrading enzymes were identified by analysing EST obtained from *M. incognita* infective juveniles. Two pectate lyase (*Mi-pel1* and *Mi-pel2*) and one polygalacturonase (*Mi-pg*) full length cDNAs were isolated. The deduced proteins contain a predicted secretion signal sequence. Transcription of the three genes was evidenced in pre-parasitic juveniles, sedentary females and males by RT-PCR. In infective juveniles the transcripts are localised in the subventral pharyngeal glands. Enzyme activities were analysed by *in vitro* tests. This study demonstrates that root-knot nematodes use a battery of cell wall degrading enzymes during parasitism.

281 Expression pattern of *ENOD40* in tomato roots upon infection with root-knot (*Meloidogyne incognita*) and cyst (*Globodera rostochiensis*) nematodes

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Early nodulin (*ENOD*) genes have been defined as genes expressed in legumes during nodule formation. Recent studies, however, revealed a number of homologues of *ENOD40s* in non-legume plants. Both in legumes and non-legumes *ENOD40s* are thought to play a role in the development of vascular tissues. *In situ* localisation of *ENOD40* transcripts in root-knot nematode-infected roots of the legume *Medicago truncatula* revealed that this plant gene is expressed in and around giant cells at 6 days post infection (dpi). To test the expression of *ENOD40* in a non-legume upon infection with root-knot and cyst nematodes, the expression of endogenous *ENOD40* promoter-*gusA* fusion was studied in nematode-infected tomato roots at 1, 3, 7 and 14 dpi. In the main root, GUS-stained areas were irregularly alternated with unstained areas. The lateral roots showed staining in the root-tip. Occasionally, GUS staining was observed in syncytia and giant cells. Sections from both GUS stained and non-stained feeding sites were investigated microscopically, and the results confirmed our macroscopic observations. Hence, *ENOD40* expression in tomato is basically unaffected by the induction of feeding sites by root-knot and cyst nematodes.

282 Molecular cloning of chitinase genes from the pine wood nematode, *Bursaphelenchus xylophilus*

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Chitinase function in nematodes, other than during egg hatch, has been the subject of interest in recent years, particularly in animal-parasitic nematodes. In this study we describe the cloning of chitinase genes from the pine wood nematode, *Bursaphelenchus xylophilus*, which is mycetophagous and phytophagous, the causal agent of pine wilt and vectored between trees by insects. Degenerate primers derived from the conserved regions of known chitinase sequences were used to amplify fragments of putative *B. xylophilus* chitinase genes. Thus, two distinct fragments that encode chitinase sequences were obtained. Using these fragments as probes, corresponding cDNA clones were identified from

our mixed-stage cDNA library and designated as *Bx-cht-1* and *Bx-cht-2*. Sequence analysis revealed that *Bx-cht-1* and *Bx-cht-2* genes putatively encode proteins composed of 371 amino acids and 567 amino acids, respectively. The deduced amino acid sequences of these proteins showed significant homologies to chitinases of other nematodes. Our further analyses aim at identifying the timing and location of the chitinase genes expression in the nematode.

283 Novel pectate lyase gene from *Globodera rostochiensis*

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A novel cell wall degrading enzyme was identified as a part of cDNA-AFLP project that compared gene expression profiles in five different stages of *Globodera rostochiensis*. One Transcript Derived Fragment (TDF), predominantly expressed in infective second stage juveniles, encoded a partial open reading frame showing high similarity to bacterial and fungal pectate lyases from the polysaccharide lyase 3 family (EC 4.2.2.2). The corresponding full-length cDNA designated *Gr-Pel2* harbours an open reading frame of 759 bp, encoding polypeptide with molecular mass 27 kDa. *Gr-Pel2* is significantly different from previously identified *Gr-Pel1* (30% identities and 46% similarities). Sequence was predicted to have signal peptide for secretion at its N-terminal end. Digoxigenin-labelled DNA probe hybridised specifically to the subventral pharyngeal glands, which are responsible for secreting all currently known cell wall degrading enzymes. Heterologous expression of *Gr-Pel2* in *Pichia pastoris* is in progress, in order to assess its biochemical properties. Identification of another pectate lyase gene shows the complexity of the pectinolytic system in nematode secretions.

284 Analysis of root-knot nematode chorismate mutase expressed in soybean hairy roots

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To gain insight into how plant pathogenic nematodes parasitise plants, we have been isolating and characterising nematode genes that are expressed in the pharyngeal region of the root-knot nematode, *Meloidogyne javanica*. One pharyngeal gland gene we had previously cloned was determined to be a chorismate mutase and was named *MjCM-1* (*Meloidogyne javanica* chorismate mutase). We determined this enzyme was secreted from the nematode as it was initiating its giant feeding cells. We hypothesised that *MjCM-1* is manipulating the shikimate pathway in the plant to the benefit of the nematode. Since the shikimate pathway in plants produces metabolites that regulate plant development and plant defence, we hypothesised *MjCM-1* may be altering one of these processes. To test this hypothesis we have expressed *MjCM-1* in soybean hairy roots using a dexamethasone inducible plant expression vector. Soybean hairy roots treated with dexamethasone express *MjCM-1* and the roots exhibit a distinct lack of lateral roots. This result indicates that *MjCM-1* can alter plant development. Metabolic profiling experiments are being conducted on the *MjCM-1* expressing roots to determine exactly which compounds are altered by this nematode enzyme.

285 Histochemical localisation of active oxygen species in *Glycine max* and *Arabidopsis thaliana* infected with the cyst nematodes *Heterodera glycines* and *H. schachtii*

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The production of Active Oxygen Species (AOS) is considered to be an initial indicator and key component of the plant defence response. AOS have been implicated in direct pathogen inhibition through cell-wall modification, necrosis, direct pathogen mortality, signal transduction leading to defence-related gene expression, and programmed cell-death. The distribution and role(s) of AOS in plant-nematode interactions have yet to be well characterised. We used a histochemical approach to study the distribution of AOS in susceptible and resistant interactions between *Glycines max* and *Heterodera glycines* and in *Arabidopsis thaliana* infected with *H. glycines* or *H. schachtii*. In roots of *G. max*, AOS were associated with the head of the migrating nematode in the susceptible interaction but not in the resistant interaction. AOS were also not

detected in either tissue once the nematode had initiated syncytium formation. In *Arabidopsis* roots, AOS were associated with nematode infection sites and possibly with developing syncytia. These observations were especially evident in the interaction between *A. thaliana* and *H. schachtii*. *Arabidopsis* roots infected with *H. glycines* exhibited similar staining patterns, but the incidence of nematode infection and feeding was lower. Patches of staining not directly associated with feeding nematodes were also observed.

286 Two novel *Arabidopsis* promoters with distinct tissue-specificities are responsive to cyst nematode infection

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We previously isolated partial soybean cDNA clones whose corresponding transcript abundances are elevated 1 day after infection with the soybean cyst nematode, *Heterodera glycines*. Following, we isolated full-length cDNAs for two of these clones, designated *Gm10.1* and *Gm17.1*. RNA blot hybridisations revealed that *Gm10.1* and *Gm17.1* mRNAs accumulated in a root-preferential manner, and that their corresponding steady-state mRNA levels increased over time following *H. glycines* infection. The predicted *Gm10.1* and *Gm17.1* gene products each shared similarities with the protein products of distinct *Arabidopsis thaliana* genes with unknown functions. Promoters of these *Arabidopsis* genes, designated *At10.1* and *At17.1*, were fused to the GUS reporter gene and transformed into soybean hairy roots and *Arabidopsis* plants. Activity of the *At10.1* promoter was detected in root and shoot apical meristems of *Arabidopsis* and in all tissues of soybean hairy roots. The *At17.1* promoter also directed GUS expression to the root meristems but additionally was detected in the vascular tissues of both *Arabidopsis* and soybean. Furthermore, the *At10.1* promoter was responsive to infection of *Arabidopsis* by *Heterodera schachtii*, whereas the *At17.1* promoter directed increased GUS expression to the infection sites of *H. glycines* in soybean hairy roots.

287 Selection for virulence in populations of *Globodera pallida*

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Four populations of *Globodera pallida* were reared continuously on hosts with resistance derived either from *Solanum vernei* or *S. tuberosum* ssp. *andigena* CPC 2802. After 12 generations these populations were assessed biologically to determine if there was any increase in virulence. Bulk DNA derived from these populations was examined using AFLPs to assess the effects of selection on inter-population variation. Additionally, from a subset of the material, DNA was derived from individual second stage juveniles and was investigated using microsatellites to examine the effects of selection on inter- and intra-population variation. Biologically, selection resulted in increased virulence, but the increase varied depending on the initial population and the clone used. Increases in virulence tended to be specific to the source of resistance. Molecular assessments showed an effect on the genetic constitution showing differences between the unselected and selected populations but showing no detectable association between polymorphic markers and virulence. The microsatellite data yielded allele frequencies that provided evidence that selection pressures had occurred in all four populations. Furthermore, a principal coordinate analysis derived from dissimilarity values based on microsatellite data separated the four populations into two host-specific groupings that were different from the original *G. pallida* parent population.

288 Expression profiling of the *Arabidopsis*-cyst nematode interaction

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Cyst nematodes infect and devastate many agricultural crops. After root penetration, cyst nematodes induce the formation of feeding cells (syncytia) within the host roots.

These syncytia are the sole source of nutrition for the nematodes. Syncytium formation is accompanied and likely mediated by nematode-induced changes in plant gene expression. With the availability of microarray technology, mRNA expression changes of thousands of genes can be monitored simultaneously in order to determine the mechanisms of biological processes-of-interest. We have used Affymetrix GeneChip technology to monitor *Arabidopsis* mRNA changes following infection by two different cyst nematode species. The sugar beet cyst nematode (*Heterodera schachtii*) readily infects *Arabidopsis* and induces syncytia. In contrast, the soybean cyst nematode (*H. glycines*) only rarely induces syncytium formation in *Arabidopsis* roots while it retains the pre-feeding behaviours of probing and penetration. Our analyses uncovered a large panel of *Arabidopsis* genes with altered mRNA levels following cyst nematode attack. Some of these changes were observed during infection with both cyst nematode species, suggesting a potential involvement in plant stress or defence functions. Other mRNA changes were specific for *H. schachtii* infection only and, therefore, are good candidates for being involved in syncytium formation or function.

289 ESTScreen: identify secretory proteins *in silico*

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The ever increasing gene sequences in databases offer unprecedented opportunity for biologists to exploit this new information source to find answers to various biological questions. The collection of a great number of EST (expressed sequence tags) is a useful starting place for identifying secretory proteins involved in nematode-plant interactions, either in the form of pathogenicity factors or avirulent proteins which are targeted by host defence mechanisms. SignalP is a robust program generally used to identify signal peptides for secretion from protein sequences, but it is not suitable for analysing a large number of EST directly. To this end, we have developed and validated a software tool, ESTScreen, which automates screening of thousands of EST either through the SignalP web server or a standalone

version. ESTScreen searches each cDNA sequence for a (user-defined) start codon, translates the sequence, submits automatically to SignalP and retrieves the output. ESTScreen then parses the SignalP prediction through a user-defined logical test and generates a FASTA file containing only EST encoding a signal peptide for secretion, which can be used in homology search and other subsequent analysis. This program can be a useful tool in the study of plant-pathogen interaction systems as well as other fields in which secreted molecules play important roles. The authors wish to thank Dr. S. Kamoun for communicating unpublished results.

290 Identification of parasitism genes from root-knot nematode *Meloidogyne incognita* using cDNA-AFLP, EST-analysis and GenEST

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The root-knot nematode *Meloidogyne incognita* is an obligatory plant parasite, which has evolved a complex feeding relationship with its host plants. Pharyngeal gland secretions play an important role in the parasitic life cycle of *Meloidogyne* spp. They are involved in migration through the root system and induction and maintenance of the giant cells. The cDNA-AFLP method is adopted to provide more insight in parasitism genes involved in the plant-nematode interaction. Gene expression profiles of various *M. incognita* stages differing in pharyngeal gland activity (*e.g.*, eggs, pre-parasitic and parasitic juveniles, adult females) are being compared in a cDNA-AFLP procedure based on the restriction enzymes *ApoI* and *TaqI*. Genes specifically expressed in the parasitic stages may represent candidate parasitism genes and will be isolated from gel. The DNA sequences of the isolated fragments will be compared with the *M. incognita* EST database using the computer program GenEST. Furthermore, the spatial expression pattern of the candidates will be analysed using whole mount *in situ* hybridisation.

291 Localisation of expression of *Le-Exp5* gene in feeding sites induced by *Globodera rostochiensis* in roots of susceptible tomato using *in situ* RT-PCR method

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The expression pattern of the tomato expansin gene *Le-Exp5* was examined on resin and agar embedded sections of syncytia induced by Ro1 pathotype of *G. rostochiensis* in roots of susceptible tomato cv. MoneyMaker. In 1 and 3 day old syncytia, strong expression of *Le-Exp5* was found in cells incorporated into syncytium and in neighbouring cells. In 7 day old syncytia, the older parts of syncytia contained relatively less *Le-Exp5* mRNA than cells situated at distal parts of syncytia that had been recently incorporated into it. Strong expression of *Le-Exp5* took place also in proliferating and dividing parenchymatous cells surrounding syncytia. These results were gained in experiments where *in situ* RT-PCR was followed by *in situ* hybridisation which confirmed the RT-PCR data.

292 Micropreparative sampling and molecular characterisation of proteins secreted by the plant-parasitic nematode *Heterodera schachtii*

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Sedentary plant-parasitic nematodes such as *Heterodera schachtii* have evolved a highly complex relationship with their host plant by redifferentiating root cells into specialised feeding sites. Proteins, produced in the pharyngeal glands of the nematode and injected into the plant cell, are thought to play a key role in this

sophisticated transformation. The key to understand this form of parasitism is the characterisation of these proteins. However, the small size of plant-parasitic nematodes makes direct analysis of their secretions extremely difficult. We present a method to isolate nematode secretions in sufficient quantities to allow direct analysis by combining two-dimensional gel electrophoresis and mass-spectrometry. So far, we have been able to identify several proteins (endoglucanases and pectate lyase) which are believed to be involved in the invasion of the plant tissue by the nematode. Other spots from the two-dimensional gels are under investigation. This knowledge will help us to elucidate one of the most fascinating forms of parasitic behaviour between two completely different organisms, namely a plant and an animal.

293 Cloning genes expressed in the pharyngeal gland cells of *Heterodera glycines* using an amplified RNA technique

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mRNA within the cytoplasm microaspirated from the pharyngeal gland cells of parasitic stages of *Heterodera glycines* was immobilised on paramagnetic beads for initial cDNA synthesis. T3 and T7 promoters were adapted to the gland cell cDNA and used to drive several rounds of *in vitro* transcription. The amplified RNA pool was used for cDNA synthesis and directional cloning into a plasmid vector for construction of a *H. glycines* gland-cell cDNA library without using PCR. Analysis of 90 cDNA clones chosen at random from the library produced 67 unique cDNA sequences. Among the 67 *H. glycines* cDNA sequences were homologues to a number of different eukaryotic genes, including genes identified in animal-parasitic nematodes and genes with potential roles in host-parasite interactions. The presence of a predicted secretion signal peptide and confirmation of expression in *H. glycines* pharyngeal gland cells are being investigated for these and additional clones being sequenced from the amplified RNA *H. glycines* gland-cell cDNA library.

294 SSH enriched cDNA libraries to compare virulent and avirulent isolates of the quarantined root-knot nematode *Meloidogyne chitwoodi*

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In north-western Europe, root-knot nematodes are a serious threat for potato production particularly with reduced use of nematicides. *Meloidogyne chitwoodi* causes significant economic losses due to reduction in tuber quality and yield of potato crops. Resistance to *M. chitwoodi* has been identified in the wild tuber-bearing *Solanum* species, *S. bulbocastanum*. Three pathotypes were identified suggesting at least two different genetic factors for virulence and resistance in

the pathogen and the host species respectively. This has important implications for the successful utilisation of resistance from *S. bulbocastanum*. Roots of *S. bulbocastanum* were infested with two isolates of *M. chitwoodi* varying in virulence. Plants were propagated in tissue culture and then transferred to soil before inoculating. The infection process was monitored by histological examination of roots allowing time points to be selected for RNA extraction. cDNA libraries were constructed from infected root tissue using the Suppressive Subtractive Hybridisation (SSH) method which enriches for rare transcripts. This enabled the construction of libraries, enriched in transcripts from the compatible and incompatible interactions, at 3 and 7 days post-infection. Both plant and nematode genes, which may be important during the host/parasite interaction, were identified.

295 Resistance to *Meloidogyne incognita* in coffee: identification of molecular markers F-AFLP in resistant genotypes

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This study is about a diagnostic system based on molecular markers that co-segregate within a gene of interest. The chosen technique was f-AFLP due to its great power of detection of genetic variability, being a dominant marker efficient for large and simultaneous sampling of a genome. A total of 16 primers combination ECO-RI (+3) and MSE-I (+3) were used to study polymorphism within a population of 40 plants F5 (H 47-82-7-925), donated by Instituto Agronomico de Campinas – IAC, segregating for the resistance to *Meloidogyne incognita* nematode in coffee tree. One hundred and twenty eight segregating loci were obtained with Mendelian inheritance by selecting only markers that segregated 1:1. These markers were submitted to a comparative analyses between the donor of the resistance factor of this progeny (*Coffea canephora* duplicated – C1330) and the plant group that is resistant and susceptible ('bulk' resistant and 'bulk' susceptible).

296 Nematode control by expression of lectins in transgenic plants

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With the advent of transgenic methodologies, an attractive method to control plant parasitic nematodes is the transfer into plant host species of genes encoding nematicidal proteins. Lectins or lectin-related proteins, for example, are believed to have toxic and/or repellent effects on nematodes. As a good host for migratory and sedentary nematodes and being considerably easier to transform than most plants, *Arabidopsis thaliana* can be used as an *in vitro* system to rapidly express transgenes and evaluate

their putative nematicidal effect. Three tropical nematode species (*Radopholus similis*, *Pratylenchus coffeae* and *Meloidogyne incognita*) could successfully penetrate and develop in *A. thaliana* under monoxenic conditions. Eight to 10 weeks after inoculation reproduction ratios of respectively 16.3, 11.9 and 24 could be observed. This *in vitro* system enables the preselection of genes or gene combinations showing the greatest potential for nematode control. Transgenic *Arabidopsis* plants, expressing lectins or lectin-related proteins in their roots, have already been screened. Results on nematode penetration and multiplication in these transgenic plants will be presented.

297 Marker-assisted selection in screening peanut (*Arachis hypogaea* L.) for resistance to the peanut root-knot nematode

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A restriction fragment length polymorphism (RFLP) marker linked to a locus for resistance to *Meloidogyne arenaria* race 1, along with visual evaluation following root staining were used to screen four breeding populations and three lines of peanut in a root-knot nematode infested field. Coan and Florunner cultivars were used as the resistant and susceptible parental controls, respectively. Genomic DNA was isolated from young leaves of these plants during the growing season, and Southern blot analysis was conducted using the RFLP probe R2430E. Only line T301-1-8 was homozygous for the resistance marker. At harvest, root systems were stained with Phloxine B, egg masses counted, and resistance phenotype scored. Field tests confirmed the RFLP marker results. Except for T301-1-8, all other genotypes displayed high levels of nematode reproduction. The RFLP probe R2430E loci linked to nematode resistance provided a useful selection method for identifying resistance to the peanut root-knot nematode.

298 Understanding the genetic and molecular basis of (a)virulence in the root-knot nematode *Meloidogyne incognita*

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Using two pairs of near-isogenic *M. incognita* lines, avirulent and virulent against the tomato *Mi* resistance gene, a cDNA-AFLP differential analysis was initiated, in order to identify genes differentially expressed between avirulent and virulent nematodes. AFLP markers were developed on cDNA templates digested with *Hind*III and *Mse*I, with all of the 256 combinations that could be generated with primers with two selective nucleotides. A total of approximately 84 000 fragments were amplified, among which 61 appeared differential (*i.e.*, present in the two avirulent lines and absent in the two virulent lines). In order to confirm this result, cDNA-AFLP was run again on the same templates, but with all of the 64 combinations that could be generated with primers with two selective nucleotides for *Mse*I and one selective nucleotide for *Hind*III. This allowed us to eliminate a number of false positive bands, and 19 fragments differential between avirulent and virulent lines were thus selected for further analysis. The next experimental steps included cloning of the full-length genes, RT-PCR experiments, *etc.* The results will be discussed in relation to the ability of the nematode to overcome or not the plant resistance gene.

299 Characterising sources of resistance to *Globodera pallida* and *G. rostochiensis* identified in the Commonwealth potato collection

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Previously untested material from Professor JG Hawkes' collection of wild species of potatoes now incorporated into the CPC was screened against the potato cyst nematodes. A total of 56 and 53% of accessions were identified as having resistance to *G. pallida* and *G. rostochiensis*, respectively, from 198 accessions. Molecular analysis of the resistant accessions identified was conducted to observe the genetic relatedness and diversity of the material, using the AFLP DNA fingerprinting technique. A

total of four primer combinations were applied, yielding 275 identifiable marker bands. Analysis of the identified marker bands was then completed using a hierarchical cluster analysis and a dendrogram produced. The dendrogram was then used to identify 16 genetically diverse species for further analysis. These species have been tested against a range of *G. pallida* populations. Histological analysis of the resistance mechanisms involved has also been conducted, focusing on the effect of the wild species on hatch, invasion, development in the root and production of eggs per cyst.

300 Host-plant response of some *Musa* cultivars from Brazil to different species of root-knot nematodes (*Meloidogyne* spp.)

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Five *Musa* cultivars commonly grown in Brazil, (triploid AAA-group, cvs Nanicão and Caipira; triploid AAB-group, cvs Prata and Terra; tetraploid AAAB-group, cv. Pioneira) were evaluated for their susceptibility to three different root-knot species (*Meloidogyne javanica*, *M. incognita*, *M. arenaria*) alone and in combinations under glasshouse conditions. Four months after nematode inoculation, plant growth parameters (*e.g.*, fresh and dry weights, number of leaves and foliar area, macro- and micro-element concentrations), final nematode populations, reproductive factor and interspecific competition were analysed. All *Musa* cultivars were found to be hosts for the three *Meloidogyne* species. The highest nematode multiplication was observed with *M. incognita* on cv. Prata. The cv. Caipira seemed the most susceptible to *Meloidogyne* infestation in terms of plant growth parameters when inoculated with a mixture of *M. javanica* and *M. incognita*. The calcium content of *Musa* cultivars is the only macroelement which increased systematically when infested with root-knot nematodes (*e.g.*, significant increase with cvs Prata, Caipira and Terra). Based on their multiplication rates and on the *Musa* cultivar, some competition between root-knot nematode species was observed.

301 Evaluation of beans and cowpea genotypes for resistance to *Meloidogyne javanica*

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Meloidogyne javanica is a major nematode pathogen in soybean in Brazil. In many regions beans and cowpeas are used in rotation with soybeans, increasing the problem. The use of nematode resistant cultivars in rotations with soybean can help reduce the damage. The reproduction of *Meloidogyne javanica* was studied on 21 bean and seven cowpea genotypes. The plants were grown on plastic tubes in glasshouse conditions, inoculated with 3000 nematode eggs and evaluated for egg production after 42 days. Tomato plants were used as inoculum efficiency check. The same genotypes were grown in a *M. javanica*-infested field and were evaluated for galling index. Despite high tolerance in field conditions, all cowpea genotypes permitted abundant nematode reproduction. The bean genotypes Aporé and Pot-51 were resistant whereas the majority was susceptible.

302 Resistance to *Heterodera ciceri*, *Meloidogyne artiellia* and *Pratylenchus thornei* in wild species of chickpea

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Chickpea (*Cicer arietinum*) is an important food legume throughout the world. *Heterodera ciceri*, *Meloidogyne artiellia* and *Pratylenchus thornei* are nematodes known to affect the crop. Chickpea germplasm evaluation has revealed the absence of resistance to these nematodes. Therefore, different lines belonging to eight wild *Cicer* species were screened for their reaction to a Syrian population of *H. ciceri* and *P. thornei*, and to an Italian population of *M. artiellia* in a glasshouse. Out of 207, 154 and 249 lines of *Cicer* spp. tested for their reaction against *H. ciceri*, *M. artiellia* and *P. thornei*, respectively, 17 of *C. bijugum*, six of *C. pinnatifidum*, and five of

C. reticulatum were resistant to *H. ciceri*. One line each of *C. bijugum*, *C. chorassanicum* and *C. judaicum*, and two each of *C. pinnatifidum* and *C. reticulatum* were resistant to *M. artiellia*. Six lines of *C. bijugum*, four of *C. cuneatum*, 16 of *C. judaicum* and one of *C. yamashitae* were resistant to *P. thornei*. Because *C. reticulatum* is crossable with *C. arietinum*, these findings indicate the possibility of introgression of resistance to *H. ciceri* and *M. artiellia* into the cultigen.

303 Characterisation and high-resolution genetic mapping of root-knot nematode resistance genes in pepper (*Capsicum annuum* L.): comparison with the tomato and potato nematode resistance gene locations

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In pepper, researches on genetic basis of root-knot nematode resistance were conducted on three genetically distant accessions: PM687, PM217 and CM334. They revealed three dominant and thermostable loci with broad-spectrum of resistance: *Me3*, *Me1* and *Me7*, and two other loci: *Mech1* (PM217) and *Mech2* (CM334) controlling the quarantine nematode *M. chitwoodi*. Comparative histological studies showed that the three broad-spectrum resistance genes suppressed nematode reproduction, but induced very different response patterns in root cells upon nematode infection. Bulked-segregant analysis was performed to identify AFLP markers linked to the genes. Fine mapping of *Me3* gave AFLP less than 0.5 cM flanking the gene. Indirect selection markers (SCAR or CAPS) were identified. *Me3* locus was localised on chromosome P9 on intraspecific pepper maps, in a syntenic region of two other nematode resistance genes, the tomato *Mi-3* and the potato *Gpa2* genes, which mapped on the short arm of the tomato and potato chromosome 12. We also identified new AFLP markers linked in coupling to *M. chitwoodi* resistance genes. The nearest were located less than 1.7 cM from *Mech1* (no recombinant individual for two markers), and at 1 cM from *Mech2* (one recombinant individual for one marker).

304 Comparison of two screening methods for early evaluation of resistance to *Radopholus similis* in *Musa* germplasm

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Plant-parasitic nematodes are one of the most important constraints to sustainable *Musa* production. In the lowlands of Central Uganda, the most common nematode species is *Radopholus similis*, while at higher elevations *Pratylenchus goodeyi* is more prevalent. The use of host plant resistance provides promising prospects towards sustainable nematode management through conventional breeding. To identify resistance, reliable screening methods need to be developed. In this study two screening methods for resistance to nematodes are compared. The first screening method is based on the inoculation of individual roots placed in a cup. The second screening method is based on the classic pot experiment, whereby the complete root system is inoculated. The newly developed cup method requires less nematode inoculum and less plant material per cultivar compared to the pot experiment. By using single roots for inoculation, the host response to nematode attack is not influenced by differences in root growth rates between *Musa* genotypes. Hybrids developed within the banana breeding programme of the International Institute of Tropical Agriculture are being evaluated for resistance against *Radopholus similis* using both methods. Nematode reproduction ratio is compared against a known resistant check (Yangambi km 5) and a known susceptible check (Valery). The results of both screening methods will be discussed.

305 Proteomic analysis of soybean cyst nematode (SCN) inoculated soybean roots

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Proteins and small molecules within a soybean root at any given time depend on genes expressed up to that time and are influenced by the genotype and the environment. Earlier, we demonstrated reproducibility of an extraction and chromatographic protocol used to compare profiles from roots exposed to either biotic or abiotic stresses. We have challenged cvs Williams 82 and Hartwig, and our SCN resistant germplasm PUSCN14 with *Heterodera glycines* and analysed the fingerprints at different time intervals following inoculation. Gross comparison of the chromatographs showed considerable similarity between the inoculated and non-inoculated samples of the same cultivar. However, analyses of peak areas (four replications) showed statistically significant ($P = 0.05$) alterations of specific peak areas due to stress. All three cultivars showed a transient increase in the peak 11 area 2 days after inoculation but returned to non-stressed levels at 4 days after inoculation. Interestingly, peak 5 showed an increase in levels 4 days after inoculation. Selected peaks were collected and analysed by mass spectrometry.

306 Chitin synthase as molecular target in the plant-parasitic nematode *Meloidogyne artiellia*

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Plant-parasitic nematodes cause serious damage to agricultural crops. Molecular biology studies are focusing on plant-parasite interactions with the aim of finding the weak point that will permit nematode control. In spite of this, several chemical compounds are still used as nematicides that are specific inhibitors of the synthesis of chitin which is present in the eggs of nematodes. Data in the literature indicate successful applications of chemical compounds suggesting that these may affect nematode-egg formation by interfering with chitin deposition in the nematode egg-shell. The prevention of chitin synthesis may be triggered by an inhibition of the proteolytic activation of chitin synthase zymogen or can be due to a direct effect on the chitin synthase. In this presentation, we show that the eggs of *M. artiellia* do contain chitin and

have chitin synthetic activity. In our laboratory we have isolated in the eggs of *M. artiellia* two different portions of chitin synthase cDNA by RT-PCR. One of the cDNA contains the motif QRRRW, which is demonstrated to be conserved in chitin synthase of different organisms. To prove that the encoded protein shows chitin synthase activity, we will express both clones and their enzymatic activity will be tested.

307 Evaluation of soybean genotypes for host suitability to *Meloidogyne incognita* race 2: an *in vivo* study

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M. incognita race 2 is predominant on soybean in South Africa. Sixty-nine soybean hybrids were evaluated for host suitability to this species in the glasshouse. Each seedling was inoculated with 10 000 eggs and larvae. Assessments were made 56 days after inoculation and RF-values were calculated. Five soybean genotypes S5995, Potties, PAN660, TXS89/10/29 and D82-3298, identified as poor hosts in glasshouse screenings, were evaluated under natural conditions in microplots and in the field to verify their resistance. Prima 2000 was included in both trials as a susceptible standard. In the microplot trial each seed was inoculated with 10 000 eggs and larvae at planting while a uniform and natural distribution of *M. incognita* race 2 occurred in the field trial. *Meloidogyne incognita* race 2 eggs and larvae were extracted and counted 90 days after planting. S5995 maintained the lowest numbers of *M. incognita* race 2 in the above-mentioned trials, followed by PAN660, TXS89/10/29, D82-3298 and Potties, and differed significantly from the susceptible control (Prima 2000). Crosses between these poor-host cultivars and Prima 2000 are underway to develop a mapping population for identification of genetic markers associated with this resistance trait.

308 SSR markers associated with resistance to *Meloidogyne javanica* in soybean

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Meloidogyne javanica is a major nematode pathogen in soybean in Brazil. The development of resistant varieties is the main goal of many breeding programmes. Progress in selection for nematode resistant lines can be speeded up by the use of molecular markers associated with genes for resistance. The objectives of this study were to use SSR markers to identify quantitative trait loci (QTL) conditioning soybean resistance against *M. javanica* and to determine their genomic location. Eighty-eight SSR markers were used to amplify the DNA of soybean lines (25 resistant and 26 susceptible) obtained from a cross between BRS 133 (susceptible) and PI 595099 (resistant), and selected in previous studies by galling number assayed in glasshouse and infested field. HSP 176, Satt 114 and Satt 423 showed significant association with resistance. The QTL analysis on the linkage group indicated the presence of at least one gene on HSP 176 locus (Lod 27.5). In all gene extensions between Satt 114 and HSP 176 (8.3 cM), the Lods were high (22.6-27.5).

309 Ultrastructure of resistant responses of selected *Solanum bulbocastanum* lines to infection with *Meloidogyne chitwoodi*

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Different genotypes of *S. bulbocastanum* varying in resistance to different isolates of *M. chitwoodi* were examined by electron microscopy. In moderately resistant combinations feeding sites were composed of two to three strongly enlarged giant cells derived from procambium. They contained condensed cytoplasm, small cytoplasmic vacuoles, enlarged nucleus with dispersed heterochromatin, electron translucent nucleoplasm and hypertrophied nucleolus. In resistant combinations two different plant responses were found. If giant cells were induced among procambial cells their cytoplasm was strongly condensed. The central vacuole was substituted by small cytoplasmic vacuoles. The nucleus was enlarged and amoeboid and the nucleolus contained many

nucleolar 'vacuoles'. If giant cells were induced among cortical or pericyclic cells they contained only paramural layer of strongly osmiophilic cytoplasm. The central vacuole was present and filled with fibrillar material. Neighbouring cells contained large starch grains. Many surrounding cells necrotised or divided hyperplastically. In absolutely resistant combinations juveniles induced slight enlargement of selected vascular cylinder cells. Their cytoplasm was located paramurally. Their central vacuoles were filled with osmiophilic granules. Later on, giant cells degenerated and the juveniles were embedded in a layer of degraded cells. Some cortical cells were enlarged and contained many large starch grains.

310 Effective and durable resistance against plant-parasitic nematodes

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The success of transgenic approaches to plant-parasitic nematode resistance will require an effective and durable defence. This work aims to define new targets that can be disrupted with a deleterious consequence on the animal. Two candidate genes have been selected as putative targets. The first is a gene encoding an aspartic proteinase isolated from an *H. glycines* library using a PCR fragment produced by degenerate primers. The second is an aminopeptidase, also isolated from an *Heterodera glycines* cDNA library by heterologous probing with *C. elegans* EST. Recombinant proteins corresponding to both genes have been expressed in order to conduct inhibitor assays. Plant derived aspartic proteinase inhibitors have been shown to be effective *in vitro* and have subsequently been transformed into *Arabidopsis thaliana* under the control of CaMV35s promoter. Infecting the transformed plants with *H. schachtii* will assess the potential of these inhibitors as a defence strategy. There are no inhibitors of aminopeptidases described from a plant source, therefore synthetic peptide and antibody phage display libraries are being screened to isolate a functional inhibitor. *In situ* hybridisation experiments indicate that the aspartic proteinase gene is expressed intestinally whereas the aminopeptidase gene is expressed in the reproductive system.

311 Tolerance of sugar beet to *Heterodera schachtii*

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The crop potential to produce high yields even under severe nematode pressure is an important economic trait in integrated control strategies. Plant tolerance is especially important for beet and tuber crops where nematode damage at the early stages can delay or destroy beet/tuber formation. Breeding of tolerant cultivars is currently limited by our poor understanding regarding functioning and inheritance of tolerance. In field studies and glasshouse experiments, a broad spectrum of sugar beet cultivars/hybrids was studied for tolerance of *Heterodera schachtii*. Three cultivars/hybrids were selected for further studies on the mechanisms of tolerance: Nematop (tolerant/resistant), Stru.1915 (tolerant, susceptible) and Penta (intolerant/susceptible). With increasing nematode pressure, yield decrease in the tolerant cv. Nematop and tolerant hybrid Stru.1915 was significantly less than in the intolerant cv. Penta. Tolerant plants suffered later from water stress and the rate of photosynthesis was generally higher. Furthermore, tolerant plants showed higher compensatory growth, and increased rooting depth. Tolerance was most pronounced under dry summer conditions and less obvious under humid conditions. The concept of using plant tolerance for managing plant parasitic nematodes will be discussed.

312 Growing resistant sugar beets; a new opportunity in fields infected with *Heterodera schachtii*

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In sugar beet rotations, *Heterodera schachtii* is now kept below the economic threshold by growing resistant varieties. Resistant oil radish, or mustard, grown in autumn is not as effective as in spring. The density dependent reduction after oil radish is 0.36 Pf/Pi compared with 0.64 Pf/Pi after fallow. Even though the reduction is quite effective, the high multiplication rate of *H. schachtii* on sugar beet overcomes the effect on the

Pi. These problems are now overcome by resistant and tolerant sugar beet varieties. With a *Pi* between 10 and 30 eggs + juveniles/g soil, the *Pf/Pi* lies between 0.9 and 0.7 while the yield is 5-20% higher with a tolerant variety than compared to a susceptible one in an infested field. On the other hand, resistant varieties earn 10% less compared with susceptible ones on nematode-free land. As few individuals of *H. schachtii* can overcome the resistance in the sugar beet, but not that of oil radish or mustard, a combination of methods will be discussed to overcome these disadvantages.

313 Durable resistance against *Meloidogyne chitwoodi* and *M. fallax*: a dream?

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Final goal of EU-project DREAM (No. QLRT-1999-1462: Durable resistance management of the soil-borne quarantine nematode pests *Meloidogyne chitwoodi* and *M. fallax*) is to deliver crops (potato as well as green manure crops) which are resistant against the root-knot nematodes *M. chitwoodi* and *M. fallax*. This poster presents the first results of a comparison of 15 different genotypes of Italian ryegrass, fodder radish and potato. These 15 genotypes were previously selected with colleagues from Plant Research International, Barenbrug Holding BV and P.H. Petersen Saatzucht. The host suitability of the selected genotypes was determined by extracting nematodes from soil before planting (May 2001) and after the growing season (November 2001). First results on *M. chitwoodi* showed that on average the genotypes of fodder radish and potato proved to be more resistant compared to all tested genotypes of Italian ryegrass. However, none of the tested genotypes reduced the populations to zero, which was found after fallow. *Meloidogyne fallax* was able to reproduce much more on most genotypes, although three potato genotypes had a very low multiplication of *M. fallax*.

314 Influence of potato cultivars with different degrees of resistance on hatching and multiplication of the potato cyst nematode *Globodera pallida*

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Hatching of eggs of five populations of *Globodera pallida* in root diffusate of six potato cultivars was studied *in vitro*. All cultivars induced hatching, regardless of their degree of resistance to *G. pallida*. The percentage of hatched eggs after 7 weeks varied between populations and cultivars, but none of the cultivars consistently induced most or least hatch for all populations. Addition of freshly collected root diffusate (not stored) after 7 weeks of experiment resulted in a new flush of hatching and made differences between induction of hatching by the cultivars even more obscure. Results on the hatching of invasive juveniles are useful for estimating of the multiplication factor of nematode populations. Multiplication of all populations was highest on the susceptible cv. Bintje and lowest on the completely resistant cv. Innovator, but varied with population for partially resistant cvs Sante, Maritiema and Cycloon. Multiplication factors were higher and less variable in closed containers than in pots in the glasshouse, which facilitated discrimination between the cultivars. Test in closed containers could be used to advise farmers which cultivar to grow in *G. pallida*-infected fields. However, correlation between multiplication in closed containers and in the field should be investigated.

315 Comparative study of two *Heterodera avenae* resistance genes from *Aegilops ventricosa*: differences in defence-enzymes induction and chromosomal location in wheat/*Ae. ventricosa* introgression lines

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Two independent introductions of genetic resistance to *Heterodera avenae* (Ha71 Spanish pathotype), from *Aegilops ventricosa* to hexaploid wheat were compared – the *Cre2* (from *Ae. ventricosa* AP-1) and *Cre5*

(from *Ae. ventricosa* #10) genes. No susceptible plants were found in the F₂ progeny from the cross between both accessions of *Ae. ventricosa* suggesting that their respective resistance factors could be allelic. However, the genes *Cre2* in H-93-8, and *Cre5* in substitution 6D/6N^V and addition 6N^V lines have been transferred to different chromosome location. The induction of several defence responses during early incompatible interaction of resistant lines carrying *Cre2* and *Cre5* genes has also been studied. Isoelectrofocusing isozyme analysis revealed changes in peroxidase, esterase, superoxide dismutase, glutathionereductase, shikimat dehydrogenase and ascorbate peroxidase activities in infected roots of resistant lines in comparison to their susceptible parents. The highest differential activity between infected and uninfected roots was found for the peroxidase system, implicated in lignification process. A DNA marker, lacking in 6N^V line, was linked to *Cre2* gene in H-93-8. Differences observed between *Cre2* and *Cre5* genes with respect to the chromosomal location, detoxificant enzymes induction, and behaviour against different pathotypes, suggests that they are different CCN resistance sources for wheat.

316 Comparative histology of feeding sites of *Meloidogyne artiellia* and *M. javanica*

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The root-knot nematode *Meloidogyne artiellia* causes considerable damage to several agricultural crops in the Mediterranean region. Microscopic examination of longitudinal root sections from two cruciferous plants, *Brassica oleacea* L. (cabbage) and *B. rapa* L. (turnip), infected with *M. artiellia*, revealed that the feeding sites consist of several large nurse cells in the central vascular tissues, which includes the companion cells. The intracellular organisation of these cells closely resembled the syncytial forms. The feeding zone cells showed hypertrophy and hyperplasia and intense vacuolisation. Also, different degrees of cell wall dissolution between contiguous cells were observed and only few cell nuclei and nucleoli were observed, all significantly enlarged and amoeboid, as compared to those in the feeding cells induced by *Meloidogyne javanica*. The feeding sites formed by *M. artiellia* differed considerably from

the multinucleated giant cells (coenocyte) formed by *M. javanica* infection on these plants. The formation of a syncytium-like feeding site by root-knot nematodes is a highly unusual phenomenon.

317 Engineering coffee for root-knot nematode resistance using cysteine and serine proteinase inhibitors

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In Hawaii, the Kona coffee root-knot nematode, *Meloidogyne konaensis*, causes severe damage to *Coffea arabica* cv. Typica. Due to the lack of resistant cultivars of *C. arabica*, genetic engineering for nematode control was explored. The modified rice cystatin gene, Ocl-ÄD86, and the dual proteinase inhibitor gene, Ocl-ÄD86/GO/CpTI, were used to produce transgenic coffee for resistance to *M. konaensis*. Leaf discs were transformed using *Agrobacterium tumefaciens* containing the cystatin constructs, then placed on G418 selection medium. The 38% that formed primary callus was presumed to be transformed. Somatic embryos have not yet been obtained from this callus because embryogenesis in coffee takes 7-9 months. In a second trial, somatic embryos were obtained from untransformed leaf discs. *A. tumefaciens* was used to transform the embryos with the two gene constructs. Secondary embryos were produced in 37% of the initial experiments under G418 selection. In addition, embryogenic calli or somatic embryos were also transformed using particle gun bombardment. Within 6 weeks, secondary embryos were produced from the bombarded embryos, while embryogenic calli only produced more calli. These results indicate that somatic embryos are the most efficient target tissue in the production of transgenic coffee.

318 Differential gene expression between avirulent and virulent *Meloidogyne incognita* isogenic lines as monitored by cDNA-AFLP

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Using two pairs of near-isogenic *M. incognita* lines, avirulent and virulent against the tomato *Mi* resistance gene, a cDNA-AFLP differential analysis was initiated, in order to identify genes differentially expressed between avirulent and virulent nematodes. AFLP markers were developed on cDNA templates digested with *HindIII* and *MseI*, with all of the 256 combinations that could be generated with primers with two selective nucleotides. A total of approximately 84 000 fragments were amplified, among which 61 appeared differential (*i.e.*, present in the two avirulent lines and absent in the two virulent lines). In order to confirm this result, cDNA-AFLP was run again on the same templates, but with all of the 64 combinations that could be generated with primers with two selective nucleotides for *MseI* and one selective nucleotide for *HindIII*. This allowed elimination of a number of false positive bands, and 19 fragments differential between avirulent and virulent lines were thus selected for further analysis. The next experimental steps included cloning of the full-length genes, RT-PCR experiments, *etc.* The results will be discussed in relation with the ability of the nematode to overcome or not the plant resistance gene.

319 Differential gene expression in nematode resistant soybean genotypes

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Meloidogyne javanica causes significant losses to soybean production in Brazil. Understanding how resistant plants respond to nematode infection at molecular level might indicate new strategies for the development of resistant genotypes. Roots of soybean genotypes, BRS-133 (susceptible) and PI595099 (resistant), were analysed for differential gene expression during nematode infection. At 5 days after germination, both genotypes were infected with nematode eggs and juveniles. Root samples were collected at 9 and 12 days. Total RNA was extracted from

the roots and the Differential Display (DD) technique was used to identify and isolate genes that differ between the genotypes during nematode infection. Differentially expressed genes were identified, isolated and cloned into pGEM-T vectors for sequencing. Differentially expressed gene sequences were used to search for homologies. Preliminary analysis identified homology with a nucleotide binding protein, a pathogenesis related protein, a low temperature and salt responsive protein, an aquaporin protein, and transcription factors.

320 Evaluation of maize genotypes for resistance against *Meloidogyne javanica* and *M. incognita* race 3

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The use of nematode resistant crops in rotations prevents losses in susceptible crops. In Brazil corn is the main crop used in rotation with soybean. The reproduction of *Meloidogyne javanica* and *M. incognita* race 3 was studied in 176 and 57 maize genotypes, respectively. The plants were grown in glasshouse conditions, inoculated with 5000 nematode eggs and evaluated after 60 days. Tomato plants were used as an inoculum efficiency check. The egg production of *M. incognita* was higher than *M. javanica* in all maize genotypes. *Meloidogyne javanica* presented low reproduction in 78 genotypes. Only the genotypes BRS 2114, P 30F80 and AG9090 were resistant to *M. incognita*, resulting in low egg production.

321 Host suitability in soybean cultivars for the reniform nematode, 2001 tests

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In 2001 glasshouse pot experiments, 139 soybean varieties from the Arkansas and Mississippi variety testing programs (119) and varieties submitted by Louisiana extension nematologists (20) and 34 breeding lines from Clemson University were tested to determine their suitability as hosts for the reniform nematode, *Rotylenchulus reniformis*. The *R. reniformis*-resistant varieties Forrest and Hartwig, the susceptible variety Braxton, and fallow-*R. reniformis*-infested soil served as controls. Total number of eggs and nematodes extracted from both the soil and roots from each pot, reproductive indices ($RI = Pf/Pi$), RI/RI of Forrest (RF), log ratio ($\log_{10}(RF + 1)$), and RF calculated from $\log_{10}(RF + 1)$ were calculated for each cultivar or breeding line in each test. Varieties with RF significantly greater than the RF on Forrest (1.00) were considered suitable hosts for *R. reniformis*. In the states variety test, 90 of the 119 varieties were suitable hosts when RF were compared and 117 when the log ratio ($\log_{10}(RF + 1)$) were compared. In the Clemson test, 23 of the 34 varieties and breeding lines were suitable hosts when RF were compared, and 24 when the log ratio ($\log_{10}(RF + 1)$) were compared.

322 Characterisation of resistance to root-knot nematodes in carrot

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Host plant resistance to the root-knot nematodes *Meloidogyne javanica* and *M. incognita* conferred by the *Mj-1* locus is being incorporated into advanced breeding lines of carrot (*Daucus carota*). Genetic analysis of F2-F4 and BC families determined that the resistance is inherited as a single dominant gene or possibly two closely linked genes. The *Mj-1* locus is derived from the variety Brasília, and it expresses very high levels of resistance to *M. javanica* and almost as high resistance to *M. incognita*. To improve the selection efficiency for the *Mj-1* locus in breeding programs, closely linked STS codominant flanking markers have been developed. Application of these

markers helped to confirm phenotypic separation of homozygous and heterozygous resistant plants. Heterozygous plants had similar levels of resistance to root-galling symptoms, but supported higher nematode reproduction compared with homozygous plants. This gene dosage effect was more apparent against *M. incognita* than *M. javanica*, and at temperatures of 28°C or higher, when *Mj-1* expression was weaker. Effective resistance was shown in field experiments when used alone and in combination with other cultural tactics, including use of rotation crops with resistance and manipulation of carrot planting date to minimise infection.

323 New sources of resistance to *Rotylenchulus reniformis* in cotton, *Gossypium* spp.

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Rotylenchulus reniformis causes yield losses to cotton (*Gossypium hirsutum*) in Mississippi, Louisiana and Alabama in the USA. Crop rotation and nematicides are marginally economic and resistance is needed but only weak resistance is reported in *G. hirsutum*, and all previous studies represent a small percentage of accessions available within *Gossypium* species. We evaluated ca 3000 primitive accessions of *G. arboreum*, *G. herbaceum*, *G. longicalyx*, *G. hirsutum* and *G. barbadense* from the US National Cotton Collection and confirmed resistance to *R. reniformis* in the diploid species *G. arboreum*, *G. herbaceum* and *G. longicalyx*. No resistance was found in more than 1500 accessions of *G. hirsutum*. Several of more than 1000 accessions of the compatible tetraploid *G. barbadense* were resistant; others were comparable to susceptible controls; none were immune. Resistant *G. barbadense* were collected in widely scattered regions: Peru, Guatemala, Mexico and St Lucia. The *G. barbadense* accession GB-713 had the highest resistance, followed by a second group (GB-49, GB-264, TX-110), and a third group that was weakly but consistently resistant (TX-1347, TX-1348). Differences in origin and resistance level suggest further enhancement of resistance may be gained through new combinations of resistance genes.

324 Effects of temperature and soybean genotype on *Heterodera glycines* males and females population

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Soybean cyst nematode resistant 'Bell' and susceptible 'S 20-20' soybean cultivars, were pre-germinated and transplanted to *Heterodera glycines* infested soil, and exposed to temperatures of 20, 25, 30 or 35°C for 10 days in growth chambers. Males were counted from plants growing hydroponically for 10 additional days and females were counted from plants growing in autoclaved sand for 30 additional days. The male:female ratios were always higher than one and higher on the resistant cv. Bell. Infection by males and females was affected by temperature and was highest between 26 and 28°C. Total male population was not affected by soybean genotype. More abnormal males (<800 µm) developed on roots of the resistant cv. Bell and shorter males developed following exposure at 35°C. The female population was significantly higher on the susceptible cv. S 20-20. At 35°C only a few females developed on both cultivars, which led to a higher male:female ratio. The number of eggs/female was higher on susceptible S 20-20 than on resistant Bell and did not differ significantly with different temperatures.

325 Response of pepper stocks to *Meloidogyne incognita* in glasshouses in the southeast of Spain

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Commercial pepper varieties resistant to *Meloidogyne incognita* did not match the standard production of glasshouses in the southeast of Spain. When methyl bromide (MB) is not used for soil disinfection, nematodes become one of the main soil-borne pathogens. The response of 75 resistant pepper stocks was tested against *Phytophthora capsici* and *M. incognita* in relation to non-

resistant varieties grown on MB disinfected soils. More than 30% of the stocks assayed had average root-knot index similar to those of MB treatments. More than 30% of the stocks had also marketable yields (8.3 kg/m² C-58 hybrid; 8.0 kg/m² C-57 and 8.2 kg/m² C-30) similar to those of MB treatments (8.9 kg/m²). Stocks grown on the same soil over 3 years led to an increase of aggressiveness in *M. incognita* populations. This was noticed by an increase in average root-knot index and the percentage of infested plants (*i.e.*, in the 1st year the C-30 hybrid had 0.1% of infested plants and 0.1 of root-knot index, while in the 2nd year 73.3% of the plants were infested and the root-knot index increased to 4.1).

326 Identification of markers to facilitate cloning of resistance genes to *Heterodera trifolii* in clover

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White clover (*Trifolium repens*) is a major legume component of dairy pastures throughout Australia. However these plants are susceptible to a variety of root endoparasitic nematodes, the most common and widespread being *Heterodera trifolii*. Nematodes can severely affect productivity and incur substantial losses to the dairy industry. There is little resistance to *H. trifolii* in white clover, although we have identified resistance in caucasian clover, *Trifolium ambiguum*. Conserved sequences of previously characterised resistance genes and their analogs are being used to identify candidate resistance genes in *T. ambiguum*. Partial sequences of resistance gene analogs (RGAs) have been isolated from legumes using DNA sequence databases and through a PCR approach with white clover. Over 200 RGA sequences from legumes have been analysed phylogenetically and 39 sequences selected to represent distinct clusters. These individual sequences were used as probes in a bulk segregant analysis of *T. ambiguum* plants that are either resistant or susceptible to *H. trifolii*. Probes that reveal polymorphism and where subsequent analysis of individuals shows a tight linkage to the resistance phenotype will be targeted. Diagnostic probes provide an entry point to screen a resistant *T. ambiguum* genomic library to identify candidate resistance genes for use in transformation experiments.

327 Identification of resistance to the oat race of stem nematode (*Ditylenchus dipsaci*) in faba bean (*Vicia faba*) in Australia

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The oat race of stem nematode (*Ditylenchus dipsaci*) was first recorded in South Australia in 1973. The principal hosts in this region are oats and faba beans and the only economic method for control is the use of resistant crops/varieties within rotations. At the commencement of research in 1994, high levels of resistance and tolerance were identified in oat breeding material and screening has contributed to the release of five resistant and tolerant oat varieties. In contrast, of the 120 advanced faba bean lines tested, only low to moderate levels of resistance were found. Variation was observed within the commercial cultivars Fiord and Ascot, however, and crosses from plants showing superior resistance were made. Lines from progeny of crosses from Ascot produced accessions with moderate to high levels of resistance and from Fiord with moderate resistance following recurrent selection from 1994 to 2000. These selections represent a significant improvement on existing material and will be used as parents to develop varieties with superior resistance for the South Australian Faba Bean Breeding Program.

328 Reaction of banana clones (*Musa* spp.) to *Meloidogyne incognita* race 3

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The aim of this work was to study under glasshouse conditions the reaction of banana to *Meloidogyne incognita* race 3. These clones were developed by a Breeding Program of Embrapa Cassava and Tropical Fruits Center and Agriculture Promotion Company. Micropropagated offspring were used and 5 days after transplant to containers filled with sterilised soil + sand

+ manure, they were inoculated with 10 000 eggs. The experiment design was completely randomised with four replicates. After 120 days, the clones were evaluated by the egg number and presence of nematodes in root, using Nanicão 122 clone as the susceptibility standard, at two levels of watering. Soil samples were collected to determine the nematode number per 200 cm³ per replicate for each clone. The Nanicão clones (90 and 122) allowed a large multiplication of *M. incognita*, compared with the other eight clones, showing susceptible reaction, but Maçã 57 showed the lowest parasite multiplication. All the other clones showed some nematode resistance, depending on the water level, and it can be seen in the results of Nanicão clones. For the root weight and aerial parts, the results showed little difference, compared with inoculated and non-inoculated plants, showing some difference between two levels of watering.

329 Resistance of watermelon (*Citrullus* spp.) germplasm to the peanut root-knot nematode (*Meloidogyne arenaria* race 1)

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Root-knot nematodes (*Meloidogyne arenaria*, *M. incognita* and *M. javanica*) are serious pests of watermelon (*Citrullus lanatus*) in the southern US and world-wide. Currently, root-knot nematodes (RKN) are controlled in watermelon by pre-plant soil fumigation with methyl bromide or other nematicides. The proposed removal of methyl bromide from the US market in 2005 has focused interest in developing alternatives for managing nematodes in vegetable crops. Resistant cultivars would provide an inexpensive, environmentally compatible alternative for managing RKN in watermelon. All accessions of *C. colocynthis* (21) and *C. lanatus* var. *citroides* (88), and approximately 10% of *C. lanatus* var. *lanatus* (157) accessions from the USDA *Citrullus* germplasm collection were evaluated for resistance to *M. arenaria* race 1 in greenhouse tests. The *C. lanatus* var. *lanatus* and *C. colocynthis* accessions were susceptible with root gall severity indices (GI) of 4.0-9.0 (scale, 1.0-9.0) and 8.0-9.0, respectively. However, 21 of 88 *C. lanatus* var. *citroides* accessions were moderately resistant (GI, 3.0-9.0). Significant genetic variability exists within the USDA *Citrullus* germplasm collection for reaction to *M. arenaria* race 1

and the *C. lanatus* var. *citroides* accessions are potential sources of resistance to *M. arenaria* race 1.

330 Multiple selection and durability of potato cyst nematode *Globodera pallida* virulence on a range of potato species

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A series of selection experiments on potato cyst nematode *Globodera pallida*, pathotype Pa1 tested the virulence response of the nematode to a range of resistant potato *Solanum* genotypes. Alleles conferring virulence against all four *Solanum* sources used in the study, i.e., *Solanum vernei*, *S. multidissectum*, *S. sanctae-rosae* and *S. tuberosum* spp. *andigena* were detected. Selection for multiple virulence against a combination of resistant sources resulted in the originally selected virulence genes being retained or lost. The mechanism of potato cyst nematode resistance differs between the *Solanum* species. This, potentially, offers a management tool for controlling potato cyst nematode levels in infested potato land through the appropriate use of resistant cultivars produced from a range of *Solanum*-resistant species. Extended selection trials on a range of worldwide *G. pallida* populations clarified that some *Solanum* species were more durable at maintaining their PCN resistance than others. After 5 years of continual selection of PCN populations on *S. tuberosum* spp. *andigena* no appreciable increase in virulence had occurred, whereas on *S. stenotomum* and *S. kurtzianum*, increased PCN reproduction rates were apparent from the second selected generation. These results have clear implications when selecting the most appropriate material for PCN breeding programmes.

331 Members of the root lipoxygenase gene family of susceptible and resistant pea genotypes exhibit distinct expression patterns when infected by cyst nematodes

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Plant lipoxygenase activity is involved in plant growth and development by formation of fatty-acid hydroperoxides and/or jasmonic acid. Moreover, lipoxygenases are known to contribute in regulating cell death and resistance to pathogenic agents. In order to understand the timing of lipoxygenase involvement during nematode infection, we characterised the expression pattern of five genes coding for distinct lipoxygenase isoforms (*LOXs*) in *Pisum sativum* resistant (line MG103738) and susceptible (cv. Progress9) to the cyst nematode *Heterodera goettingiana*. The expression pattern of the same *LOX* genes has also been evaluated in pea roots following wounding in the absence of the parasite. An increased expression of each lipoxygenase isoform, in both resistant and susceptible roots, 2 h after wounding with a peak of transcript abundance at 6 h was observed. Furthermore, in infected roots *LOX* expression was followed at 24, 48, and 72 h after nematode infection. The *LOXs* of susceptible and resistant pea roots exhibited distinct expression patterns indicating the occurrence of gene down- and up-regulation. Intriguingly, some *LOX* members were clearly repressed during nematode infection in the resistant genotype.

332 The influences of different soybean cultivars on the dynamics of *Heterodera glycines* race 3

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Nine cultivars were used to study the dynamics of soybean cyst nematode (SCN) *Heterodera glycines* race 3 for different soybean cultivars in the field of Shenyang Agricultural University, Liaoning province in China. The results indicated that the numbers of SCN second stage juveniles (J2) in the rhizosphere of Liaodou 10 reached its highest peak, 60.4 J2, in 100 cm³ soil, on 24 May. The cyst population of soil around the root of Liaodou 10 and Kaiyu 10 had a peak on 6 June (23.3 and 30.0 cysts in 100 cm³ soil, respectively), which was 1.5 to 2.0 times the number on Peking. There was a peak of J2 in the root of soybean on 24 May for the resistant cultivars PI90763, Harbin small black soybean, Yingxian small black soybean and Franklin, but the peak value was much lower than that of Liaodou 10 and Kaiyu 10. From the dynamics of J2, J3 and J4, soybean LiaoK89102 was

resistant to invasion by SCN, PI90763 was resistant to development of SCN, Liaodou 10 and Kaiyu 10 were susceptible cultivars.

333 Selection of root-knot nematode resistant sugar beet from field plantings

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Sugar beet is a favoured host for numerous species of nematodes, including *Meloidogyne* spp. Resistance to root-knot nematodes was identified 7 years ago, and since then it has been introgressed into cultivated sugar beet. Preliminary observations on several breeding populations were conducted in field plots infested with either *M. incognita* or *M. javanica* at UC Research and Extension Centers, Irvine and Parlier, California. In resistant progeny families, more than 50% of the plants produced healthy taproots that exhibited no root-knot symptoms. In comparison, none of the susceptible control plants were free from galling. Significant reductions of approximately 45% or more in root weights occurred when these susceptible control plants were grown in infested soil. Susceptible sugar beet suffered a higher sensitivity reaction to prolonged temperature (>38°C) stresses and secondary pathogenic invasions than the resistant counterpart. Glasshouse inoculation screenings provided reliable classification of resistant genotypes, but no index of full growth potential of the plants. Our results indicate that a productive root-knot nematode-resistant sugar beet line with elite root yield, taproot conformation, and sucrose content would be developed more readily when resistant parents were grown and selected from nematode infested fields.

334 Resistance of *Aegilops geniculata* Roth to cereal cyst nematode (*Heterodera avenae*) and root lesion nematode (*Pratylenchus thornei*)

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Wild wheat relative *Aegilops geniculata* represents a valuable source of genetic variation for improvement of disease and pest resistance in cultivated wheat (*Triticum aestivum*). A better understanding of the resistance responses of this species against cereal cyst nematode (CCN) and root lesion nematode (RLN) is needed. *Ae. geniculata* accessions originating from different eco-geographical regions possessing useful biotic and abiotic stress resistance traits were evaluated for their reaction to two populations of CCN *Heterodera avenae*: Ha41 originated from France and E125 from Syria, and to one population of RLN *Pratylenchus thornei* from Mexico. Results obtained confirmed the potential value of *Ae. geniculata* as a source of CCN resistance for wheat improvement. They also demonstrated the interest of this species as a source of moderate resistance to *P. thornei*. Promising *Ae. geniculata* accessions possessing resistance traits to cereal cyst and/or root lesion nematodes in addition to other abiotic/biotic stresses were identified and are presently used in wide hybridisation programme. They were crossed with susceptible high-yielding bread and durum wheat CIMMYT cultivars as well as with Chinese Spring in order to transfer this resistance into cultivated wheat.

335 Durable resistance against *Meloidogyne chitwoodi* and *M. fallax* (EU-DREAM)

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The EU-funded project QLRT-1999-1462 DREAM (Durable Resistance Against *Meloidogyne*) aims to contribute to sustainable production systems by developing a strategy for durable resistance management for these soil-borne pests. This study focuses on the two polyphagous quarantine-organisms *Meloidogyne chitwoodi* and *M. fallax*, which are an important economic threat and for which no adequate durable alternative exists. The objective will be achieved by integrating expertise in breeding, nematology, botany and molecular biology into one project, novel by its European dimension. The project combines three areas of research: *i*) identification and

incorporation of resistance in important arable crops: potato, pepper, ryegrass and fodder radish, *ii*) study of variation in nematode virulence and of durability of the resistance, and *iii*) optimising of production systems by rotation schemes. The main results expected are: resistant germplasm, well characterised pathogen collections, reliable selection and breeding methods, knowledge on the stability of resistance, molecular markers linked to resistance and (a)virulence, knowledge on resistance mechanisms and genes, genetic maps, and advice about improved rotation schemes. More information and first results can be found at www.eu-dream.nl.

336 Molecular characterisation and morphometrics of cereal cyst nematodes in Syria and Turkey

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Cereal cyst nematodes (CCN) are becoming serious cereal disease in the rainfed areas of the Mediterranean basin. A survey of cereal fields in Syria and Turkey has shown that the main cereal cyst nematodes *Heterodera avenae*, *H. filipjevi* and *H. latipons* occur frequently. Characterisation of these nematodes was conducted on 14 populations using PCR-RFLP analysis of the ITS regions of rDNA and on 12 morphometric characters of cysts and juveniles tested through a principle component analysis (PCA). Restriction patterns obtained with four endonucleases (*Hae*III, *Hinf*I, *Ita*I, *Pst*I) enabled differentiation of the three cited species plus the chick-pea cyst nematode *Heterodera ciceri*. Principal component analysis applied to the morphometrical parameters followed by a direct hierarchical classification confirmed the molecular diagnostics but demonstrated a substantial variability in the *H. latipons* group.

337 Ultrastructural and histological changes induced by *Rotylenchulus reniformis* in resistant and susceptible upland cotton

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Several events that may determine the degree of susceptibility of cotton plants to reniform nematode occur at the feeding site. The objective of this work is to describe histological and ultrastructural modifications induced by *Rotylenchulus reniformis* in resistant and susceptible upland cotton (*Gossypium hirsutum*) roots. Susceptible (Deltapine50) and resistant cotton materials under evaluation were inoculated with reniform nematode in

the glasshouse. Fifteen days after inoculation, roots were washed and prepared for observation. Tissues were fixed in Karnovsky and post-fixed in 1% osmium tetroxide. They were pre-stained in 0.5% uranyl acetate, dehydrated, and embedded in Spurr. Thin sections were prepared and stained for observation under transmission electron microscope. Additionally, thick sections were prepared and stained for observation under light microscope. Both susceptible and resistant plants, formed syncytia with cell wall perforations, dense cytoplasm, increased endoplasmic reticulum, and increased size of nucleus. Changes that appear to be induced in plants with a higher level of resistance include a layer of necrotic cells surrounding the syncytia and prominent cell wall appositions in syncytium component cells near the necrotic layer.

338 Morphometric variation of *Rotylenchulus reniformis* geographic populations from cotton-growing regions in the United States

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The variability of morphological characters among populations of *Rotylenchulus reniformis* associated with cotton plantations in the United States is not known. A study was conducted to determine the range of variability of diagnostic characters in males and immature females among 12 populations from different cotton-growing regions in the United States (two from Alabama, two from Arkansas, one from Florida, one from Georgia, two from Hawaii, one from Louisiana, one from Mississippi, one from South Carolina, and one from Texas). Specimens were extracted from soil by the centrifugation-flotation technique, and were heat narcotised. Nematodes were mounted in water and measured with the aid of a compound microscope. Analysis of variance was used to compare the characters measured among populations. Morphometrics of males and immature females from the different geographic regions of the southeastern United States were similar, but differed from those from Hawaii. The effect of the host on the range of variability of diagnostic characters was also determined by comparing measurements of the populations grown on tomato (Rutgers), cotton (Deltapine 50), and soybean (Braxton).

339 The cellular structure of the female reproductive system of *Meloidogyne* spp. compared with other nematode species

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Gonads from living young females, belonging to 15 *Meloidogyne* species and 80 other species, were extruded to study the cellular structure of the female genital structure. Within the genus *Meloidogyne*, the spermatheca is always spherical and formed by a variable number of thick, lobe-like cells, which makes it different from any other known nematode genus. Members of this genus are strongly unified by this characteristic anatomical feature. Nevertheless a remarkable intrageneric variability is demonstrated; most species have 16 to 18 spermatheca cells with interlaced cell boundaries while *M. microtyla* and *M. ichinohei* have more spermatheca cells with atypical cell boundaries and the spermatheca cells of the *M. fallax* specimens are clustered together forming lobes. While most species were studied with light microscopy, the gonads of *M. incognita* were studied thoroughly using scanning and transmission electron microscopy. This allowed us to determine exactly the structural separate gonoduct components and have a better insight in their action and function.

340 Eggshell ultrastructure of *Heterodera glycines*: histochemical localisation of chitinous components

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The function of the nematode eggshell to protect the developing embryo is well conserved; however, the structure of the eggshell is varied. The most common structure that is found amongst nematodes is a tri-layer ultrastructure composed of a lipid layer, a chitin layer, and a vitelline layer. Previous literature on the ultrastructure of the *Heterodera glycines* eggshell has claimed that there is no vitelline layer but rather two chitinous layers. It is our contention that the outer chitin layer described in

past literature is actually a vitelline layer. Histochemical analysis has demonstrated a lack of chitinous material on the eggshell exterior. Microscopy observations of the eggshell shows a waxy appearance and osmotic staining that is consistent with that of the vitelline layer found in other nematodes. Lectin localisation has also shown that the eggshell continues to develop past fertilisation with delivery and integration of eggshell precursors. Further, a 'zipper like' structure associated with the vitelline layer of the eggshell for juvenile emersion was observed. Based on our findings we propose that *H. glycines* follows the common three-layer structure of other nematodes and is not an exception as originally thought.

341 AFM profiling of putative hydrogen bond patterns on nematode cuticle surface

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Atomic Force Microscopy (AFM) is a non-invasive high-resolution probe microscopy useful in the study of biological surfaces in their quasi-native state. Applications to nematode cuticle from dried specimens showed that atomic resolution studies are feasible, allowing the identification of structural arrangements of single collagen chains. AFM scanings were applied to air or ethanol dried *Xiphinema diversicaudatum* specimens. After scanning stabilisation during long-run sessions at room temperature and pressure, atomic resolution images allowed the reconstruction of molecular level details. These included ordered patterns of drop-like clouds linking parallel atom chains interpreted as putative H-bonds. Their length, shape and dimensions, as well as the ratio with the other particle measurements, fit quantum models of charge distribution as well as Bohr's 'hanging drop' model of H-bonds. These are the first atomic resolution data obtained from nematode cuticle and the first imaging of a putative chemical bond produced by AFM. The potential of this technology in high resolution studies of biomolecules is discussed.

342 Morphobiometrical and biochemical characterisation of *Heterodera zae* Portuguese populations

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Three populations of *Heterodera* from different locations in Portugal were isolated from fig tree rhizosphere and corn roots. Morphological and morphometrical analysis revealed that all populations presented the two main diagnostic characters of the corn cyst nematode, *Heterodera zaeae*: i) the unique two level bullae with characteristic arrangement and orientation; and ii) stylet mean length usually smaller than 20 μm . The morphology (LM, SEM) and morphometry of cysts and second-stage juveniles are given and the intrapopulation and intraspecific variability are discussed and compared with other populations. Furthermore, a characteristic and reproducible phenotype of nonspecific esterase activity was detected, by native polyacrylamide gel electrophoresis, in the three populations. The occurrence of *H. zaeae*, an economically significant parasite and originally identified from corn (*Zea mays* L.), is reported for the first time in Europe (Portugal).

343 A comparative study of amphimictic populations of lesion nematodes with two lip annuli from citrus, coffee, yam, and banana in Brazil

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A collection of 17 amphimictic populations of *Pratylenchus* spp. with two lip annuli collected from *Dioscorea cayenensis* Lam., *Musa* sp., *Coffea arabica* L. (one from each), and *Citrus limonia* L. Osbeck (13 populations) has been maintained on their hosts in a glasshouse at the UNESP Campus at Jaboticabal, SP, Brazil. A comparative study was done on all populations with the exception of eight from citrus, using 21 morphologic, morphometric and derived variables obtained using SEM and light microscopy. The populations from yam and banana were identified as *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven. All others pertain to a new

species found on citrus in São Paulo State that is being described and named *P. jaehni*. A cluster analysis of stylet length, width and height of the stylet knobs, a, b, c, c' and V parameters for these populations was consistent with the identifications.

344 Scanning electron microscopy of the juvenile stages of some Wilsonematinae (Plectidae)

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Lip regions of *Tylocephalus auriculatus* and *Ereptonema arcticum* undergo extensive morphological transformations during the nematodes developmental stages, from a simple *Plectus*-like appearance to a much more complex set of anterior structures. We present the first scanning electron microscopy observations of these changes. Relationships within Wilsonematinae based on morphological cladistics suggest a straightforward hypothesis of increasing complexity from *Plectus*, which could be considered ancestral, to the highly ornamented condition of e.g., *Tylocephalus* and *Wilsonema*. However, small subunit (SSU) rRNA sequence data are not in congruence with this morphological hypothesis, in that *Tylocephalus* and *Wilsonema* diverged before species of *Plectus*. We discuss the probable homologies of various structures, and the implications of different phylogenies on character polarity within Plectidae.

345 An improved SEM technique for preparing perineal patterns of *Meloidogyne* spp. and for studying host pathogen interactions of sedentary nematodes

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Pieces of galled root were fixed in 0.05 M potassium phosphate buffer, pH 7.4 during 5 days. Subsequently, the pieces were washed in pure buffer and partially dissected

under the dissecting microscope, inside the buffer, to expose the posterior portion of females, juveniles inside the roots, giant cells or other details of the anatomical changes in the root caused by the nematodes. After that, the pieces were washed three times again in the buffer and post-fixed overnight in 2% osmium tetroxide. Next, the pieces were dehydrated in ethanol, dried in critical point dryer using CO₂, mounted onto the stubs with the structure of interest facing up using adhesive copper tape, sputtered with 30 nm of gold, observed and electron-micrographed with a scanning microscope JEOL JSM 5410, operated at 15 kV. By this technique, perineal patterns are better preserved and there is no need to cut the females in lactic acid. Juveniles inside the roots, details of the giant cells, and details of the disruption of the root tissues were documented.

346 Ultrastructure of the feeding site of *Gracilacus latescens* Raski, 1976 in timber bamboo roots

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Female *Gracilacus latescens* remain attached by the stylet to the surface of timber bamboo roots. Transmission electron microscope observations of the nematode feeding site showed accumulation of callose-like material enveloping the stylet. The enveloped stylet extends from the point of insertion in an epidermal cell wall into the lumen of two to three cells in the sclerenchyma and cortex. The callose deposit enveloping the stylet increases in thickness with each additional cell wall perforated by the stylet. Possible lignin deposit around the callose is observed in perforated sclerenchyma cells. Cross sections of the enveloped stylet revealed the stylet tightly encased in the callose, which protects the affected cells and also anchors the stylet. A syncytium originates from the innermost cell reached by the enveloped stylet and expands into the inner cortex and stele. Cell wall dissolution and pit fields are characteristics of the syncytium.

347 SEM observations on the coastal marine nematode *Haliplectus bickneri* (Chitwood, 1956) Swart et al., 1993

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The free-living marine nematode *Haliplectus bickneri* (Haliplectidae: Leptolaimina) was detected in high numbers at coastal foredunes in the Netherlands and examined by field emission electron microscopy. This proved to be very useful as most external Haliplectidae features are strongly reduced. The head region is tapering and includes: six labial-, six anterior cephalic- and four posterior cephalic papilliform sensilla; a minute stoma with 12-fold vestibulum and circular amphids. The coarsely annulated cuticle has eight longitudinal rows of body pores, between head and tail end. Annules are ornamented with fine longitudinal striae; additionally a double transverse row of punctations is present between the annules. In males a long ventral cuticular rim runs from mid-body towards four small pre-cloacal papillae, located closely together near the cloaca. The short conoid tail is provided with a nipple-like tail tip and includes a spinneret with three caudal-gland openings. The variability of some of the characters and the present position of the Haliplectidae within the Chromadorida are discussed.

348 Morphological variability of bullae in vulval cones of cysts of *Heterodera glycines* Ichinohe, 1952 (Nematoda: Tylenchida) evaluated with scanning electron microscopy

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The morphology of bullae in two populations of *H. glycines* (races 1 and 3), collected from different localities in Argentina, was studied. These cuticle protuberances were located at or under the underbridge; their size and shape were variable. Shapes varied from round

to finger-like; the round bullae were of different sizes and the finger-like ones showed variable length and thickness. Few cysts had bullae of a single type; in most specimens of both populations the two different shapes were present simultaneously. The number of these structures varied from scarce to highly abundant depending on the individuals, the latter configuration being the most commonly observed. Although this character was variable, no relationship with a particular population was established. Some of the different configurations observed would correspond to the expected pattern for other species. The considerable variability of this character requires observation of several specimens to be able to determine the tendency of these structures in the population under study.

349 Discrimination among second stage juveniles of *Heterodera glycines* Ichinohe, 1952 of different origin and races

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Second stage juveniles of four populations of *Heterodera glycines* from Argentina and Brazil, belonging to races 1 and 3, were compared based on the study of their morphometrical characters. An analysis of variance showed significant differences among populations with respect to all the characters considered, except for the lip region height. The possible relationships among populations were evaluated through a linear discriminant analysis. Five out of the 16 characters considered resulted as the most significant in separating populations: body length, body diameter, pharynx length, and ratios a and b. Although the four populations studied showed a certain relationship in the discriminant space, it was possible to differentiate them. In the first canonical axis, populations from Argentina and Brazil were separated; the second axis showed differences between representatives of both races considered. The results obtained show that individuals belonging to different populations and/or races of *H. glycines* may represent different biological entities.

350 Comparative structural, functional and expression analyses of the *flp-11* gene in nematodes

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Panagrellus redivivus has characteristics in common with *Caenorhabditis elegans* (size, habitat, behaviour) yet it has been assigned to a different clade. *Caenorhabditis elegans* possesses 22 genes (designated *flps*) that encode FMRFamide-related neuropeptides (FaRPs), and immunocytochemical analyses have revealed that some 50% of the nerves in both worms are FaRPergic. Using chromatographic methods, two FaRPs were isolated and structurally characterised (AMRNAVLRamide and NGAPQPFVRamide) from *P. redivivus*. Functional assays using *Ascaris suum* revealed that NGAPQPFVRamide inhibits body wall muscle and vulval function whereas AMRNALVRamide inhibits body wall muscle and stimulates vulval function. Single copies of both peptides are encoded by *C. elegans flp-11a*, and molecular analysis of the orthologous gene from *P. redivivus* revealed that AMRNAVLRamide and NGAPQPFVRamide were also co-encoded on a single gene (designated *prflp-1*) along with the novel peptide, AAGMRNALVRamide. The *C. elegans flp-11* gene has been reported to be expressed in three paired head neurones and a tail neuron. *In situ* hybridisation analysis of *prflp-1* revealed that expression was confined to a single unpaired head neuron (RIS-like) with no staining occurring in the tail. Although *flp-11* and *prflp-1* are orthologous neuropeptide genes, the encoded peptides appear to fulfil different roles in *P. redivivus* and *C. elegans*.

351 Morphological and morphometric variations of *P. loosi sensu lato*

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Morphological and morphometric variations of 11 populations of *P. loosi sensu lato* were examined. Extensive morphological and morphometric variations existed. Guatemala, Iran, Japan-Kagoshima, Japan-Shizuoka, Sri Lanka-Passara and Sri Lanka-Talawakele populations closely fit the morphometrics of *P. loosi sensu stricto*. Scanning electron micrographs of West Africa cowpea and West Africa sorghum populations did not show similarities to *P. loosi sensu stricto*. The Florida population exhibited morphological similarities to both *P. coffeae sensu stricto* and *P. loosi sensu stricto*. The Florida, Guatemala, Iran, Japan-Kagoshima, Japan-Shizuoka, Sri Lanka-Passara and Sri Lanka-Talawakele populations along with *P. coffeae* population were separated by principal component analysis of their female and male morphometrics. The populations did overlap with no obvious clusters of populations probably because of the considerable variability of the morphometrics. The intraspecific variability observed in the *P. loosi* populations could be attributed to geography, host species and host nutrition, agronomic practices and origin of nematodes. Possible existence of distinct biological pathotypes/strains *P. loosi* is highlighted. The *P. loosi* populations were conspecific with each other and demonstrated a '*P. loosi* species complex'.

352 SEM observations of the lip region in species of the genus *Discolaimus* Cobb, 1913 (Dorylaimida: Qudsianematidae) with comments on their taxonomic interest

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The genus *Discolaimus* Cobb, 1913 is characterised by its peculiar lip region, expanded (*i.e.*, markedly wider than the adjacent body) and with the oral field more or less sunken, showing a sucker-like appearance. It is a rather homogenous genus whose species are mainly separated by morphometric features, so that their identification becomes a difficult task. SEM studies of the lip region

of four species (*D. major* Thorne, 1939; *D. agricolus* Sauer & Annells, 1985; *D. labiatus* Peña-Santiago *et al.*, 2002; *D. mariae* Peña-Santiago *et al.*, 2002) collected in Andalucía Oriental (SE Iberian Peninsula) have revealed an interesting interspecific variability affecting several features: presence/absence of perioral elevated liplets, oral field divided in six primary radial sectors but sometimes with another six smaller interradian sectors, and outer margin divided in six, 12 or 18 lobes; moreover, both sectors and lobes may be separated by more or less deep transversal or longitudinal grooves respectively. These results make possible a new approach to the taxonomy of the genus that is briefly discussed.

353 TEM observations on early moulting of anterior body in *Xiphinema dentatum* (Dorylaimida: Longidoridae)

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Transmission Electron Microscopy (TEM) observations on transverse sections of the anterior body in moulting *Xiphinema dentatum* juveniles revealed the following: anterior tissues are withdrawn from the head cuticle very early during moulting. The space in between is simultaneously filled with a liquid substance, produced by the epidermis. The epidermal cells form large chords in the body cavity, particularly laterally, and extend sheath-like epidermal outgrowths between body cuticle and somatic muscles. The moulting liquid passes *via* chords underneath the body cuticle. Anteriorly, the epidermal tissue assumes a tubular form during its separation from the old cuticle. At that time, it is not yet covered by new epicuticle, showing that in *X. dentatum* separation of the old cuticle precedes new cuticle formation. The two amphidial linings and 16 linings of papillary nerves are left in front of the tissues together with remnants of the latter. These linings encircle the old cheilostoma wall and form what was previously described as the sleeve-like membranous structure around the old cheilostoma.

354 Anomalies of genital organs in *Xiphinema dentatum* (Dorylaimida: Longidoridae)

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During a survey of *Xiphinema dentatum*, numerous individuals from a single field population in Yugoslavia were observed under dissecting microscope. Seven were found with an abnormal genital system, and these were permanently mounted and studied under light microscope. Normal males are rare, sometimes with fully developed diorchic tract, but usually with only a copulatory apparatus. Of 137 males, one is abnormal, having a normal copulatory apparatus, two abnormal testes and lacking the seminal duct. Normal females have two opposed, equally developed genital branches, opening through a slightly anterior vulva ($V = 42.0 \pm 1.3\%$). Of some 42 000 females, six were found to be abnormal. One has the genital system anatomically normal, but abnormally posterior ($V = 71.0\%$). Two females have two normally developed systems, one system in a normal position ($V = 45.9$ and 43.8% , respectively), the other posterior ($V = 62.1$ and 65.3% , respectively). Another two females lack central parts of the system, the remaining distal parts being fused proximally. The sixth female has a normal posterior branch, while the anterior one is abnormally branching at *pars dilatata uteri* into two distal oviducts with ovaries, one ovary being degenerated.

355 SEM studies of the pseudolabia and cervical armature of a female *Heth* (Rhigonematida: Hethidae)

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Heth Cobb, 1898 is an unusual genus of Rhigonematida found in the posterior gut of tropical diplopods from the American and Australasian regions. The species studied here originated in a rhinocricid diplopod from Trinidad, West Indies. Sexes are dimorphic, the female bearing a complex pair of pseudolabial plates arching over the oral aperture. The pseudolabia are subtrapezoid in shape and are attached laterally, the remaining free margins being fringed with pectinate cuticular processes. These 'combs' probably act as filters during feeding, restricting the size of ingested food particles. Posterior to the pseudolabia is

a cervical collar bearing numerous, posteriorly directed, spines. The collar is deflected posteriorly on either side of the midlateral line to form a pair of cuticular lappets, the free margin of each lappet bearing a fringe of cuticular spines. Posterior to the lappets are two pairs of lateral spines, their bases arranged more or less in tandem. In the species studied, the transverse striae of the cervical region bear retrorse posterior margins. A number of stud-like somatic papillae are also visible. Variations in form and detail of the female cervical armature are of major diagnostic importance in specific determination within the genus *Heth*.

356 Morphometry of *Pratylenchus* populations from coffee, banana, ornamental plant and citrus in Brazil

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The morphometric variability of four *Pratylenchus* populations from different hosts and regions of Brazil identified as *Pratylenchus coffeae* was studied. These populations were extracted from roots of coffee, citrus (São Paulo State), *Aglaonema* (Rio de Janeiro State) and banana plant (Minas Gerais State) and were cultured on alfalfa callus tissues. Baermann-extracted specimens of each population from callus were killed by heat and fixed with 4% formalin. Temporary slides were made and examined in light microscope. Body length (L), excretory pore to head end, stylet length, relative distance of vulva from anterior end (V%), body length/greatest body width (a), body length/distance to anterior and to junction of pharynx and intestine (b), body length/tail length (c), tail length/body width at anus (c') were obtained. There were statistically significant differences in all parameters studied.

357 Cuticular diversity within the genus *Ogma* (Criconematidae)

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Females and juveniles of the genus *Ogma* display the most spectacular and diverse cuticular ornamentation. Some of the differences in the structure of a few species are illustrated. Cuticular structure plays an important role in the identification of nematode species. Until recently the cuticle of only females were studied and recorded, but it is becoming more important to take note of the fact that the cuticular structure of the different juvenile stages within a species differ from those of the female and even from each other in most species. These differences can cause problems in a population where more than one *Ogma* species is present because, if the diversity in species is not known, wrong identifications can be made. It is therefore important to become familiar with and study these structures and to include their descriptions in all descriptions of species as far as is possible. The SEM is a handy tool to illustrate the cuticle variation in this genus.

358 Statistical analysis of morphometrics of *Longidorus* species (Nematoda: Dorylaimida)

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Longidorus currently has 132 valid species based primarily on morphometrics. Many *Longidorus* species have a high degree of variability within their morphometrics which leads to considerable overlap between species and increases the potential for misidentification. One way Anova, Student's t and principal components were applied to compare seven species of *Longidorus* found in Arkansas (*L. breviannulatus*, *L. crassus*, *L. diadecturus*, *L. fragilis*, three undescribed species) in order to screen for the most useful morphometrics in species discrimination. Canonical analysis and hierarchical cluster analysis based on female average morphometric characters including body length, distance from vulva opening to head end, lip width, odontostyle length, pharynx length, body diameter, tail length and diameter, were used to examine the morphometric relationships and create dendrograms among 130 published *Longidorus* species (*L. heynsi* Andrassy, 1970 and *L. tardicauda* Merzheevskaya, 1951 with insufficient data were not included) and 94 populations from Arkansas and other locations by using Jump 4.0 softwares. Six major clusters were produced for all *Longidorus* species. Cluster analysis may lead to identification of unidentified *Longidorus* species.

359 List of soil nematodes from a non-tillage field in Tsukuba, Japan, with a comment on the Maturity Index and species diversity

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A list of soil nematodes from a non-tillage (organic) field established in 1981 at the National Institute for Agro-Environmental Sciences, Tsukuba, is given compared with those from a conventional (inorganic) field and a grove nearby. The soil type of the land concerned is andosol. Barley and soybean were cultivated in a double-cropping system on the fields. The non-tillage field was amended with manure made yearly from litter from the grove. Nematodes in 20 g soil were extracted by Baermann funnel method in October, 1999 and counted to each distinguishable taxon under compound microscope at magnification of 100×. Nematode identification was done with specimens mounted on slides in glycerin. The total number of species detected from the non-tillage field so far was 55, though the number of species identified to the species level is rather small. The number of species from the conventional field and grove were smaller and larger than from the non-tillage field, respectively. This tendency was the same in Maturity Indices calculated from the data, but Shannon's H' indices were largest in the non-tillage field. Abundance of *Monhystrella postvulvae*, *Prismatolaimus* sp., *Wilsonema othophorum*, *Bastiania* sp., *Alaimus* sp. and *Xiphinema* sp. characterised the non-tillage field. In contrast, the conventional field had greater *Pratylenchus penetrans* and *Cephalobus* sp. populations.

360 The relationship between soil type and soil texture with the spatial distribution of *Heterodera glycines*

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In order to test the potential of managing soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe site-specifically, the spatial relationship between SCN distribution and soil texture was explored under field conditions. Single-core soil samples were collected

at planting and at harvest using a modified nested sampling design from two fields in Michigan. Samples were analysed for SCN cysts and soil texture. A strong correlation between cyst density and soil texture was found. Coarse soil with more than 60% sand, less than 20% silt and less than 20% clay had consistently more cysts than finer soil. As defined by the USDA-recommended texture triangle, this composition corresponds to sandy loam, loamy sand and sand. Previous work showed SCN preference for sandy soil types, but it is important to note that only a portion of the area defined for sandy loam in the texture triangle is favourable for SCN. Our results indicate there is a potential for site-specific management of SCN based on soil texture (particle size) rather than soil type.

361 Structure of nematode communities in nine types of ecosystems

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Nematode communities were analysed in nine ecosystems: winter wheat in crop rotation – standard tillage; winter wheat in crop rotation – reduced tillage; corn in monoculture; sugar beet; potato – chemical plant protection; potato – integrated plant protection; alfalfa; grassland; poplar forest. Sampling was done in 1997 and 1998, in spring and autumn, to the depth of 15-20 cm. Nematode communities were analysed to genus level. Greatest number of nematodes and number of genera were 2843 nematodes/100 g of soil in alfalfa treatment, and 30 genera in poplar treatment. Lowest was in row crops (216 and 12 genera in sugar beet). Analyses of nematode trophic structure showed greatest abundance of bacterial feeders and plant parasitic nematodes in all treatments, and lowest abundance of omnivorous nematodes and predators. The greatest abundance of predators was determined in poplar forest. Positive correlation between quantity of humus and total number of nematodes was determined but a negative correlation between amount of K₂O and number of genera. Statistically very significant differences occurred between treatments in analysing MI, PPI and PPI/MI and showed the most disturbed nematode communities in row crops, the less disturbed in poplar forest, grassland and alfalfa; and intermediate group was wheat with reduced and standard tillage.

362 Effects of soil pH on *Heterodera glycines* reproduction and soybean chlorosis

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Soybean cyst nematode, *Heterodera glycines*, is a destructive pest on soybean in Minnesota, a state in the North Central region in the United States, where soybean is a major crop. The objective of this study was to determine the effect of soil pH on *H. glycines* reproduction and iron-deficiency chlorosis of soybean in fields. A survey was conducted in a 20 acre area in a commercial soybean field in Waseca, Minnesota, to monitor soil pH, density of *H. glycines*, and severity of iron-deficiency chlorosis of soybean in 2001. Positive correlation between either two of the three variables was significant. Glasshouse studies confirmed that *H. glycines* reproduction on susceptible soybean increased with increasing soil pH from 5 to 8. The results suggest that *H. glycines* may increase severity of iron-deficiency of soybean at higher soil pH in Minnesota fields.

363 Relationships between nematode and microbial communities and carbon transfers in grassland soils

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At an upland field site, plots of an established *Festuca* – *Agrostis* pasture and of a newly sown monoculture of *Lolium perenne* have been treated for 3 years with nitrogen and lime or with pesticide (chlorpyrifos). These treatments were designed to produce contrasting degrees of diversity in communities of soil animals and microbes as part of our research on the relationships between grassland management practice and the diversity of biological communities in soil. Nematode communities have been described by trophic composition and by

ecological indices based on proportions of five groups on the coloniser-persister scale. These data are analysed in relation to variations in root growth and microbial community structure and activity over 3 years. In additional plots, the ¹³C fixed by photosynthesis of a 24 h pulse of ¹³CO₂ applied to the herbage, was detected in nematodes extracted from underlying soil at 8, 22, 48 and 69 days after the pulse. There was more ¹³C in nematodes from untreated controls than from limed plots. These differences are consistent with a greater carbon flux in soils treated with lime, but could not be related to differences between the nematode communities.

364 A new nematode genus (Rhabditida: Panagrolaimidae) from pitcher plants

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Measurements, line drawings and scanning electromicrographs are provided of a new nematode species and genus, isolated from pitcher fluid of *Nepenthes* sp. from Thailand. The new genus differs from all known nematodes in having two opposing and offset spermathecal pouches at the junction of oviduct and uterus. It also differs from all known Rhabditida in having four cephalic setae instead of papillae. Phylogenetic analysis of small subunit rDNA sequence data robustly places the new genus within Panagrolaimidae, as sister taxon to *Panagrellus*. These unusual nematodes resemble *Panagrellus* in e.g., body size (1.8–2.5 mm in females, 1.3–1.8 in males) and in the monodelphic, prodelphic female reproductive system with thickened vaginal walls and prominent postvulval sac. However, they differ from *Panagrellus* in the characters mentioned above, in the presence of liplets around the mouth opening, in their comparatively longer stegostom, and in the shape of the male spicules. Because of its aberrant characters, inclusion of this new genus in Panagrolaimidae will entail changes to the family diagnosis.

365 Ecophysiological studies on pine wilt disease occurring in coastal dunes

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The spreading manner of pine wilt disease has been compared between two 20 × 20 m experimental plots established in Japanese black pine forest distributed over coastal dunes in Tottori University Arid Land Research Center. One of the plots was fertilised every year since 1996, and the other one remained with no treatment and served as a control. The number of pine trees killed by pine wilt disease was far more in the fertilised plot than in the control one, suggesting that the fertilisation might have promoted the death of pine trees by pine wilt disease. To reveal the reason why the fertilisation promoted the pine wilt disease, mycorrhizal relationship has been examined as a possible cue to explain the difference. Fertilisation might suppress mycorrhizal synthesis which has been regarded to work as absorbing organ for pine trees especially when under water stress. Diversity and constitutions of mycorrhizae formed on young pine seedlings were compared between the two plots, and among three groups of pine seedlings with different watering regimes. We conclude that mycorrhizal relations play an important role to make pine trees survive the epidemic wilt disease.

366 Effect of soil pH on *Heterodera glycines* population density in Brazil

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Previous observations in growers' fields infested with the soybean cyst nematode (SCN), *Heterodera glycines*, suggested that SCN populations remained higher for long periods in soils with high pH. Therefore, experiments

were conducted in three regions from 1995 to 2000, comparing two pH levels, under two crop rotation systems using corn; one (pH5 to 5.5 in CaCl₂) representing the recommended pH range, and another (6 to 6.5) representing a high pH level. In all experiments there was a trend of higher SCN population density in plots with higher soil pH. Under these conditions, 1 year rotation with corn reduced the SCN population, but its incidence was still higher in plots with higher soil pH.

367 Edaphic microfauna in woody crops from Mediterranean environments

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Microfauna associated with several vineyard and olive crops grown in a continental Mediterranean climate under various soil management systems on the Spanish Central plateau were studied. Nematode trophic groups and microarthropod populations in plots under different cropping systems (non-tillage, traditional tillage, covers with clover, weeds or vetch) were studied in comparison with two controls (soil from an abandoned cereal crop and a hedge of wild vegetation). Plant-parasitic and bacteria-feeder nematodes had the highest populations in the hedge control and in plots with clover cover, while their populations were lower under non-tillage. Predators were abundant in grape: under traditional management, under a covering of runners and in the control group. However, fewer predators were found in non-tillage plots, where omnivorous predators abound, in olive with clover cover and in the control group with natural vegetation. Collembola had a higher population in all alternatives in plots with clover cover and control groups. Acari were frequent in vineyards, except in weed-covered and non-tilled plots and along the edge, appearing in weed-covered olive and the control group. Coleoptera appeared in all but non-tilled olive plots while Diptera were in vineyards, except those under tillage, and the control group, where only Thysanoptera were present.

368 Diversity of soil nematodes in an alpine dwarf-shrub heath in Austria: first results of a 2 year study

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The soil nematode community was investigated in an alpine dwarf shrub heath in the Central Alps (1930 m) near Obergurgl, Austria. The vegetation on the acidic soil consisted mainly of *Vaccinium gaultherioides*, *V. myrtillus* and *Rhododendron ferrugineum*. The aim of the study was firstly to investigate the nematode biodiversity of alpine environments. Secondly, it was designed to serve as a basis for investigations on the use of nematode communities as an indicator for the global climate change. A total of 216 soil samples was taken in July, August and September 2001. All nematodes extracted from bulk samples were identified and counted. Nematodes of all five feeding groups were encountered. A list of species, species abundance, diversity indices and the maturity index are presented. The nematode community is apparently typical for alpine regions and the most dominant genus was *Rotylenchus* sp. (Tylenchidae). Further plant-feeding families were Pratylenchidae and Paratylenchidae. Within the predators, Mononchidae and Anatonchidae were encountered and the bacterial feeders were represented by the Prismatolaimidae, Plectidae, Cephalobidae and Teratocephalidae. Genera of the omnivorous families Dorylaimidae and Aporcelaimidae were identified, along with genera of the fungivorous Aphelenchidae, Aphelenchoididae and Nordiidae.

369 Current uncertainties in the estimation of soil nematode diversity and its use in soil quality monitoring

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Estimation of soil biodiversity and monitoring of soil quality are two of the major priorities of the international environmental agenda, although their quantification is not free from uncertainties. This contribution analyses these uncertainties. In an Acrisol under a continental

Mediterranean climate, the study of nematofauna took the following issues into account: *i*) land use, *ii*) various soil horizons, *iii*) seasons, and *iv*) extracting methods. Results show that an estimation of the taxonomic biodiversity and individual abundance patterns is not possible, without taking all the above-mentioned items into account. Different extracting methods show distinct scenarios, as well as respect for seasons and soil depth. Sampling the upper 15 cm of the A horizon involved a great abundance of soil individuals, biomass and biodiversity. It is also demonstrated that in some instances the B horizon (not usually sampled for soil biodiversity and soil monitoring studies) has more individuals and taxonomic diversity than the A horizon. Thus, from the data obtained, it seems that both the theoretical assumptions and the standard techniques currently used for soil biodiversity and soil-monitoring studies do not show the complexity of soil nematofauna. Therefore, we need to improve present methods to develop more relevant research on these topics.

370 Nematode abundance, diversity and trophic structure in relation to spatial heterogeneity of a natural fen

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The abundance, diversity, and trophic structure of nematode communities of a natural tall-sedge fen in Poland were studied. The sedge association was of a tufty structure due to the dominance of *Carex paradoxa*. Soil samples were taken in the spring (May) and in the summer (July) within and outside of the sedge tussocks. It was found that nematode communities were more abundant and more diverse within than outside of the tussocks and the differences were more pronounced in the spring than in the summer. Both habitats differed also in nematode trophic structure and in the relative importance of the trophic groups. No omnivorous and predatory nematodes were found in the soil between tussocks on the spring sampling. Bacterial-feeding nematodes predominated in the two habitats but, while within the tussocks, on both sampling dates they contributed to about 60% of the total nematode numbers in the soil between tussocks, bacterial-feeding nematodes constituted 94 and 73% in spring and summer, respectively. The variation of nematode community parameters in such a short distance was

explained by the differences in some soil characteristics of the two studied habitats.

371 Phylogenetic analysis of the phoretic association between *Bursaphelenchus conicaudatus* (Nematoda: Aphelenchoididae) and *Psacotheta hilaris* (Coleoptera: Cerambycidae)

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The phoretic relationship between the nematode *Bursaphelenchus conicaudatus* and the yellow-spotted longicorn beetle, *Psacotheta hilaris*, collected at various districts in Japan were investigated. All ten subspecies of the beetle examined were infected with the nematodes at a high proportion suggesting the universality of this phoretic association. Molecular analysis based on the partial base sequence of cytochrome oxidase subunit I in the mitochondrial DNA revealed that the phylogenetic relationship among ten subspecies of the beetle was similar to that among associated nematode isolates obtained from each beetle subspecies. Intraspecific vector replacements were supposed to occur in the isolates of *B. conicaudatus* on two islands. The phoretic association between the nematodes and the beetles was assumed to be on the way from non-specific facultative commensalism to specific obligative commensalism.

372 Influence of diverse agricultural systems on the population dynamics of free-living, plant-parasitic and entomopathogenic nematodes

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The population dynamics and densities of soil-inhabiting nematodes were measured in different agricultural systems at the Center for Environmental Farming systems near Goldsboro, NC, USA. Systems studied included old-field succession, forestry, integrated crop/animal, organic, and cotton in conventional vs no-till production. Numbers of omnivorous and predacious nematodes

were greatest in forestry, successional and organic systems in July and September compared to conventional cropping systems ($P = 0.01$). The population densities of fungivorous and bacterivorous nematodes were greater in organic systems compared to other systems at the July sampling, but not in September ($P = 0.01$). Temporal variation in population maximum may influence conclusions about the relationships of trophic groups to one another. The use of organic amendments to supply nutrients in the organic system may be responsible for high numbers of free-living nematodes in these treatments. Cotton in no-till production had greater numbers of bacterial feeding nematodes than conventional tillage ($P = 0.10$). The presence of various species of plant-parasitic nematodes was related to the crop grown, and was little influenced by tillage system. The entomopathogenic nematodes *Steinernema glaseri*, *Steinernema carocapsae* and *Heterorhabditis bacteriophora* were detected in all treatments. Highest numbers of entomopathogenic nematodes were detected in the succession and forestry treatments.

373 An essay of detection of zoogeographic units of the territory in base to nematode fauna distribution: the case of a protected area in SE Iberian Peninsula

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The identification of biotic regions, i.e., series of operational geographic units (OGU) that share a similar fauna, leading to the division of the territory into operational zoogeographic units (OZU), was one of the objectives of a nematological project addressing the taxonomy and distribution of dorylaimid and mononchid nematode fauna of the Sierra Mágina Natural Park (SE Iberian Peninsula). Sixty OGU of the UTM (Universal Transversal Mercator) 2 × 2 km square grid were classified in base to the presence/absence of 138 nematode species using a quantitative analysis protocol. The results

obtained suggest that the studied area may be divided into seven biotic (zoogeographic) regions with their respective biotic boundaries. The biotic regions have been environmentally characterised, with altitude, slope, and sand and clay contents the main factors influencing the division. The analysis also produced a faunistic characterisation of the biotic regions. These results, which represent a new approach to the study of nematode distribution, are discussed.

374 Nematode community and trophic structure along a sand dune succession

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Changes in below-ground nematode communities, in terms of abundance, diversity, trophic structure, sex bias and adult : juvenile ratio were related to edaphic factors from sample sites representing differing successional stages. Nematode abundance increased along a 1 km transect of increasing succession, from sandy beach through active dune systems to mature dunes. As the succession progressed, the adult nematode sex ratio changed from male biased to highly female biased. Consequently, the adult : juvenile ratio decreased as the nematode community became more female dominated. An assessment of nematode diversity using both univariate and multivariate analysis produced contradictory results. Univariate indices yielded inconsistent results with different indices suggesting lower diversity at different successional stages. Multivariate analysis clearly separated the beach and dune heath samples from the other samples and each other and, to a lesser extent, separated the fore dune samples from the grey and yellow dune. Omnivorous nematodes represented the largest trophic component of the nematode community in all successional stages of the terrestrial sites. A trend was apparent of increasing numbers of omnivorous nematodes along the succession in relation to increasing organic matter and/or litter accumulation.

375 Analysis of nematofauna in some coastal dune ecosystems of Sicily

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A survey of nematodes of some coastal dune ecosystems in Sicily was carried out both from a faunistic and an ecological point of view. The species composition was very peculiar, with many new species, many taxa typical of dunes or rare, and some others typical of coastal marine and brackish environments. The ecological analysis was conducted more deeply in two nature reserve sites, namely Vendicari and Irminio river-mouth, with different environmental characteristics. Shannon diversity index, species richness, evenness, maturity index, frequencies of c-p categories and of trophic groups were calculated at each station. The results were compared with those from some other coastal sites with a different degree of environmental disturbance. Both biodiversity and maturity were higher in the stations where dunes were better preserved, but the differences among the sites were not as high as one would expect, probably because dune nematodes are adapted to survive in such highly changing environments.

376 Restoration of soil nematode fauna (dorylaims and mononchs) in the Guadiamar River basin (SW Iberian Peninsula) 3 years after a mining accident

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As a consequence of the mining accident which occurred at the Guadiamar River basin (SW Iberian Peninsula) in April 1998, part of the river banks were covered with toxic mud containing heavy metals, and a research project focused on the faunistic characterisation of the area and control of the recolonisation process was undertaken. Nematode fauna, particularly dorylaims and mononchs, was included in the study. A few well-conserved and non-polluted natural areas (biocentres), and a series of experimental plots located in the river banks, have been surveyed over two seasons (spring and autumn) during 2 years (2000 and 2001). One hundred and twenty seven

soil samples were collected, but only 99 have been studied at present. More than 100 species constitute the fauna of the region, indicating a remarkable faunistic potential of this geographical area. Concerning the recolonisation process, two distribution patterns, one spatial and another temporal, have been detected and are also discussed.

377 Soil nematode community as a valuable bioindicator of soil ecosystem recovery after mining spillage in southern Spain

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In April 1998, an amount of about 5hm³ of toxic sludge, with high concentrations of heavy metals, was released along the Guadiamar river system as a consequence of the tragic Aznalcollar accident in southern Spain. In order to evaluate the environmental impact produced by the heavy metal pollution on soil health conditions, we carried out field and laboratory studies. Ten control and 27 polluted soil samples were taken along the Guadiamar riverside in three sampling periods (June 2000, December 2000 and June 2001). For each soil sample, values of diversity and trophic indices were calculated, and the concentration of heavy metals (Pb, Zn, Ni and Cu) was analysed. Soil nematodes were identified, at least to the genus taxonomic level. In addition, short-term toxicity bioassays were conducted in triplicate using adults of *Aphelenchus avenae* and *Cephalobus persignis* collected at the control area. Results from field studies showed that concentrations of heavy metals were significantly higher at polluted sites than at control sites. Conversely, values of biological indices were significantly much lower at polluted sites. Results from short-term toxicity bioassays revealed that Pb and Cu are mainly responsible for the observed environmental impact on the soil nematode community. In spite of the fact that concentrations of heavy metals remained similar among sampling periods, values of biological indices exhibited a clear tendency to increase since the mining spillage.

378 Nematodes as indicators of waste water treatment

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In this paper we present the results of a study carried out on 28 waste waters of Andalucía (Spain) in order to assess the presence of nematodes in the environs of the treatment process with special attention to sludge samples that can constitute a suitable environment for development of these organisms. Nematodes were present in all the waste water and 21 species belonging to 21 genera were identified. These species are *Alloionema appendiculata*, *Eudorylaimus intermedius*, *Brevibucca saprophaga*, *Cervidellus cervus*, *Pelodera serrata*, *Cruzinema tripartitum*, *Diploscapter coronatus*, *Factor similis*, *Mesorhabditis spinula*, *Neodiplogaster pissodis*, *Panagrobelus incisus*, *Paroigolaimella coprophaga*, *Pelloditis friderici*, *Pelodera teres*, *Protorhabditis filiformis*, *Rhabditis maupasi*, *Rabditis producta*, *Rhabditophanes schneideri*, *Tylocephalus auriculatus*, *Anguillula aceti*, *Tylopharinx phoetida* and *Xiphinema pachtaicum*. Species were grouped according to their feeding habits and their community composition were studied. The results obtained provide new information on the occurrence of populations and role of these organisms as indicators.

379 Dispersal ability of *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) and inhibition by *Pinus densiflora* branches

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To determine the degree of inhibition against the nematode dispersal by live *Pinus densiflora* trees, four *Bursaphelenchus xylophilus* isolates of different virulence and one *B. mucronus* isolate were inoculated separately on the upper cut ends of 2.5 and 5.0 cm long living branch sections. An increase in live section length induced an 80% decrease in the number of passing nematodes irrespective of isolates. That value was higher than 45.2% for more susceptible pine species and lower than 100% for a resistant, interspecific hybrid. Further, to determine the among-isolate difference in vulnerability to inhibitory effect of the live trees on nematode dispersal, the five

isolates were inoculated on the upper cut ends of boiled and live branch sections 5 cm long. The vulnerability to inhibitory effect of the live trees differed among isolates. Two experiments showed that virulence had no relation to intrinsic and realised dispersal rates in *B. xylophilus*. Analysis of spatial distribution of *B. xylophilus* 24 h after inoculation on 25 cm long branch sections showed the random dispersal with diffusion coefficient of 40.73 and 109.4 cm²/day for live and boiled branches, respectively.

380 Rhizodeposition and soil microbial communities in clover plants infected with the clover cyst nematode, *Heterodera trifolii*

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Below ground herbivory can alter the distribution of plant-derived soil organic matter and may impact on soil microbial diversity and functioning. Invertebrate root feeding organisms include the larval stages of many insects as well as ecto- and endoparasitic nematodes. The process of root herbivory is likely to have significant impacts on soil microbes that rely on root-derived carbon as an energy source. *Heterodera trifolii*, the clover cyst nematode, is a sedentary endoparasite of clover roots. Our objective was to determine how plant growth and rhizosphere carbon availability change when roots are infected by parasitic nematodes and how changes in the quantity and quality of rhizosphere carbon influence the structure and function of the soil microbial community. Clover was grown in microcosms in sand with or without *H. trifolii*. Rhizodeposits were washed from the sand periodically for chemical analysis. These solutions also were applied to plant-free grassland soil to evaluate the response of the microbial communities. Changes in rhizosphere chemistry were linked to microbial responses and will be discussed. The results contribute to understanding the indirect effects of plant-parasitic nematodes on the rhizosphere environment and microbial diversity and function in grasslands.

381 Ecology of plant-parasitic nematodes, their host plants and antagonists in European coastal sand dunes: training opportunity for ecologists and agricultural biocontrol researchers (*EcoTrain*)

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EcoTrain aims to examine the regulation of plant-parasitic nematodes in natural ecosystems, which processes contribute most to nematode control, and how predictable they are. The concepts and technology resulting from this study will encourage research into multitrophic interactions in natural soils, which seriously lags behind research on aboveground multitrophic interactions. Basic insights resulting from EcoTrain will also benefit research on the biological control of nematodes in production systems, which is currently based mainly on trial and error. Specific objectives of EcoTrain are: *i*) to examine and compare potential mechanisms that control population densities of plant parasitic nematodes in nature, *ii*) to bridge and stimulate scientific co-operation between phytopathologists working in production systems and ecologists working in natural environments, *iii*) to set up a trans-European network of agronomists and ecologists with a mutual interest in soil phytopathology and *iv*) to stimulate biological control of plant-parasitic nematodes in agriculture by learning how this works in nature. EcoTrain will use coastal sand dunes of Europe as a natural model system. These dunes provide one of the best case studies on interactions of natural plants and their parasitic nematodes. The natural occurrence of dominant dune grasses in monospecific stands and the heavy soil disturbance makes sand dunes a good model for agricultural production systems. Website address: <http://www.nioo.knaw.nl/projects/ecotrain/index.htm>.

382 Seasonal change of *Caenorhabditis japonica* on the shield bug, *Parastrachia japonensis*

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Caenorhabditis japonica, a species closely related to the *C. elegans* group, is associated with the shield bug, *Parastrachia japonensis* showing provisioning behaviour. To elucidate the ecology of *C. japonica*, the nematode population on the bug was investigated at a deciduous forest of Hinokuma Mountain, Saga, Japan from March 2001 to February 2002. During the period of reproductive diapause of the bug, until early May, dauer juveniles

of *C. japonica* were exclusively found as clusters under the scutellum of almost all female bugs investigated while those were rare on male bugs. During the mating, oviposition and egg guarding periods of the bug, from early May to June, nematode population on the bug remained nearly unchanged. When the first stage nymphs hatched in late June, the nematode became undetectable from female bugs. Dauer juveniles appeared on the bug at the second stage and were always detectable on nymphs of both sexes until the bugs became adults in late July. Thereafter the number of dauer juveniles on female bugs gradually increased while those on male bugs decreased and became almost undetectable in February of the next year. Ecological relationships of *C. japonica* to the life cycle of *P. japonensis* will be discussed.

383 Survey of the pine wilt nematode and its vector insects in the autonomous community of Galicia, northwestern Spain

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Surveys have been carried out to detect *Bursaphelenchus* spp. in the Galician Community because the pine wilt nematode *B. xylophilus* was reported in Portugal. Forest areas in this region represent almost 12% of the forest areas in Spain, with conifers at 7% and *Pinus pinaster* at over 25%. Besides, Galicia is one of the main routes in Spain for imported timber. Several samplings were taken between 1999-2001. The first time, 1249 samples were collected from pine trees with or without symptoms, taken every 5 km². The other samplings were gathered according to European Community rules, 2179 samples being analysed from pine trees, sawmills and imported wood. Species were identified mainly on the basis of morphometric characteristics and, when necessary, by PCR-RFLP techniques. Four species of *Bursaphelenchus* were found: *B. eggersi* in four samples from *P. pinaster* and *P. radiata* with symptoms of decline, *B. sexdentati* and *B. cf. eremus*, once each, associated with a declining pine tree close to sawmills, and *B. mucronatus* at four sawmills on *P. pinaster*, imported timber from the Ukraine and France. The vector insect for *B. xylophilus*, *Monochamus galloprovincialis*, was also collected on imported wood from sawmills.

384 Population dynamics of the potato cyst nematode (*Globodera* sp.) in two potato varieties cultivated in the Balearic Islands

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The biology and population dynamics of the potato cyst nematode (PCN) have been studied in two commercial

potato varieties cultivated in Sa Pobla-Muro (Balearic Islands, Spain) in order to arrive at locally relevant solutions for their appropriate management. The Maris Peer short cycle potato variety is usually planted in November and harvesting starts in February, while cv. Marfona, a large cycle potato variety, is usually planted in January and harvesting starts in May. In both, the highest level of second-stage juvenile hatching takes place 2 months after planting and the highest level of root invasion occurs a month later, with the plant showing damage due to the nematode infection from this time on. In cv. Maris Peer, nematodes cannot complete the whole cycle due to the early harvest, while the nematode cycle is shorter in cv. Marfona because of the warmer temperatures, thus giving a greater rate of reproduction. In the same way, the effect of PCN life cycle on host-plant development and crop loss has been studied to know the PCN population densities in which most plants can tolerate invasion and damage, without it adversely affecting potato yield.

385 Survey of plant-parasitic nematode genera in Al-Qassim area, Saudi Arabia

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An extensive survey was carried out in Buriedah City and the ten governorates of Al-Qassim area Saudi Arabia. The study was conducted to examine and evaluate the distribution of plant-parasitic nematode genera, the degree of nematode infestation and plant nematode associations. Twenty one nematode genera were found in this survey: *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus*, *Pratylenchus*, *Heterodera*, *Tylenchulus*, *Rotylenchulus*, *Hemicriconemoides*, *Macroposthonia*, *Trichodorus*, *Paratrichodorus*, *Paratylenchus*, *Trophonema*, *Hoplolaimus*, *Xiphinema*, *Longidorus*, *Hemicyclophora*, *Aphelenchoides*, *Ditylenchus*, *Aphelenchus* and *Tylenchus*. The most common genera in almost all governorates are *Meloidogyne*, *Helicotylenchus*, *Tylenchorhynchus* and *Pratylenchus*. The frequency of nematode occurrence and the population densities varied greatly between governorates. *Meloidogyne* and *Helicotylenchus* dominated on fruit trees, while *Tylenchorhynchus* and *Pratylenchus* dominated on field crops. The population densities of *Meloidogyne* were higher on vegetable crops, especially tomato and eggplant. Unayzah, Al-Mezneb, Al-Asyah governorates and the city of Buriedah

are infested with the highest nematode genera. The greatest number of nematode genera is associated with date palm trees. *Trophonema* is recorded in this study for the first time in Saudi Arabia and *Rotylenchulus* for the first time in Al-Qassim area.

386 The cereal nematode, *Heterodera avenae*, in Al-Qassim area, Saudi Arabia

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The cereal nematode, *Heterodera avenae*, is a major limiting factor in wheat production in the Al-Qassim area, Saudi Arabia. *Heterodera avenae* was first reported in a wheat field of the Al-Bekeria governorate of Al-Qassim in 1987 but, over the last 5 years, it has spread in the major wheat fields of most of the Al-Qassim governorates. Because of the lack of an economic alternative, wheat is grown continuously and such field practice allowed the cereal cyst nematode population to rise above damaging thresholds. In some heavily infested fields of Al-Qassim, the nematode population has reached 250 juveniles/g. Nematode control relies on the use of non-fumigant nematicides. Application of oxamyl nematicide as a foliar spray after wheat emergence is a common practice and often results in increases in the grain yield, but it has not reduced the nematode population below damaging thresholds for the next season. Recently, ploughing the top soil two to three times during summer time (solarisation), rotation with alfalfa or a vegetable crop, and application of organophosphate nematicides as a seed dressing have given encouraging results and have been recommended as a management practice for *H. avenae* control in wheat fields of the Al-Qassim area, Saudi Arabia.

387 Nematodes from the dorylaimid and mononchid orders associated with Canary Island crops

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In order to ascertain the effect that agricultural practices produce on the most representative species of dorylaimids and mononchids in the Canary Islands, the nematode collection at the Department of Agroecology of the Environmental Sciences Center (CSIC) was reviewed, where the majority of environments cultivated in Spain are represented. A total of 750 samples were studied. Nineteen species of the dorylaimid order were found, the most frequent being: *Aporcelaimellus obtusicaudatus* and *Belondira tarjani* (3.9%), followed by *Discolaimus texanus*, *Eudorylaimus leuckarti*, *Nyngolaimus brachyuris* and *Oxydirus oxycephalus* (2.6%). Of the samples studied, seven species were identified from the mononchid order, the most frequent being, in order: *Mylonchulus sigmaturus* (36%), *Clarkus papillatus* (13.1%) and *Mononchus truncatus* (3.9%). The dorylaimids demonstrated greater diversity while the mononchids presented greater frequency, since the latter nematodes are more susceptible to changes caused by anthropic action. Nematodes from the dorylaimid and mononchid orders are of interest because they are bioindicators of the level of alteration that agrarian systems have undergone.

388 Plant-parasitic nematodes associated with a sugar beet rotation system in central Portugal: possible implications on production

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Today, at least 37% of the world's sugar is produced from sugar beet. In many growing areas, nematodes are amongst the most important pests; capable of causing irreversible damage and unacceptable economical crop loss. Population dynamics of plant-parasitic nematodes is essential to anticipate crop damage caused by these pathogens and parasites. The Coruche region in the province of Ribatejo, Portugal, is the location of a major sugar beet production region. A preliminary survey and study was conducted during 2001 to evaluate the presence of taxa and their relative abundance from three fields where a sugar beet-corn rotation system is in use. The main phytoparasites collected belonged to the genera *Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Tylenchorhynchus*, *Trichodorus* and *Heterodera*. Two sampling seasons were established: autumn and spring; 200 g soil samples were collected for nematode extraction, which was performed according to two

methods: Baermann funnel and centrifugal flotation. Nematodes were counted using an inverted microscope and photographed with a digital camera, from a compound microscope. *Helicotylenchus* sp. appears to be particularly numerous, and may be mainly responsible for causing a negative effect on the crop.

389 Host range study of an undescribed *Meloidogyne* sp. isolated from groundnut

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The reproduction of a new *Meloidogyne* sp. (93-13) isolated from groundnut in Collingsworth County (Texas) was tested against a range of different plants species and compared to a population of *Meloidogyne arenaria* race 1. Pea cvs Progress and Early Snap were moderate hosts (RF = 1.57 and 1.18). The bean cvs Bountiful and Blue Lake 274 had low reproductive rates (RF = 0.69 and 0.75) of *Meloidogyne* sp. 93-13, whereas Pea cv. California Blackeye 5 and 46, and Clemson Purple (RF = 0.05, 0.02 and 0.09) were resistant. Genetically modified (Roundup Ready) soyabean cvs HBKR 4660, 4855, 4920, 5588, 5920, 6020, 6600, and conventional soyabean cultivars HBK 4891, 5990, 5991, were also tested. The best soyabean with resistance to the two species of root-knot nematodes is HBKR 4660, followed by HBKR 6020 with high levels of resistance to *M. arenaria*, and HBK 5991 with intermediate levels of resistance to *M. sp.* 93-13. A comparison between the conventional and the genetically modified soyabeans did not show any significant difference ($P = 0.05$) in any of the variables evaluated.

390 Cotton nematode distribution and density in the USA

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In 1986 a nematode survey was started in the state of Mississippi. A beltwide program was initiated in 1989 under the direction of the National Cotton Council and with industry support. The purpose of the survey was to determine nematode species and population densities. Yield losses were also determined as each

state used a standard treatment of control. Yield losses were determined by using nematicides compared to an untreated control. The survey has been completed on more than 60% of the cotton producing counties and parishes in the USA. The major species found in the survey are root-knot, reniform, lance, and sting nematodes. The root-knot nematode is found in most areas where cotton is grown. The reniform nematode is prevalent in the south-east and mid-south sections of the cotton belt. The lance and sting nematodes are concentrated in the southeast. The survey maps show the three major species present and their relative population levels. These maps are updated annually and can be seen on the National Cotton Council web site at <http://www.cotton.org/cf/nematodes>.

391 First records of *Bursaphelenchus* species in Thailand

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The damage caused by the pine wood nematode (*Bursaphelenchus xylophilus*) in several East Asian countries (Japan, China, Taiwan) and its easy spread by the timber trade provoked scientific interest in tree-inhabiting *Bursaphelenchus* species. Thailand is a country with high temperatures which make it favourable for the development of pine wilt caused by *B. xylophilus*. Pine forest occurs naturally in small tracts in some upland areas at altitudes between 800 and 1800 m. Native pines are *Pinus merkusii* and *P. kesiya*. Wood samples collected in North Thailand were examined for the presence of *Bursaphelenchus* spp. The species found were *Bursaphelenchus hylobianum*, *B. mucronatus* as well as two undescribed species. The pine wood nematode was not present in these samples. However, the occurrence of *B. mucronatus*, a species closely related to *B. xylophilus* and transmitted by the same vectors (*Monochamus* spp.), indicates the possible establishment of *B. xylophilus* in case of its introduction to Thailand. Special quarantine attention should be paid to wood imports (sawn coniferous timber, logs, wooden packaging material) from countries where the pine wood nematode occurs.

392 Nematode genera associated with mango in Zulia State, Venezuela

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We have identified nematode genera associated with mango (*Mangifera indica* L.) in Zulia state. Forty samples of roots and soil from production farms in Mara and Urdaneta Municipality were processed with a combination of Oostenbrink funnels and Baermann pans. Roots were ground at low speed. Results showed the presence of *Hemicriconemoides*, *Xiphinema*, *Meloidogyne*, *Hoplolaimus*, *Rotylenchulus*, *Tylenchus*, *Trichodorus* and *Tylenchorhynchus* in Mara-Urdaneta, with the highest frequency for *Rotylenchulus*, *Hoplolaimus* and *Meloidogyne*. A brief description is reported and identification key is proposed.

393 Nematode pests of potato in the mesothermic valleys of the Andean region, Santa Cruz Department, Bolivia

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The mesothermic valleys in the Andean region of the Santa Cruz Department occur at altitudes of 1400 to 2500 m a.s.l. Potato production, both with *Solanum tuberosum* var. *tuberosum* and *S. tuberosum* var. *andigena*, has increased in these warmer mid-altitude valleys. Intensification of potato cropping systems has led to increased severity of a complex of potato diseases including nematodes. Over 70 farm fields were examined in seven valleys. All the three main nematode groups: the root-knot nematodes, *Meloidogyne* spp. (mainly *M. incognita* and *M. javanica* but also *M. hapla*), the false root-knot nematode, *Nacobbus aberrans*, and the potato cyst nematodes, *Globodera* spp. (mainly *G. pallida* but also *G. rostochiensis* plus *G.(?)tabacum*) were

found. *Meloidogyne* species occurred in over 30% of farms; *Nacobbus* in 20%; and *Globodera* also in over 20%. Seed tubers are moved from the traditional potato growing areas in the higher and cool altiplano and both *Meloidogyne* and *Nacobbus* were found in these seed tubers. Thirty parasitic species were found on potato and another undescribed species of *Globodera* was also identified, occurring in large numbers on the roots of *Brassica rapa*.

394 Nematode genera associated with merey (*Anacardium occidentale*) in Zulia state, Venezuela

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In order to identify the main nematodes associated with merey (*Anacardium occidentale*) in Zulia state, a survey of nematodes was performed in Mara municipality farms and 60 soil and root samples were taken. The samples were processed with a combination of Oostenbrink and Baermann funnel equipment. Results showed the presence of *Dorylaimus*, *Aphelenchus*, *Tylenchus*, *Helicotylenchus*, *Tylenchorhynchus*, *Criconemoides*, *Hoplolaimus* and *Xiphinema*, with *Aphelenchus* as most common followed by *Helicotylenchus*. Finally, a brief description is reported and an identification key is proposed.

395 Plant-parasitic nematodes associated with semeruco in Zulia State of Venezuela

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Plant-parasitic nematodes affect production in most crops in Mara Municipality Zulia state. In order to evaluate the nematodes in semeruco (*Malpighia glabra* L.) crops, a nematological sampling was performed where 60 soil and roots samples were analysed. Samples were processed with a combination of Oostenbrink and Baermann funnel

equipment. Roots were ground at low speed. Seven genera were identified: *Meloidogyne*, *Criconemoides*, *Discolaimus*, *Dorylaimus*, *Tylenchus*, *Hoplolaimus* and *Acrobeles*. The genus *Meloidogyne* was found to be the main genus. A brief description is given and an identification key is proposed.

396 *Heterodera mediterranea*: biological observations with emphasis on factors affecting egg hatching and differential responses of olive cultivars to infection

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The influence of temperature and olive root exudates on *Heterodera mediterranea* egg hatch and the effects of *H. mediterranea* on the growth of two olive cultivars (Arbequina and Picual) were investigated. Egg hatch occurred over a temperature range of 10-30°C and was optimal at 20-25°C. There were no differences in egg hatch between sterile deionised distilled water or root exudate dilutions (undiluted, diluted 1:1, and 1:2) of Arbequina and Picual at 20°C. *Heterodera mediterranea* reproduced on both olive cultivars in growth chambers at 25°C. Soil and root final nematode populations, as well as total number of cysts per plant and reproduction rate, were significantly higher in Arbequina than in Picual. Shoot dry and root fresh weights, and increases of shoot height, trunk diameter and numbers of nodes were significantly suppressed by infection with 10 000 eggs + second stage juveniles/pot in Arbequina but not in Picual. *Heterodera mediterranea* completes its life cycle (embryogenic and postembryogenic development) in about 60-70 days on olive roots. Syncytia formation and disorder of root stellar structure are the main anatomical changes induced by the parasite on olive cvs Arbequina and Picual roots.

397 Survey of nematodes in Andean potato fields of northwest Argentina

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A germplasm collecting expedition was carried out during the autumn of 2001 to collect Andean potato cultivars (*Solanum tuberosum* ssp. *andigena*) in the province of Jujuy, Argentina (23°S). Local farming practices usually involve the purchasing or bartering of varieties in market places but in a few cases some varieties are sold in major centres. As some nematodes are a potential threat for the cultivation of the potato, the detection of the species present in the potato-growing areas is needed in order to establish quarantine methods. Twenty-five sites situated at altitudes ranging from 2370 to 3885 m were sampled and nematodes extracted from 100 cm³ of soil with modified centrifugation-flotation technique. Sixty-five genera of nematodes were found in the samples. The plant-parasitic nematodes were mostly represented by *Pratylenchus* sp. (69%), *Meloidogyne* sp. (65%) and *Nacobbus aberrans* (61%). Four new localities were added to the distribution of *Globodera tabacum* in the region. The bacterial-feeders were represented mainly by *Chiloplacus* (77%), *Rhabditis* and *Seleborca* (42%); fungivores by *Aphelenchus* (96%), *Ditylenchus* (85%) and *Aphelenchoides* (57%); predators by *Aporcelaimellus* (54%) and omnivores by *Ecumenicus* (35%). The genera *Bitylenchus*, *Butlerius*, *Cervidellus*, *Crassolabium*, *Dolichorhynchus*, *Enchodelus*, *Labronemella*, *Seleborca*, *Stegelleta* and *Zeldia* are new records for Argentina.

398 Nematodes of citrus in open nurseries and orchards in São Paulo State, Brazil

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A survey of key-nematodes of citrus was conducted in open nurseries and orchards in São Paulo State Brazil. Totals of 2518 samples from 595 nurseries in 99 counties, and 1078 samples from commercial orchards in 86 counties were examined. The nematodes were separated from 100 cm³ of sampled soil by centrifugal flotation and from 10 g of sampled roots by maceration in a blender followed by centrifugal flotation. The citrus nematode (*Tylenchulus semipenetrans* Cobb) was found in 34% of the nurseries and in 72.5% of the commercial

orchards. Putative populations of *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven were found in seven nurseries and ten orchards. These populations pertain to a new species of lesion nematode that is being described and named *Pratylenchus jaehni*. Maps of the distribution of these nematodes throughout the main region of citrus production in São Paulo State were prepared.

399 Nematode problems in Castilla y Leon (Spain), a continental Mediterranean climate

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Plant-parasitic nematode problems were analysed in the region of Castilla y Leon, northwestern Iberian Peninsula. Nematode groups, in the order of their importance, are: i) cyst nematodes, represented by *Globodera rostochienis* and *G. pallida* in potato, *Heterodera schachtii* in sugar-beet, *H. avenae* in cereal, and *H. humili* in hops; ii) the root-knot nematode *M. incognita* in sugar-beet and potato; iii) virus vector nematodes, among which *Xiphinema index* stands out, of interest in vineyards; iv) bulb and stem nematodes, *Ditylenchus dipsaci* in garlic; v) the endoparasitic nematodes *Pratylenchus*, *Pratylenchoides* and *Zygotylenchus*; vi) ectoparasitic nematodes, where *Macroposthonia xenoplax* stands out for causing chlorosis in grape and fruit trees (*Prunus* spp.). Species from the *Amplimerlinius*, *Anguina*, *Aphelenchoides*, *Bitylenchus*, *Criconema*, *Criconemella*, *Criconemoides*, *Crossonema*, *Helicotylenchus*, *Hemicriconemoides*, *Hemicycliophora*, *Hoplolaimus*, *Longidorus*, *Macroposthonia*, *Merlinius*, *Ogma*, *Paratrichodorus*, *Paratylenchus*, *Quinisulcius*, *Rotylenchus*, *Scutellonema*, *Serriespinula*, *Trichodorus*, *Tylenchorhynchus* and *Xenocriconemella* genera were also found. Various control alternatives are indicated, such as preventative practices and crop rotation, which regulate the development of pathogenic nematode populations as well as the appearance of phytopathological problems. These alternatives must be followed by the use of resistant plants and trap crops within an integrated production system.

400 *Heterodera glycines* distribution within a field in no-till production over time

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Heterodera glycines (SCN) is a major pest of soybean causing significant yield losses when present and when management techniques to reduce egg population density are not used. A study investigated the distribution of SCN at planting and harvest in a claypan soil field in central Missouri under no till soybean production. Comparisons were made in the nematode distribution in 1999 and 2001 years of soybean production. Nematode distribution in the 2 years was compared to yield, topsoil thickness, elevation, and soil nutrient maps. Nematode distribution was most closely correlated with topsoil thickness (*i.e.*, depth to the claypan). Lower *H. glycines* population density occurred in areas with shallow topsoil thickness.

401 Tobacco rattle virus and its associated vector trichodorid nematodes in Portugal

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Tobacco rattle virus (TRV, genus *Tobravirus*), transmitted by trichodorid nematodes, occurs as a wide range of serologically distinguishable strains that cause diseases in various economically important crops. The virus and its associated vector nematodes occur world-wide, being particularly prevalent in Europe and North America. In Portugal, an investigation is being conducted to identify TRV strains occurring in association with their natural vector species. The project has focused on potato growing areas in northern and central Portugal, with soil sampling biased in favour of areas where TRV-like symptoms have been reported. Nematodes were extracted from soil and phenotypically identified. TRV was recovered from nematodes in virus transmission studies, and identification confirmed using sap transmission tests, a leaf squash method with EM, ELISA, ISEM, and RT-PCR. Trichodorids were recovered frequently from potato fields, and the species identified were: *Trichodorus*

lusitanicus, *T. primitivus*, *Paratrichodorus anemones*, *P. hispanus*, *P. minor*, *P. pachydermus*, also three new undescribed species and an unidentified *Paratrichodorus* species. TRV occurred in four of 58 (7%) soil samples in association with trichodorids: one in the north and the others in the centre of the country. Molecular characterisation of the TRV isolates and their associated vector trichodorids is presently in process.

402 Nematodes associated with coniferous woods in Spain

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Nematodes associated with coniferous woods in Spain were studied in 2000-2001. Sampling was coordinated by the Spanish Ministry of Agriculture, Fisheries and Food, with the cooperation of the official forestry, plant health services and diagnostic laboratories of several autonomous communities. Seven nematode genera were found on conifer trees. *Laimaphelenchus penardi*, the most widespread species in the Peninsula and on the Balearic Islands, was found associated with *Abies alba*, *Pinus nigra*, *P. pinaster* and *P. pinea*; *Cryptaphelenchus* spp. and *Deladenus* spp. are also widespread, but their species are under study. Seven species of *Bursaphelenchus* were found, *B. sexdentati* being the most widespread in Peninsular Spain associated with *Pinus* spp. *Bursaphelenchus eggersi* and *B. mucronatus* appeared in northern Spain associated with *Abies alba* and *Pinus pinaster*, and on *Pinus halepensis* and *Pinus* spp., respectively. Other species are more localised: *B. fungivorus* on *P. pinaster* in the Cazorla mountains, Andalusia; *B. leoni* and *B. teratoespicularis* restricted to the Balearic Islands on *Pinus halepensis* in Mallorca and Ibiza; *B. pinasteri* appearing once on *Pinus pinaster* in Extremadura; *Aphelenchoides daubidaensis*, *A. jonesi* and *Ektaphelenchus macrobulbosus* on *P. pinaster* and *P. nigra* in the west, and finally, *Seinura* spp. on *Pinus* spp. from northeastern Spain.

403 Nematode problems in the region of Murcia (Spain), a Mediterranean model

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Plant-parasitic nematode problems in the region of Murcia in the southeastern Iberian Peninsula were analysed. The nematode groups, in the order of their importance are: i) the root-knot nematodes, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, which affect horticultural crops and fruit trees; ii) virus vector nematodes, among which *Xiphinema index*, of interest in vineyards, stands out; iii) the citrus nematode, *Tylenchulus semipenetrans*, found in citrus orchards and vineyards; iv) cyst nematodes, represented by *Heterodera cruciferae* in broccoli, *H. latipons* in oats and *H. schachtii* in chard; v) the endoparasitic nematodes *Pratylenchus* and *Pratylenchoides*; and vi) ectoparasitic nematodes, among which *Macroposthonia xenoplax* stands out for causing chlorosis in grape vines and fruit trees (*Prunus* spp.). Species were also found from the genera *Criconea*, *Criconemella*, *Criconemoides*, *Crossonema*, *Hemicriconemoides*, *Helicotylenchus*, *Merlinius*, *Paratylenchus*, *Rotylenchus* and *Tylenchorhynchus*. Various control alternatives are indicated, especially preventative practices to regulate the development of pathogenic nematode populations and to prevent the appearance of phytopathological problems, followed by the use of resistant plants and biofumigation, all within an integrated production system.

404 Distribution of *Globodera tabacum* in the La Vera region of Spain

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La Vera is the principal tobacco growing area in Spain. Every year 11 000 ha of flue-cured and 2500 ha of burley tobacco are produced in monoculture. The most important phytonematological problem in the crop is caused by root-knot nematodes. However, in August 2001 *Globodera tabacum* was first detected over a K326 flue-cured variety field. To know the incidence of these cyst nematodes in this tobacco region, a survey of *Globodera* populations was carried out during February 2002. 82 soil samples were gathered in fields where tobacco was cultivated in 2001. Soil was analysed for nematode abundance (cyst and second juveniles). *Globodera* is widely distributed

across the region. Abundance and distribution of this nematode in the La Vera tobacco area is shown.

405 Characterisation of cereal cyst nematodes, *Heterodera* spp., in Norway

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Cereal cyst nematodes (CCN), *Heterodera* spp., are common pests of cereal crops in Norway. Fifteen populations were investigated morphologically, through biotests, and through electrophoretic studies, using isoelectric focusing and silver staining of proteins. In the biotest the populations were grouped by their virulence on barley differentials (resistance genes) Varde (*Rha*), Emir (*Rha* "E"), Ortolan (*Rha1*), KVL 191 (*Rha2*), Siri (*Rha2*) and Morocco C.I. 3902 (*Rha3*). Eleven populations expressed themselves as *H. avenae* pathotype Ha11. These were all fairly similar morphologically and electrophoretically. The biotest further suggested two populations belonging to pathotype Ha12. One of them was morphologically and electrophoretically similar to *H. avenae*; the other different in both respects, indicating a possible undescribed species. Two other populations appeared to be *H. filipjevi*, pathotype 'West'. One population under study was different in all aspects, morphologically, in the biotest and biochemically. Earlier studies in Norway have also indicated the occurrence of CCN pathotype Ha51. Thus, the situation in Norway regarding the CCN is very complicated.

406 First year interim results of investigations on the nematode fauna in the pedobiocoenosis of grape phylloxera (*Dactylophora vitifolii*) infested vines (*Vitis vinifera*) in selected Austrian vine production areas

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Investigations about the potential correlation between the occurrence of plant-parasitic nematodes and the increased grape phylloxera infestation of vines in Austrian vineyards are being carried out within a 3 year research project. Roots and soil adhering to the roots were investigated. In 2001, more than 100 samples in total were collected from eight selected vineyards in Lower Austria and Burgenland at monthly intervals from May to August. Subsamples of both highly phylloxera-infested and uninfested grapevines were checked for the presence of free-living and plant-parasitic nematodes, applying standard extraction methods. The predominant nematode genera, which were regularly detected and identified in infested as well as in uninfested root samples, were *Aphelenchus* sp., *Aphelenchoides* sp. and *Helicotylenchus* sp. Plant-parasitic nematodes from eight families and free-living nematodes from 14 families were identified from soil samples. Prospects for the investigations in 2002 are presented.

407 A survey study of phytoparasitic nematodes and the associated host plants in Egypt

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Information concerning the occurrence and distribution of phytoparasitic nematodes is very important to assess their potential to cause economic damage to many crop plants. A nematological survey was conducted to determine the genera and species of phytoparasitic nematodes and the associated host plants in Egypt. The results indicated the occurrence of about 56 genera and 180 species of phytoparasitic nematodes associated with many cultivated plants, grasses and weeds. Many of these nematodes, e.g., *Criconemella* spp., *Helicotylenchus* spp., *Heterodera* spp., *Hoplolaimus* spp., *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Pratylenchus* spp., *Rotylenchulus reniformis*, *Tylenchorhynchus* spp., *Tylenchulus semipenetrans* and *Xiphinema* spp. are considered limiting factors in crop production in Egypt.

408 Comparison of boarding ability between *Bursaphelenchus xylophilus* and *B. mucronatus* on their vectors, *Monochamus alternatus* and *M. saltuarius*

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Bursaphelenchus xylophilus, the causative agent of pine wilt disease, has been introduced from North America to Japan and displaced the closely related, native nematode, *B. mucronatus*, in the disease infestation area because the two nematode species occupy the same niche in the pine forest ecosystem. Both nematode species enter the tracheal system of newly emerged adult beetles in the genus *Monochamus* within pupal chambers of the beetles in xylem. To compare the boarding ability of two nematode species on the beetles, one of the plausible processes of nematode displacement, post-diapause larvae of *M. alternatus* or *M. saltuarius* were placed individually in artificial pupal chambers of *Pinus densiflora* bolts inoculated with *B. xylophilus* or *B. mucronatus*. *Bursaphelenchus xylophilus* load on beetles was significantly greater than *B. mucronatus* load for the two *Monochamus* beetles, although there was no difference in the abundance of dispersal juveniles around the pupal chamber between the two nematode species. These results showed an extremely low ability of *B. mucronatus* to board the beetles compared with *B. xylophilus*.

409 Acquisition, retention and transmission of tobnavirus particles by trichodorid nematodes

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Tobnaviruses are naturally transmitted by plant-parasitic *Trichodorus* and *Paratrichodorus* nematodes. Trichodorids are assumed to acquire virus particles upon sap ingestion from virus infected root cells. Subsequently, these particles are retained on the cuticle lining the nematode pharyngeal wall and thereafter are released into further cells upon salivation of pharyngeal secretions. Real-time observations of living trichodorids feeding on roots of tobacco seedlings revealed that although attached cells are

customarily killed, a 5-10% proportion is probed, the cell walls are apparently perforated; however, the nematodes depart prior to sap ingestion, thus leaving these cells alive. Additionally, transmission electron microscopy study revealed differences in the sites of retention of serologically distinguishable tobnavirus strains in the pharynx of different (*Para*)*trichodorus* vector and non-vector species, *i.e.*, it revealed three different patterns of retention. Subsequently, immunogold labelling with antisera designed specifically to identify these tobnavirus strains from one another and from other viruses, provided unequivocal evidence of the tobra-viral identity of virus-like particles observed within the pharyngeal lumen of the examined trichodorid species.

410 Distribution and temporal population dynamics of *Meloidogyne incognita* and *Thielaviopsis basicola* in an Arkansas cotton field

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Thielaviopsis basicola (Berk. & Broome) Ferraris, causal agent of black root rot of cotton, and the root-knot nematode, *Meloidogyne incognita*, frequently occur together in cotton fields in Arkansas. Where both organisms occur together, black root rot in seedlings is significantly more severe and seedling mortality is greatly increased. Infection of cotton seedlings by the nematode appears to broaden the temperature range at which *T. basicola* can infect the seedlings, and the nematode provides the fungus access to the vascular tissue of the plant that is not normally invaded in the absence of the nematode. A 6.6 ha cotton field in southeastern Arkansas, with a history of severe seedling disease and root-knot

nematode damage, was divided into 512 grids (4 × 30 m) and sampled prior to planting a cotton crop (April 2001) and immediately after cotton harvest (October 2001) to determine the population densities of both *T. basicola* and *M. incognita* in each grid. In addition, nematode populations were monitored in 80 arbitrarily selected grids at monthly intervals during the growing season. Spatial and temporal relationships of these organisms and their relationship to various edaphic factors are described.

411 Presence of *Heterodera trifolii* Goffart, 1932 (Nematoda: Tylenchida) in Argentina

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The analysis of roots of *Trifolium repens* and of the soil around them, from the locality of Malargüe, province of Mendoza, Argentina, permitted the identification of representatives of the nematode *Heterodera trifolii*. White females with masses of eggs and cysts were observed on roots; second-stage larvae were extracted from the soil. Morphological and morphometric studies performed on these specimens confirm the species identity, which is cited in Argentina for the first time.

412 Nematodes in international quarantine legislation

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The Society of Nematologists currently has a USDA funded project to list and rank exotic nematodes of regulatory concern to the USA. A list of nematodes, which are regulated by international quarantine legislation in 2002, was compiled using the APHIS/USDA data base, and was compared to Kahn's 1982 list of the ten most frequently regulated nematodes. In general, in the past two decades the number of countries regulating nematodes has increased. For example, 51 countries regulated *Globodera rostochiensis* in 1982, but 106 countries regulate it in 2002. Worldwide, *G. rostochiensis* is the most regulated

nematode and ranks second only to potato wart fungus, *Synchytrium endobioticum* (108 countries) in a list that includes regulated insects pests and plant pathogens. The rank for many nematodes has changed the past two decades, which indicates that regulations are not static. For example, in 1982 *Aphelenchoides besseyi* ranked tenth and was regulated by nine countries, but in 2002 it ranked second with 70 countries regulating it, primarily to protect rice crops. In contrast, in 1982 11 countries had regulations to exclude *Meloidogyne javanica*, but in 2002 no country listed it as a prohibited nematode.

413 Biogeographical characterisation of Trichodoridae in the Iberian Peninsula

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The existence of two faunistic groups have been found on analysis of the distribution patterns of the 18 species from the family Trichodoridae that have been found in representative crops and environments of the Iberian Peninsula. The first one represented by the autochthonous species, *Paratrichodorus hispanus*, *Trichodorus azorensis*, *T. beirensis*, *T. giennensis* and *T. lusitanicus*, is present in uncultivated and cultivated areas; *T. azorensis*, *T. beirensis* and *T. giennensis* have been found very localised, while *P. hispanus* is widespread in Spain and northern Portugal and *T. lusitanicus* is common mainly in southern but also found in central Portugal. The second one is defined by the plant-parasitic and virus vector species, *P. minor*, *P. pachydermus*, *P. teres*, *T. primitivus*, *T. sparsus* and *T. viruliferus*, in which *P. anemones* and *T. similis* could also be included, in spite of their very localised presence. *Paratrichodorus anemones*, *P. pachydermus*, *T. similis* and *T. viruliferus* could be regarded as characteristic species from temperate environments, while *P. minor*, the most widespread species in subtropical crops, has also been found in the Canary and Madeira Islands. On the other hand, *P. teres*, *T. giennensis*, *T. similis*, *T. sparsus* and *T. viruliferus* have only been found in Spain, while *P. acutus*, *P. allius*, *P. nanus*, *P. porosus*, *T. azorensis* and *T. orientalis* appeared very localised only in Portugal, *P. acutus*, *P. porosus* and *T. azorensis* appearing only in the

Azores and Madeira Archipelagos. Climatic, vegetation and soil type influence are discussed.

414 Association of *Melilotus albus* Desr. with a population of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 in the province of Mendoza, Argentina

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A population of *M. javanica* was detected in the locality of Malargüe, province of Mendoza, infecting roots of *Melilotus albus*. The plant was at the flowering stage at the time of analysis. Its aerial portion had a normal aspect. However, a great number of galls containing females of the nematode were observed on roots. In some galls, the posterior end of females protruded beyond the tissues; in other galls, females were inside them. Internally, giant cells were observed in the central cylinder, which reduced the vascular tissues and also caused important anomalies in their arrangement. The nematode develops its life cycle completely, producing an egg mass. It is concluded that the nematode – host relationship is close, and that the plant represents a good host for this species of the genus *Meloidogyne*. This weed is cited as an alternative host of a phytophagous nematode in Argentina for the first time.

415 Population dynamics of the pine wood nematode, *Bursaphelenchus xylophilus*, in dead pine trees

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Experimental results of inoculation tests and observations on naturally infected pine trees demonstrated that the pine wood nematode was detected rarely in wood at the time when disease symptoms appeared, such as the cessation of oleoresin exudation from wounds. The nematode population in wood of a diseased tree increased

as the disease advanced more. Nematode reproduction was rapid and a huge population developed in the wood of a diseased tree. About 50 million nematodes were calculated to be in the stem wood of a dead tree 10 m tall and 15 cm diameter at breast height. Nematodes were distributed throughout the dead tree. Spatial distribution patterns of nematodes varied considerably among dead pine trees. Aggregated distribution was demonstrated in the stem. Nematode populations in dead trees declined gradually after reaching a maximum level, about 3000 nematodes per g of dry wood, and were almost extinct in July of the following year. In this stage of the population dynamics, dispersal third stage juveniles appeared and the proportion of them in the whole population increased gradually.

416 Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, in Swedish potato fields and their relationships to other European populations

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Sixteen Swedish potato cyst nematode populations were compared with 19 populations from the Netherlands, United Kingdom, Germany and Norway as to genetic and virulence variability through AFLP analysis and biotests. Obtained data indicate a large heterogeneity, and the present occurrence seems to be the result of several older and more recent introductions. Swedish Ro1/Ro4 populations of *Globodera rostochiensis* came from heavily infested fields, had a general European appearance, and may be supposed to be very old introductions. Members of virulence group Ro2/3 of this species were genetically more heterogeneous but similar to continental populations. They may have been introduced later. Swedish *G. pallida* populations of virulence group Pa2/3 could according to the AFLP analysis be divided into two subgroups, one similar to a British population and the other to continental populations. *G. pallida* populations belonging to the 'British' group were pure populations and probably fairly old introductions. Pa2/3 populations of the 'continental' group were, with one exception, found in the starch potato district where they had been selected during intensive cultivation of cultivars with *Solanum tuberosum* ssp. *andigena* resistance. They may be fairly recent

introductions. Norwegian populations of both species seemed to have backgrounds similar to the Swedish ones.

417 Nematodes from an urban forest in Sofia, Bulgaria

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Generic composition, trophic structure, diversity and Maturity indices of nematode assemblages were studied at Knyaz Borisova Gradina City Park. The study site was a 60-90 year-old mixed deciduous-coniferous forest dominated by *Quercus pedunculiflora* C. Koch. Litter and soil samples: decomposition layer, ca 0.5-1 cm, and humus layer at three depths: 0-5, 5-10 and 10-25 cm were collected. Ninety nematode genera were registered. Generally, nematode abundance was low and differed between samples and depths. Bacterial feeders were the most common, abundant and diverse (36 genera) group, followed by plant feeders (22 genera). A clear difference between litter and soil nematode assemblages was revealed in regard to almost all parameters studied. Nematode communities from decomposition and humus layers were similar in taxonomic and trophic group structure. In soil layers plant-feeding nematodes prevailed, omnivores were well represented while bacterial feeders decreased with depth. No clear layer dependent patterns in generic distribution were found. Maturity Indices of soil communities were high and their values increased gradually with depth. Nematode soil assemblages were characterised by high diversity and maturity, which could/might be related to the age, heterogeneity and size of the forest studied although it is situated in an urban environment.

418 Tourist flow effect on Nematoda of Brazilian sandy beaches

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This study is an attempt to evaluate the impact of human activity on the Enseada Beach during summer, comparing it with the preserved Una Beach, through the study of the

taxon Nematoda. At each beach, sampling was undertaken at three points along three profiles with a copper core of 28 mm diameter to a depth of 20 cm. Each sample was divided into layers of 5 cm. Samples for sedimentological analysis were collected and temperature, salinity, pH and slope profile measurements were made. Nematoda mean density at the Enseada Beach ranged from 719.5 ind. $\times 10 \text{ cm}^{-2}$ in December 98 to 1299 ind. $\times 10 \text{ cm}^{-2}$ in June 99. At the Una Beach mean density ranged from 751.8 ind. $\times 10 \text{ cm}^{-2}$ in February 99 to 1570.1 ind. $\times 10 \text{ cm}^{-2}$ in November 99. On the Enseada Beach in February 99, at the points where human density was higher, Nematoda was found deeper in the sediment. In March 99 Nematoda was concentrated in the first layer of the sediment. Nematoda is being identified at genus level to seek for variation in vertical and temporal distribution.

419 Nematodes of Haughton Crater

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Haughton Crater has been called Mars on Earth due to presumed similarities with craters on Mars. Sixteen km in diameter, Haughton Crater is located on Devon Island, Nunavut, Canada approximately 640 km south of the north magnetic pole. Plant communities in the crater are frequently found in isolated patches surrounded by a coarse, rock and gravel substrate. The nematode communities within these 'micro-oases' are surprisingly diverse. To date 75 species have been identified. Compared with other nematode communities, the micro-oasis nematode community is dominated by predators and omnivores and includes an unexpectedly large number of males. Outside the micro-oases, a less diverse community exists, with 58 species identified to date, including 27 species not observed within the micro-oases. Overall nematode abundance ranged from 22 to 4129 nematodes per 100 cm³ of soil. All nematodes observed in this study were revived from an anhydrobiotic state. The diversity and abundance of nematodes in Haughton Crater sharply contrasts with the depauperate communities of the Antarctic dry valleys.

420 Phytoparasitic nematodes associated with the rhizosphere of lulo, mora, tree tomato and granadilla plants in Colombia

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Nine genera of phytoparasitic nematodes were found associated with the rhizosphere of lulo or naranjilla (*Solanum quitoense* Lam.), tree tomato or tamarillo (*Cyphomandra (Solanum) betacea (betaceum)* (Cav.) Sendt), granadilla (*Passiflora ligularis* Juss.) and mora (*Rubus glaucus* Benth.) in different Colombian regions. The genus *Meloidogyne* was both more prevalent and abundant in all the locations sampled. *Meloidogyne incognita*, *M. javanica*, *M. hapla* and *M. arenaria* were identified in the rhizosphere from lulo and tree tomato whereas *M. incognita* and *M. hapla* were found in the rhizosphere of granadilla. On the other hand some of the above mentioned nematode species were found in soil borne on mora plants but *M. incognita* and *M. javanica*, *Helicotylenchus* sp. and *Paratylenchus* sp. do not reproduce in this plant. *Trichodorus*, *Hemicyclophora* and *Pratylenchus* do reproduce in mora plants. Other important genera associated with all the fruit plants studied were *Xiphinema*, *Paratylenchus*, *Hirschmanniella* and *Criconemella*. A strong interaction was found between *Meloidogyne incognita* and *Fusarium solani* which reduced development and growth parameters of granadilla plants.

421 Host status of the common weeds of banana establishments to banana nematodes in Uganda

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Host status to the banana nematodes *Radopholus similis*, *Helicotylenchus multicinctus* and *Pratylenchus goodeyi* was studied in 13 common weed species, in a greenhouse experiment. Banana nematodes were recovered from soil, roots or both in banana and nine weed species, but nematode population densities indicate that these weed species are poor hosts of the banana nematodes. *Helicotylenchus multicinctus* was the only nematode

species recovered from soil and occurred on banana and only six weed species, at population densities much lower than that of banana. From roots, *R. similis* was recovered from banana only while *H. multicinctus* was present on banana and nine weed species and *P. goodeyi* was present on banana and only two weed species, namely, *Digitaria velutina* and *Eleusine indica*, at a very low count compared to banana. No nematodes were recovered from *Tagetes minuta*, *Cyperus esculentus*, *Senecio disfolius* and *Digitaria scalarum*, indicating that these four weed species are non-hosts of banana nematodes.

422 The occurrence of burrowing nematodes *Radopholus* spp. on durian and coffee in Western Highland of Vietnam

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Burrowing nematodes associated with durian (*Durio zibetinus*), coffee (*Coffea* spp.), banana (*Musa paradisiaca* var. *sapientum*), rambutan (*Nephelium lappaceum*) and avocado (*Persae americana*) have been recently recorded in Western Highland Vietnam. The nematodes are widely distributed on durian orchards and coffee plantations; only a few specimens were extracted from root samples of banana, avocado and rambutan. In the infected regions, *Radopholus* was not detected on corn (*Zea mays*), black pepper (*Piper nigrum*), mango (*Mangifera indica*), citrus (*Citrus* spp.), pineapple (*Ananas sativa*), sugarcane (*Saccharum officinarum*) and papaya (*Carica papaya*). Nematodes caused decline and death of trees in many durian nursery gardens and newly replanted orchards. Nematode densities reached several thousands of individuals per g for root samples. Nematodes also induced some damage in coffee plantations (*C. arabica* and *C. robusta*). Morphological and morphometrical comparisons of all nematode stages revealed only slight differences between *Radopholus* populations from durian and coffee and from *R. similis*. However, the molecular analysis based on sequencing of ITS-rDNA showed substantial nucleotide differences

between these Vietnamese populations and *R. similis* and supports their separate taxonomic status.

423 The frequency and diversity of the cyst and lesion nematodes on wheat in the Turkish Central Anatolian Plateau

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In June 2000 a survey was conducted on the Central Anatolian Plateau, the major winter wheat growing region of Turkey. The objective was to understand the distribution of two economically important cereal nematodes, cyst (*Heterodera* spp.) and lesion (*Pratylenchus* spp.) Thirty soil and plant samples were collected around the milky dough stage from wheat sampled about every 50 km. Seventy two percent of the root samples and 83% of the soil samples contained cysts, and in approximately 40% of soil samples, one or both lesion nematodes were found (*P. thornei* and/or *P. neglectus*). Cereal cyst nematode was identified to species level using both traditional morphology and a RFLP PCR based molecular method. None of the samples contained the most common cereal cyst nematode documented, *H. avenae*. Instead, 40% of the samples contained *H. latipons*, 32% *H. filipjevi* and 28% a mix of both species. Regarding species identification, in about 10% of the samples the molecular diagnostics did not align with the morphological assessment. Furthermore, 60% of samples contained root-rotting fungi (*Fusarium* spp. and/or *Bipolaris* spp.).

424 First report of *Meloidogyne partityla* on pecan in Georgia

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Tree decline was observed in a commercial pecan orchard in Georgia in 2000. Affected trees exhibited dead branches in the upper canopy, stunted growth, and feeder roots with small galls and associated egg masses typical of root-knot nematode infection. All declining trees that were examined had root systems infected with *Meloidogyne* sp. Identification of this nematode was based on two procedures. First, in Georgia, females were removed from root galls of declining trees and identified by determining the esterase phenotype as compared to standard root-knot nematode species including *M. partityla*. Secondly, galled roots were sent to New Mexico State University in Las Cruces, where mitochondrial DNA from specimens was extracted and compared to standard root-knot nematode species. Specimens were identified as *M. partityla* at both locations. This is the first report of *M. partityla* from Georgia, and the third report of this nematode outside of South Africa. *Meloidogyne partityla* may be endemic to North America and not South Africa. It is believed that this nematode entered South Africa on pecan rootstock imported from the USA between 1912 and 1940. Determining the distribution of *M. partityla* within the pecan-growing regions of Georgia and North America is warranted.

425 The occurrence and geographic distribution of *Xiphinema* and *Xiphidorus* species (Nematoda: Longidoridae) in Brazil

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The occurrence and geographic distribution of *Xiphinema* and *Xiphidorus* species were investigated during a national survey of 16 Brazilian states between 1999 and 2001. Eighty-two soil samples were collected from different habitats, including crop plants, grassland, savanna (cerrado) and typical Amazonian forest vegetation. Fourteen *Xiphinema* species (*X. brasiliense*, *X. costaricense*, *X. elongatum*, *X. ensiculiferum*, *X. ifacolum*, *X. krugi*, *X. longicaudatum*, *X. paritaliae*, *X. vulgare*, *X. surinamense*, *X. brevicolle* and three distinct morphotypes of *X. americanum sensu lato* tentatively identified as representing *X. diffusum*, *X. oxycaudatum* and *X. peruvianum*) were found in all surveyed states. Also, *Xiphidorus yepesara yepesara*, *X. yepesara parthenus*, *X. minor* and *X. balcarceanus* were identified from two states; however, recognition of *X. yepesara parthenus* as a subspecies of *X. yepesara* requires confirmation. The most frequently occurring species were *X. krugi* (46%), *X. brasiliense* (22%) and *X. vulgare* (22%). *Xiphinema longicaudatum* and *Xiphidorus balcarceanus* constitute new records for Brazil.

426 *Bursaphelenchus* species found in maritime pine in Portugal

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In March 1999, the Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhner, 1934) Nickle 1970, was found associated with dead maritime pine (*Pinus pinaster* Ait.) for the first time in Portugal and in Europe. Official authorities implemented an intensive survey in the region where the nematode was found and then extended it to the rest of the country. As a result, this quarantine organism was confirmed to be confined to a 30 km radius area in the Setúbal region, near Lisbon. During the survey other species of *Bursaphelenchus* were found associated with *P. pinaster*, such as *B. mucronatus*, *B. leoni*, *B. tusciae*, *B. hofmanni*, *B. hellenicus*, *B. teratospicularis*, *B. hylobianum*, *B. sexdentati* and *B. pinophilus*. The identification was based on observations

of morphological characters under optical and scanning electronic microscopes.

427 Nematode assemblages from a polar arctic desert

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Preliminary results are presented on the generic structure and several ecological parameters of nematode assemblages in relation to five types of microhabitats from a polygonal polar desert on Bol'shevik Island, Severnaya Zemlya Archipelago. Among 33 genera encountered, more than half were common to the majority of microhabitats (all or four types). Nematode abundance was high in *Deschampsia borealis* and black crust microhabitats (average 81 and 69 specimens/g) and low in bare soil (eight specimens/g). Bacterial feeders were the most diverse and dominant trophic group (15 genera), followed by omnivorous and plant-feeding nematodes (five and six genera, respectively). Predatory and fungal-feeding nematodes were represented in low percentage. This general pattern differed only for assemblages from bare soils where trophic groups were almost equally distributed. Again, nematode communities of bare soils had the lowest diversity and generic richness. The first gradient revealed by an ordination procedure was from nematode assemblages of higher plant vegetation and black crust, to these of moss communities. Along the second gradient nematode communities of bare soils were separated as the most specific group. The genera *Acromoldavicus* and *Boreolaimus* represent new geographical records.

428 Plant-parasitic nematodes associated with mango varieties on two rootstocks in Mara municipality of Zulia state, Venezuela

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Parasitic nematodes on mango (*Mangifera indica* L.) cvs Haden, Tommy Atkins, Irwin, Gleen, Keitt, Ford and Palmer, grafted on Sinamaica and Bocao rootstocks, were identified from 64 soil and root samples taken at the Hortofruticola Center in Zulia state. Nematodes were extracted using Oostenbrink and Baermann funnel equipment. The varieties grafted on Sinamaica rootstocks had *Hoplolaimus*, *Trichodorus*, *Pratylenchus*, *Hemicriconemoides*, *Rotylenchus*, *Helicotylenchus*, *Xiphinema*, *Tylenchus* and *Meloidogyne*. The varieties grafted on Bocao rootstock had *Hoplolaimus*, *Helicotylenchus*, *Rotylenchus*, *Xiphinema*, *Trichodorus* and *Meloidogyne*, with the most frequent genera as *Hoplolaimus*, *Hemicriconemoides* and *Rotylenchus*. A brief description is reported and identification key proposed.

429 Plant-parasitic nematodes associated with papaya (*Carica papaya*) in different producing areas of the State of Bahia, Brazil

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In the State of Bahia, papaya production (*Carica papaya*) is distributed in all microregions whereas Extremo Sul and Extremo Oeste have the major production. Although plant-parasitic nematodes are a great problem in papaya, there are no data on nematode occurrence and damage in papaya plantings in the State of Bahia. This work aimed to identify the occurrence of plant-parasitic nematodes by collecting soil samples in commercial plantings in the Extremo Sul of Bahia, as well as in Iaçú and in an experimental area of the Federal University of Bahia, in Cruz das Almas. The extraction of nematodes from soil was carried out by the sieving and centrifugation method, and from roots samples by the Baermann method. Plant-parasitic nematodes were reported considering the frequency and incidence for each homogeneous area. In general, *Meloidogyne* sp., *Rotylenchulus* sp.; *Helicotylenchus* sp., *Criconemella* sp., *Tylenchus* sp. and *Pratylenchus* sp. were present in all production areas. The largest diversity was observed in Porto Seguro and the largest frequency for all areas by *Meloidogyne* sp., *Rotylenchulus* sp. and *Helicotylenchus* sp. More detailed

studies are suggested, as well as correlation studies of population level with other soil diseases and damage level.

430 Specialities of zone geographic distribution of soil nematodes on the territory of Russia

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The soil nematode fauna of different geographic regions of Russia (Karelia, Moscow and Voronezh regions and Kalmykia) were investigated during 1992-1997. The base of the nematode fauna of different soils of these regions was the orders Tylenchida, Dorylaimida and Rhabditida. The orders Enoplida and Chromadorida were characteristic of hydromorphic soils. The role and diversity of nematodes from the order Mononchida increased from north to south. The percentage of other nematode orders varies in different soils. The abundance of nematodes rises in latitude-zone row of soil types in sequence: podzol (Karelia); podzolic soils (Moscow region); chernozem soil (Voronezh region), and decreases significantly in chestnut soils of Kalmykia. The nematode fauna of intra- and azone soils of Karelia was less abundant than those of zone soils of that region but increases in comparison with the zone soils sequentially from north to south in other investigated regions. Thus, the nematode fauna of moist intra- and azone soils was six to ten times more than those of zone soils of Kalmykia.

431 Histological alterations in Caricaceae roots caused by a mixed population of *Meloidogyne incognita* Race 1 and *Rotylenchulus reniformis*

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Histological alterations on *Carica papaya*, *Vasconcellea cauliflora* and *V. pubescens* inoculated with a mixed population of *Meloidogyne incognita* Race 1 and *Rotylenchulus reniformis* were studied. Segments of roots from each species were fixed in Craff III and dehydrated in a tertiary butyl alcohol series, embedded in paraffin, cut in 15 μ m sections and stained with quadruple Triarch's

stain modified by Suárez *et al.* All species of Caricaceae showed giant cells, nuclei and nucleoli and egg mass typical of *Meloidogyne*. Furthermore, the same species of plant showed also the vascular parenchyma cells forming a syncytium induced by *Rotylenchulus*. These cells are hypertrophied with granular cytoplasm, enlarged nuclei and prominent nucleoli. This research allowed demonstration that both nematode species can establish their feeding site in the same plant.

432 *Meloidogyne incognita* races distinguished by sweet potato differential cultivars and their distribution in the Kyushu Okinawa region, Japan

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To use cultivars of sweet potato with resistance to *Meloidogyne incognita* effectively, occurrence and distribution of resistance breaking races were examined. Four populations of *M. incognita* were collected from different fields and inoculated to 24 major sweet potato cultivars. Based on the reproduction rates of these nematode populations, five differential cultivars, Norin No. 1, Norin No. 2, Tanegashima-murasaki 7, Elegant Summer and J-red were selected. A total of 97 *M. incognita* populations were collected from sweet potato fields in central to southern Kyushu and Okinawa. Seven races were identified from these populations. In Kumamoto prefecture central Kyushu, race SP1 that reproduces only on Norin No. 1 represented 81% of 26 examined populations. In Miyazaki and Kagoshima prefecture southern Kyushu, SP 2 that reproduces on both Norin No. 1 and Norin No. 2 was predominant. In Okinawa, SP 4 that reproduces on four of the differential hosts but not on J-Red represented more than half of the 12 examined populations.

433 Association of *Meloidogyne konaensis* and the nutritional status of *Coffea arabica*

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The relationship of population densities of *Meloidogyne konaensis* to nutritional status of roots and leaves of coffee

cv. Typica in a naturally infested commercial field in the Kona district on the island of Hawaii was assessed. The numbers of *M. konaensis* and the concentration of K + Ca + Mg in the coffee roots were inversely related. Other inverse relationships were between the numbers of *M. konaensis* and concentrations of Mg (roots), Cu (roots and leaves) and Al (roots). Even though concentrations of P, K, Mg, K + Ca + Mg, Mn, and Zn increased in soil between February (dry season) and May (wet season), concentrations of P, K, Mg, K + Ca + Mg, Cu, B decreased in the coffee roots, and Zn and Al content decreased in the coffee leaves. Nematodes plus nutrient deficiencies and imbalances in the coffee trees are causing coffee decline.

434 Plant-parasitic nematodes associated with *Ammophila arenaria* (L.) Link in Portuguese coastal sand dunes

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Portuguese sand dunes extend for 450 km, more than half of the coastline. *Ammophila arenaria* (marram grass) is the dominant, and eventually the only, natural plant species in mobile dunes. The unmatched contribution of *A. arenaria* to sand fixation and dune formation is related to its extensive root and rhizome system and with its vertical growth, stimulated by sand deposition. In stabilised dunes, where no sand accumulation occurs, this species declines in vigour and growth. This behaviour, named 'the *Ammophila* problem', has been largely studied from nutrition, ageing, genetic differentiation to interspecific competition, among others, but is still unanswered. Recent studies relate this degeneration to the presence of pathogenic soil organisms, like parasitic nematodes, while others believe that the cause is the absence of mycorrhizal fungi. In the presentation, we provide an overview of plant-parasitic nematodes present in the roots and rhizospheric soil of *A. arenaria* in two dune systems of the Portuguese coast. The data shows a higher number and diversity of nematodes in the north population, where plants are more vigorous, although only two of the three species known to parasitise *A. arenaria*

are present. Some highlight plans for further research will also be presented.

435 Reaction of passion fruit genotypes to root-knot nematode, *Meloidogyne javanica*

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Considering the widespread distribution and the losses the root-knot nematode, *Meloidogyne javanica*, causes to different crops in the Cerrado region of Brazil, the reaction of 11 genotypes (EC-2-O Hybrido, Vermelhinho, IAC-comp. Hybrido, MSC, Roxo Australiano, Seleção DF, Longão PR-2, Vermelhão, Redondão PR-1, Roxo Fiji e Itaquiraí) of passionfruit (*Passiflora edulis* f. *flavicarpa*), and *P. edulis*) were evaluated under glasshouse conditions. Five 17-day old seedlings of each genotype were inoculated with initial inoculum of approximately 5250 eggs and second stage juveniles per plant per pot containing one kg of soil, and an equal number of plants were maintained as uninoculated checks. Tomato variety Santa Cruz was included as a susceptible check. Eighty days after nematode inoculation, indices of root-knot galls, indices of egg masses, final population densities of nematodes in soil and roots, and the percentage increase or decrease in dry vine weights of the inoculated plants were compared with the uninoculated controls. The gall index for different genotypes varied from 4.6 to 5.0 and the egg mass index from 0 to 2 and the reproduction factor from 0.001 to 0.061. The reproduction factor for susceptible tomato varied from 16.95 to 39.48. All genotypes tested were highly resistant to *M. javanica*.

436 Detection of entomopathogenic nematodes and their possible use under IPM in Pakistan

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In Pakistan, studies on biology, taxonomy and efficacy of entomopathogenic nematodes (EPN) were initiated in 1996. Since then, during extensive surveys, over 1000 soil samples have been collected from diverse habitats of Pakistan with emphasis on Sindh Province. Nematodes were collected using the *Galleria mellonella*

soil trap method. Three isolates of *Steinernema* and 59 of *Heterorhabditis* were detected, of which 22 isolates were identified as *H. indica*. Out of the identified EPN, two are new species; one has been described as *Steinernema pakistanense* (Shahina *et al.*, 2001), while the other *Steinernema* species is in the process of description. Pathogenicity of new indigenous EPN, *S. pakistanensis*, was tested against seven insect pests of common occurrence and agricultural importance under laboratory conditions and confirmed on *Helicoverpa armigera*, *Earias insulana*, *E. vittella*, *Papilio demoleus*, *Leucinodes orbonalis*, *Etiella zinkenella* and *Holotrichia consanguinea*. Mortality was higher (up to 100%) on early stages of insect larvae (*H. armigera*) as compared to fourth and fifth stage larvae. Search for more indigenous EPN is continuing and, under the National IPM Programme, field-testing of promising species will be undertaken during the year 2002. The farmers will be trained for conservation of natural enemies including EPN through the farmer field school (FFS) approach particularly for the cotton crop, which receives the largest amount (60%) of pesticides in Pakistan.

437 Association of plant-parasitic nematodes of the family Longidoridae with fruit crops in Poland

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In 453 soil samples, collected from root vicinity of apple, pear, plum, peach, sour and sweet cherry, walnut, hazelnut, black and red currant, gooseberry and raspberry, six species of *Xiphinema* and eight species of *Longidorus* were found. Equally common *Xiphinema* species were *X. diversicaudatum*, *X. brevicolle* and *X. vuittenezi*. Another three species were found only once or twice. The most common *Longidorus* species was *elongatus*, followed by *L. attenuatus* and less frequently *L. euonymus* and *L. leptcephalus*. The other four species occurred sporadically. Almost all species showed preference for some hosts and some of them also for soil type and soil pH. *Xiphinema diversicaudatum* preferred black currant, pear and sour cherry, *Xiphinema brevicolle* – walnut and sour cherry, while *Xiphinema vuittenezi* preferred raspberry and red currant. *Longidorus elongatus* was commonly associated with pear, peach, sour cherry, walnut, gooseberry and plum. *Xiphinema diversicaudatum* showed preference for heavy soils,

while *Xiphinema brevicolle*, *Xiphinema vuittenezi* and most *Longidorus* species preferred light sandy soils; *X. diversicaudatum* and *brevicolle* preferred soil pH 4.5-6.5 while *vuittenezi* and *Longidorus elongatus* did not show preference for soil pH, and other *Longidorus* species preferred soils of pH 5.6-6.5.

438 Elaboration of database of nematodes detected in imported plant germplasm in Brazil in 1981 to 2001

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Plant introduction has been one of the most effective actions for agricultural development in the tropics. Coffee and soybean in Brazil, banana in South and Central America, sugarcane in South America and Caribbean and grass pastures in Latin America, are good examples of successful introductions. But the germplasm movement involves the risks of introduction of exotic pests that are sometimes carried by seeds and propagated vegetative materials. EMBRAPA's post-entry quarantine is inspecting against pests, including nematodes, all the plant material that has been introduced into Brazil for research purposes. In this context, the nematological laboratory did the inspection and registered all the nematodes found associated with germplasm from different species of plant and different countries. Now, a computational database is under construction to make extremely fast, easy and more efficient the recovering these data. Other researchers that need to access such data for their work can contact the Embrapa Genetic Resources and Biotechnology Center and we will help them with the information that is available.

439 Characterisation of *Meloidogyne incognita* reproduction on yellow nutsedge, purple nutsedge and chile pepper

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The objective of this research was to determine if either yellow or purple nutsedge altered *Meloidogyne incognita* reproduction compared to susceptible chile peppers. Greenhouse experiments were conducted in 2000 and 2001, with 240 pots planted with chile pepper (*Capsicum annuum*), yellow nutsedge (*Cyperus esculentus* = YNS), purple nutsedge (*C. rotundus* = PNS), chile + YNS, or chile + PNS. Six pots from each treatment were harvested at 3-4 day intervals from 21 through 45 days after inoculation with 5000 eggs of *M. incognita*. Root systems (chile) or roots, rhizomes and tubers (YNS, PNS) were divided and eggs extracted from half. Egg masses on the remaining half were stained with phloxine B. The number of egg masses per root, eggs per egg mass, and total egg production per root system was measured for each plant and sample date. Chile supported much greater nematode reproduction than YNS or PNS, producing twice as many egg masses per gram of root and twice as many eggs per egg mass. Competition between chile and either nutsedge had no effect on egg mass density or numbers of eggs per egg mass in any host. Reproduction occurred on roots but not rhizomes or tubers of either nutsedge.

440 Weeds as hosts to root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*)

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Pratylenchus neglectus and *P. thornei* occur throughout the broadacre, dryland cereal cropping zone of south-eastern Australia. Hosting ability of nine monocotyledon (grass) and 20 dicotyledon (broad-leaf) weed species common to cropping rotations was tested in the glasshouse. Nematode multiplication rates (P_f/P_i) were assessed relative to wheat cv. Machete (susceptible) and triticale cv. Abacus (resistant). Weeds were classified as good (susceptible), poor (moderate) or non (resistant) hosts. Grasses differed in susceptibility to *P. neglectus* compared to *P. thornei*. For each nematode, two grass species were susceptible, three moderate and four resistant. Multiplication rates on broad-leaf weeds were lower than on the grasses. Six broad-leaf species were

susceptible to *P. neglectus* and 14 resistant; one was moderate for *P. thornei* and 19 resistant. Growth of susceptible weeds will allow increase and/or persistence of nematodes in cropping soils, compromising use of management strategies (particularly rotation with resistant crops) employed by growers to reduce nematode densities and alleviate crop losses. Monitoring susceptible weeds in crops, pastures and fallows will aid nematode management and control decisions.

441 PWN-CD: information database for the pinewood nematode *Bursaphelenchus xylophilus*

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The main objective of this project is to develop an information database with the inclusion of text (research articles, keys, bibliography), images (jpeg files), video clips (mpeg files), in order to collect in one physical support (CD-ROM) all relevant information related to the pine wood nematode (PWN), *Bursaphelenchus xylophilus*. The first volume presented here covers the taxonomy of the genus *Bursaphelenchus*. All major papers with the original species descriptions have been scanned in 'pdf' format. An updated and broad bibliography has been included. In the future, a taxonomical key, based on classical dicotomous keys but with a better and more intuitive presentation, will be developed. The final product is PC and Mac compatible. Future editions/updates will be developed, in particular related to the nematode vectors and the bioecology of the nematode-vector-plant. Ultimately, researchers, technicians and political decision-makers will have at their disposal a practical and readily available source of information on this extremely important pest and pathogen.

442 A checklist of nematodes from plant and soil in Portugal

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In southern Portugal the grassland ecosystem known as 'montado' has a particular ecological and social

importance due to its extensive area and also because it is where cork and holm oak grow. Integrated in a PAMAF project a study has been conducted to evaluate changes caused by some agricultural practices and its effects on the nematode communities associated in this type of ecosystem. Nematode diversity in grasslands is usually high. Nematode identification of the specimens found in the 'montado' of the Evora region (southern Portugal) is in progress, and some represent new genera/species reports for Portugal. Related to the taxonomic work, a compilation of all plant and soil nematode genera/species (non animal-parasitic) and related references from Portugal will be presented.

443 Plant-parasitic nematodes of maize (*Zea mays* L.) in low input agriculture in Kenya

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Different farming systems existing in Kenya have varied cultivation practices supporting diverse nematode genera and species. A survey conducted during the short rains in eight localities within two districts in Central Highlands of Kenya identified 12 genera of plant parasitic nematodes associated with maize. Both soil and root samples were collected from the rhizosphere of maize at the tasseling stage and assayed for nematodes. Main nematode genera recovered from soil samples in decreasing order of prominence were *Pratylenchus*, *Meloidogyne*, *Paratylenchus*, *Tylenchus*, *Ditylenchus*, *Heterodera* and *Scutellonema* spp. The main nematode genera found in maize roots in order of decreasing prominence were *Pratylenchus*, *Hemicycliophora*, *Tylenchorhynchus*, *Tylenchus*, *Ditylenchus*, *Paratylenchus* and *Meloidogyne* spp. In both root and maize samples, *Pratylenchus* was the most predominant genus occurring in 100 and 96% of soil and maize root samples, respectively. A coefficient of determination ($r^2 = 0.83$) was obtained between number of *Pratylenchus* spp. recovered from the soil and that from the root. Soil samples collected in 63% of the localities had above critical infestation level of two *Pratylenchus* spp./cm³ of soil. Root necrosis differed significantly with locality 7 showing the highest root necrosis.

444 Biofumigation tests: changes in the population of *Globodera* spp. and in the enzymatic activity of soils added with organic amendments

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Small biofumigation tests were carried out in pots with soil naturally infected by *Globodera* spp., using different amendments as fumigants: seaweed of the genus *Gelidium*, remains from gardening (grass, dry leaves) and potato peel (*Solanum tuberosum*). Soil without amendment was used as control. The treated soil was watered, sealed with black plastic, and kept under controlled conditions of humidity and temperature for 30 days. The value of the ratio final/initial population of the *Globodera* spp. was calculated, as was the variation in ureasic, phosphatasic and deshydrogenasic activity at the end of the treatment. The nematode population fell by 34% for the soil amended with *Gelidium*, 56% for the samples with grass and potato peel, compared with 5% for the control. This fall in the population is caused by the liberation of nematicide substances by the amendments and an increase in the chitinoic microflora and total edaphic biomass, reflected in the increase in enzymatic activity of the amended soil in relation to the control.

445 Poultry litter for the management of root-knot nematode on pumpkin in Puerto Rico

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Accumulation of solid wastes from human activities and agro-industries is a serious problem in Puerto Rico that represents an environment hazard and leads to significant pollution of soils, waterways, and lakes. The proper use and disposal of these materials in agricultural soils, through their application for management of phytonematodes, could be very useful for finding solutions to this

problem. Poultry litter was evaluated under field conditions for the management of *Meloidogyne incognita* on pumpkin (*Cucurbita moschata*) cv. Soler. The amendment was applied to a root-knot-infested soil at rates of 0, 7.3 and 14.5 kg/plant; in addition, a treatment with phenamiphos at 1.5 gai./plant, was included to determine the effectiveness of the amendment. The experiment was conducted during two cropping seasons. Final soil and root populations of root-knot nematode were determined during each cropping season. Results showed that poultry litter reduced population densities of *M. incognita*, and improved plant development, and crop yield.

446 Evaluation of the efficacy of mixing tops of forage grasses with soil to control *Meloidogyne incognita* and *Heterodera glycines*

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To control *Meloidogyne incognita* and *Heterodera glycines*, *Brachiaria brizantha*, *B. decumbens*, *Panicum maximum* cv. Guiné, *Andropogon gayanus* cv. Planaltina, and soybean tops were mixed one *per se* with soil and left there to deteriorate. In trial 1, 8 g of the grass tops were each mixed into soil in 2.5 l pots, and 30 days later one indicator tomato or soybean seedling was established per pot. *Meloidogyne incognita* eggs and egg masses and *H. glycines* females were counted respectively 45 and 30 days later. In trial 2, infested pots were planted with the grass seedlings: half were cultivated for 30 days and their tops mixed with the soil; in the other half cultivation lasted 30 more days and evaluation was done as indicated. Results in trial 1 showed no treatment effects on nematodes survival. In trial 2, only 37 females of *H. glycines* were found per root system of *B. decumbens* while 689 and 547 were counted in soybean roots cultivated for 60 and 30 days, respectively. *Andropogon gayanus* cv. Planaltina was the only grass among all tested to allow *M. incognita* to reproduce. But overall there was no effect of mixing any of these forage grasses with soil on the efficiency of the rotation in controlling *M. incognita* and *H. glycines*.

447 Effect of repeated use of organic amendments and biofumigation plus solarisation on glasshouse pepper nematodes in the southeast of Spain

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When methyl bromide (MB) is not used for soil disinfection, *Meloidogyne incognita* becomes one of the main soil-borne pathogens in the southeast of Spain (Murcia and south of Alicante). Nematode incidence after several organic amendments (7 kg/m² fresh sheep manure (FSM) + 3 kg/m² chicken manure; FSM at 7 kg/m² + 0.5 kg/m² soybean flour; EFO at 7 kg/m² of + urea at 25 kg/m²) was compared with MB treatments in commercial and experimental glasshouses. The effect of the reiterated use of FSM and chicken manure was also studied. The three amendments had root-knot index and *M. incognita* incidence similar to MB treatments. However, marketable yield was significantly lower for urea (8.1 kg/m²) and chicken manure (8.6 kg/m²) than for soybean flour (9.6 kg/m²) or MB (9.7 kg/m²). Marketable yield of 1st year biofumigation-solarisation treatments was significantly lower than those of the 2nd (11.3 kg/m²) and 3rd year (11.2 kg/m²) and that of MB treatments (11.2 kg/m²). Similar response was observed for the root-knot index (1st year, 4.5; 2nd year, 1.8; 3rd year, 1.3; MB, 1.5) and the percentage of infested plants (1st year, 90.0%; 2nd year, 43.3%; 3rd year, 40.0%; and MB, 36.7%).

448 Nematode-suppressive effects of composts against *Meloidogyne javanica* on tomato

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Suppressive effects of two composts, cattle manure compost (CMC) and grape marc compost (GMC), on the root-knot nematode *Meloidogyne javanica* were tested in pot and *in vitro* experiments. No root-galls were found on tomato roots grown in soils containing 10 or 25% (v/v) CMC, and very few were found on those grown in soil containing 50% GMC. Significant reductions in galling index were also found in tomato plants grown in soils containing smaller concentrations of this compost. Chemical analysis of the composts and leachates from the soils showed that the CMC contained higher concentrations of N-NH₄ and higher EC values than the GMC, while the latter had higher N-NO₃ concentration. The water extract of the CMC showed high nematicidal activity to the nematode juveniles and less activity toward the eggs *in vitro*. The extract of the GMC showed weaker nematicidal activity to the juveniles and eggs. Washing composted soils with excess water before nematode inoculation and tomato planting led to better plant growth, but the nematode-suppressive effect was lowered. These results suggest that high nitrogen concentrations, especially N-NH₄, and high EC values contribute to the nematode suppressiveness of the composts.

449 Assessment of DiTera to control plant-parasitic nematodes in vineyards in Chile

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During the 1999/2000 and 2000/2001 seasons, trials were carried out to evaluate the performance of the biological nematicide DiTera for the control of plant-parasitic nematodes affecting grapes, *Vitis vinifera* L., mainly *Xiphinema index*, *X. americanum* s.l. and *Meloidogyne* spp. Trials were carried out in the central region on vineyards for wine production, and for table grape production in a northern region of Chile. Grape varieties include Sauvignon Blanc, Cabernet Sauvignon and Thompson Seedless, respectively. DiTera was applied through a drip irrigation system at the rate of 40 l/ha at the beginning of spring root flushing and results were compared with a chemical standard and an untreated control. The effect of the treatments was assessed taking into account variations in number of nematodes per 250 cm³ of soil, (*Pf/pi*), and also pruning weight. The final results showed no statistical differences between the chemical standard and the DiTera treatments; however with respect to the untreated check, significant differences ($P = 0.05$) were noted, with control percentages between 77.4 and 91.5% at 30 days after applications. Yield and pruning weight also were significantly different ($P = 0.05$) in respect to the untreated plants. DiTera offers a new biorational option for control of nematode populations on grapes in Chile.

450 The toxic and the antagonistic effects of marine algal natural products on the embryonic development, juvenile development, and survival of nematodes

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The nematicidal activity of the methanol extracts of ten species of marine brown algae collected from the Red Sea coast were tested against different nematode species, including some free-living and plant-parasitic species. The test was conducted on the adult stages of the free-living nematodes, the adults and/or the juveniles of the parasitic species, as well as the egg-masses of

the plant-parasitic species. The results revealed that there are recognisable nematicidal potentials of the tested algal extracts. Some of the tested extracts caused more than 90% mortality of the tested nematodes. The egg-hatching test, which was conducted on the plant-parasitic species only, proved that some algal extracts were able to prevent or disrupt the embryonic-development of the eggs in the tested nematode species.

451 The fungicide effect of the biological nematicide 'Novibiotec 7996'

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Novibiotec 7996 was registered in Tunisia as a biological nematicide ovicide, efficient in different nematode species control. It was tested *in vitro* and under plastic shelters against *Pythium* sp. associated with cucumber crops, and in open field conditions on citrus and mango orchards infested, respectively, with *Fusarium*, *Lasiodiplodia* and *Ceratocystis* sp. in the sultanate of Oman. The Novibiotec 7996 comparative mode of action on nematode eggs and fungi organs will be presented and discussed.

452 *In vitro* pathogenicity of *Monacrosporium robustum* to *Rotylenchulus reniformis*

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The reniform nematode (*Rotylenchulus reniformis*) is an important pathogen of many of the most important Brazilian crops such as soybean, cotton, beans, cowpea, passiflora, papaya, tomato, lettuce, among others. The successful biological control of pests in the upper parts of the plants and the environmental concern about the continuous use of nematicides have the effect of promoting the interest in biological control of nematodes, increasing considerably the economic potential of this activity. Among the nematophagous fungi with potential for commercial utilisation as agents of biological control of nematodes are the species of *Monacrosporium*. In this study, the predatory

capacity of *Monacrosporium robustum* to the reniform nematode (*Rotylenchulus reniformis*) was evaluated *in vitro*. Concentrated suspension of J2, J3, J4, males and young females were transferred to the cultures of the fungus in Petri dishes with 2% water-agar and incubated at 26°C in the dark. In periods of 24, 48 and 72 h after that it was observed that the percentage of captured nematodes were, respectively, 81.3, 99.87 and 100%. The adhesive branches as the capture structure of the fungus and captured nematodes were also documented by scanning electron microscopy.

453 Selection and diversity of *Pasteuria penetrans* isolates in relation to *Meloidogyne* spp. from coffee

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Pasteuria penetrans isolates from different geographical areas were tested for the ability of endospores to attach to second-stage juveniles and to colonise females of different populations of *Meloidogyne* spp. from coffee and other crops. Our results confirm that spore attachment test cannot be used as the only criteria for screening for the efficiency of the bacterial isolate against nematodes. The percentage of females infected with endospores and the endospore concentration/ml in 100 females were the best approaches for screening the highly aggressive isolates. Using these parameters, it was possible to select one isolate Pp12 for the three populations of *M. paranaensis* and the isolate Pp10 for the four races of *M. incognita* from coffee. For the other species *M. hapla*, *M. exigua*, *M. graminicola* and *M. mayaguensis*, the isolates were not compatible. In this study, there was clear evidence that the greatest parasitism occurred when the isolates of *P. penetrans* were exposed to species of *Meloidogyne* genetically close to those from which the bacteria populations were originally isolated. RAPD analysis was applied to fingerprint the genomes of *P. penetrans* isolates. Twenty 10-mer oligonucleotide primers of arbitrary sequence were used on this study. A high level of homogeneity was observed among the isolates.

454 Distribution of *Pasteuria* spp. associated with plant-parasitic nematodes in Korea

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A survey was conducted on the distribution of *Pasteuria* spp. associated with plant-parasitic nematodes in glasshouses in Korea from 1996 to 2000. Among the 372 soil samples assayed, 20 samples had plant-parasitic nematodes with *Pasteuria* spp. endospores attached. Nematodes found with *Pasteuria* endospores attached, host plants, and their localities were: *Meloidogyne* spp. on oriental melon at Sungju, Cheju, and Yeosu; *Helicotylenchus* sp. on white potato and carrot at Cheju; *Meloidogyne* sp. on soybean at Cheju; *Heterodera* sp. on unknown host plant at Chujung; and *Aphelenchus* sp. on garlic and radish at Cheju. Morphological characteristics of endospores from *Meloidogyne* spp. observed under electron microscope were the same as the previous descriptions of *Pasteuria penetrans*.

455 Biology and ultrastructure of a novel bacterial parasite of nematodes

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A novel gram-negative bacterial parasite of free-living and plant parasitic nematodes was discovered in southern Italy. The bacterium is a septate rod with a unique shape resulting from the flattening of both cell ends. It attacks vermiform stages producing a lethal disease. Parasitised nematodes may be observed easily at low magnification because of the creamy-yellow colour they assume. After adhering to the host, the bacterium eventually penetrates its body, giving rise to a multiplicative phase spreading the infection and filling the whole nematode body with cells. The bacterial cells are released at nematode death and cadaver decomposition, infecting new hosts in the soil through direct contact and cuticle adhesion. Atomic force microscopy (AFM) ultrastructural data of live cells

showed that the bacterium is covered by an adhesive matrix responsible for attachment. SEM and TEM were used to investigate the bacterium ultrastructure, and showed elongated cells measuring $4\text{--}5 \times 0.5 \mu\text{m}$. Attempts to culture the bacterium on common nutritive media failed. The pathogen was discovered in association with four distinct *Meloidogyne* spp. populations in southern Italy, but it was also observed in free-living Cephalobidae. The bacterium appears to be a novel invertebrate pathogen and will probably represent a further tool in nematode biological management.

456 Attachment tests of *Pasteuria penetrans* to clonal lines of *Meloidogyne incognita*

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Two single egg mass lines of *Meloidogyne incognita* were developed and from each of these lines freshly hatched second-stage juveniles (J2) were collected from three individual egg masses. Three separate replicates of 20 individual J2 from each egg mass were exposed to endospores of a population of *Pasteuria penetrans* originally isolated from a population of *Meloidogyne javanica* and the endospores adhering to each J2 were counted. One population of *M. incognita* became encumbered with between nine to over 100 endospores per J2 while in the other population this ranged from zero to less than 70 endospores per J2. The distribution of the numbers of endospores attaching to individual J2 was therefore very different, and in one population of *M. incognita* the numbers of endospores were highly skewed with the majority of J2 having fewer than ten endospores and one or two J2 having more than 30. The experiment was repeated and similar results were obtained. The results will be discussed in relation to clonal variation and their implications.

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458 The influence of *Tagetes patula* on *Pratylenchus penetrans* and the growth of rose rootstocks

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The influence of growing *Tagetes patula* cv. Single Gold or Italian ryegrass, and of applications of aldicarb (0.9 g a.i./m²), matalaxyl (0.075g a.i./m²), or 12-months fallow was investigated on *Pratylenchus penetrans* in a sandy soil. Nematode numbers decreased significantly after *Tagetes* but increased after Italian ryegrass. The fungicide had no effect on the nematode while the effect of aldicarb was only temporary. Fallow resulted in a decrease of *P. penetrans*. To estimate the effect of the treatments, the stem diameter and plant weight of subsequently grown rose rootstocks *Rosa dumetorum* cv. Laxa were measured. Significant increase in both plant parameters was obtained after both *Tagetes* and aldicarb. The other treatments had no effect.

459 Field evaluation of the effect of *Verticillium chlamydosporium* on *Meloidogyne arenaria* race 3

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The concern and restriction on nematicide use require the development of alternative nematode control strategies. The nematophagous fungus *Verticillium chlamyosporium* has shown potential as a biological control agent for root-knot nematodes in pot and microplot experiments. A 2-year field experiment was conducted at Coimbra, Portugal, to study the effect of *V. chlamyosporium* on *Meloidogyne arenaria* race 3. Tomato cv. Joker, susceptible to the nematode, and cabbage, as a poor host, were used in the summer and winter seasons, respectively. The experiment compared the use of *V. chlamyosporium* with the nematicides oxamyl and methyl bromide. Nematode population densities, gall index and number of eggs g⁻¹ root were higher after the tomato crop. The fungus was able to colonise only the tomato roots and was detected in soil and in nematode eggs only after 2 years. Under the conditions of this experiment, the fungus was not effective in decreasing nematode levels. A longer period would be needed for the fungus to be established and effective against root-knot nematodes. Further research, exploring environmental factors that also affect survival and proliferation of the fungus in soil, is needed to increase colonisation of soil and roots by the fungus.

460 A *Pasteuria*-like organism on an entomopathogenic nematode parasite of *Diaprepes abbreviatus*

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During a field study of endemic entomopathogenic nematodes in Florida, *Pasteuria*-like spores were frequently found adhering to 3rd stage juveniles (IJ3) of *Steinernema* sp. emerging from cadavers of the weevil *Diaprepes abbreviatus*. Cadavers with emerged IJ3 were maintained in 2 ml water in sealed Petri dishes and observed periodically. Numbers of unattached spores in the water increased over time. Spores were never found attached to the IJ3 sheath (2nd stage cuticle), whereas most exsheathed IJ3 were encumbered with several hundred spores. A few (<1%) ensheathed IJ3 had one to several spores attached

to the cuticle within the sheath. Twenty-six days after egression, numerous unattached spores were observed between the 2nd and 3rd stage cuticles of some ensheathed nematodes; however, no spores were observed within the bodies of those IJ3. When spore-encumbered IJ3 were used to infect *D. abbreviatus* larvae, no spores were detected on juvenile or adult male or female nematodes up to 96 h after insect death. However, moulted cuticles of IJ3 with attached spores were recovered from dissected insect cadavers. Studies are ongoing to determine the life cycle of the organism relative to the nematode and insect.

461 Efficacy of *Pochonia chlamyosporia* var. *chlamyosporia* and *Paecilomyces lilacinus* on biocontrol of *Heterodera schachtii* on sugar beet

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Different isolates of *Pochonia chlamyosporia* var. *chlamyosporia* and *Paecilomyces lilacinus* were highly effective in controlling nematodes on water agar. A glasshouse experiment was conducted using *P. lilacinus* and four isolates of *P. chlamyosporia* var. *chlamyosporia* (Po1, Po3, Po4, Po5). Seedlings of sugar beet were planted in steam-sterilised soil and, at the same time, 100 g cereals infected with different fungi, and 5 g oxamyl were incorporated into the soil of appropriate treatments. After 27 days, plants were inoculated with 4000 juveniles of *H. schachtii* and pots were arranged randomly in a glasshouse for 2 months. There were significantly fewer cysts on the roots treated with fungi and oxamyl than untreated nematode-infected plants; the smallest numbers observed were on Po1 treated roots. Fresh weight of these plants significantly increased with addition of fungi and oxamyl. Nematodes multiplied nearly 22-fold on untreated plants, 11-fold on *P. lilacinus* and Po3 treatments and between two to five-fold on plants treated with Po1, Po4 and Po5, respectively. Percent control of *H. schachtii* numbers (eggs/g soil) were 46% for Po3, 52% for *P. lilacinus*, 78% for Po4, 87% for Po1 and 90% for Po5, respectively.

462 Production of *Pasteuria penetrans* 'in vivo' in soils with different textures and in the sand

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The mass production and attachment of *Pasteuria penetrans* to *Meloidogyne javanica* second stage juveniles (J2) were evaluated in soils of different textures and in river sand, in the glasshouse. Tomato seedlings were transplanted to pots containing those substrates. The upper 5 cm layer of the substrate was infested with 10⁵ endospores/g soil of *P. penetrans*, and each plant was inoculated with 2000 J2. Seventy days later, the plant roots were harvested, dried, weighed and ground. The number of endospores/g of root, and per plant were estimated. Soil samples were collected from upper layer and from the bottom of each pot, dried, and infested with 600 J2 of *M. javanica* to evaluate the endospore percolation of *P. penetrans*. More endospores/plant were produced in sandy soils compared to clay soils or river sand. Negative correlation was observed between *P. penetrans* production per plant and the soil clay content. Soils with lighter texture allowed higher attachment of *P. penetrans* to juveniles of *M. javanica* than heavier ones. Most of the endospores were leached to the bottom of the pot by the percolating water in the sand.

463 Nematicidal activity of selected plant tissues to *Xiphinema americanum*

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Freeze-dried leaf and root tissue of six plants including edible mum (*Chrysanthemum coronarium*), black-eyed Susan (*Rubeckia hirta*), marigold (*Tagetes minuta* 'polynema'), sunn-hemp (*Crotalaria juncea*), castor bean (*Ricinus communis*) and sesame (*Sesamum indicum*) were assayed for nematicidal activity against the dagger nematode (*Xiphinema americanum*). The tissue was pulverised and mixed with dry sterile sand in concentrations that ranged from 0.5-5.0 mg/cm³ sand for roots and 5.0-20.0 mg/cm³ sand for leaves. Nematodes were transferred to 2 ml sample cups in 250 µl sterile distilled water followed by 1.5 cm³ of the plant/sand mix. The cups were sealed and incubated for 24 h at 24°C. All nematodes were recovered and the numbers of alive and dead nematodes

were analysed by probit analysis. Chrysanthemum roots were the most toxic with an LC50 of 1.0 mg/cm³ followed by black-eyed Susan with an LC50 of 3.5 mg/cm³. Sesame roots showed some activity but no mortality was observed in the marigold, castor bean or crotalaria root bioassays. Leaves of marigold and black-eyed Susan had LC50 values of 8.0 and 9.0 mg/cm³, respectively, while leaf tissue of the remaining plants showed little toxic activity.

464 Occurrence and biocontrol potential of potato-associated bacteria

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Plant-associated bacteria are able to improve plant growth and health, but little is known about their diversity and antagonistic potential against disease complexes consisting of plant parasitic nematodes and soil-borne fungi. In a cooperative project, bacteria were isolated from the rhizosphere, endorhiza, phyllosphere and endosphere of potato cv. Cilena at Bonn and Rostock, and studied for their antagonistic potential against *Verticillium dahliae*/ *Rhizoctonia solani* (Rostock) and *Meloidogyne incognita* (Bonn). The bacteria were characterised based on their fatty acids using standard procedures for GC-FAME and the Sherlock Microbial Identification System (MIDI). Bacterial richness and diversity were determined to compare population dynamics depending on location, habitat and growth stage. Up to 10% of the culturable bacteria showed antibiosis against *V. dahliae* and *R. solani*. Out of these isolates, 20% gave significant control of *M. incognita* in greenhouse experiments. Main bacterial genera containing isolates with antagonistic activity were *Curtobacterium* and *Streptomyces* for the rhizosphere and phyllosphere and *Pseudomonas* for the endosphere and endorhiza. In 2001, samples were taken at three different growth stages (establishment, flowering, maturity). Differences in bacterial community structure and antagonistic activity will be shown. The potential of indigenous antagonistic bacteria to suppress nematode/fungal diseases complexes is discussed.

465 Study of combined use of antagonist plants and arbuscular mycorrhizal fungi on *Meloidogyne incognita* on tomato

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Organic disease management programmes are being increasingly researched in order to limit the environmental pollution caused by standard agricultural methods. Currently, much research is focusing on nematode control using antagonist plants with nematicidal properties such as aromatic species which produce essential oils with acknowledged nematicidal and fungicidal properties, and the *Tagetes* spp. with a known suppressive effect on parasitic nematodes. Glasshouse trials were set up to evaluate the combined effects of inoculation with the mycorrhizal fungi *Glomus mosseae* or *Glomus manihotis* and the presence of either the aromatic herb *Lavandula dentata* or *Tagetes minuta* on tomato infected with the root-knot nematode *Meloidogyne incognita*. The results show significant differences in nematode reproduction between mycorrhizal and non-mycorrhizal plants with an important reduction in nematode levels in the plants inoculated with arbuscular mycorrhizal fungi and cultured in the presence of antagonist plants.

466 Occurrence of *Rhizobium radiobacter* associated with *Heterodera avenae* eggs in Tunisia

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Populations of cereal cyst nematode, *Heterodera avenae*, collected from various cereal regions in Tunisia were associated with several bacterial and fungal strains. Scanning electron microscopy study has demonstrated the occurrence of a bacterium within the cyst and eggs of the population nematode collected from Zaghuan region. This bacterium has been purified through micro-culture of disinfected eggs on agar media. The bacteria was then identified using amplification of 'rrs' gene coding the ARNr 16S as *Rhizobium radiobacter* synonymous

with *Agrobacterium radiobacter*. The role and importance of the bacteria-nematode relationship on the host are discussed.

467 Isolates of the generalist parasite *Pochonia chlamydosporia* have specific interactions with their nematode hosts

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Pochonia chlamydosporia is an opportunistic, facultative parasite of cyst and root-knot nematode eggs, fungi and molluscs, but individual isolates demonstrate host specific interactions. Genetic variation was examined in a collection of 54 isolates from diverse geographical regions and several nematode hosts using ERIC PCR. In cluster analysis, isolates formed groups on the basis of their original host; those from cyst nematodes varied more than those from *Meloidogyne* spp. The fungus produces an alkaline serine protease, VCP1, during infection of nematode eggs. The gene encoding VCP1 was sequenced and cDNAs from six isolates from different nematode hosts examined. Translated cDNA sequences in isolates from cyst and root-knot nematodes consistently had two amino acid polymorphisms at positions 65 and 99 in the binding region of the enzyme. Such differences may contribute to host preference. Isolates from cyst or root-knot nematodes were added alone and together to soil in which tomato or potato plants infected with *Globodera pallida* or *Meloidogyne incognita* were grown. Isolate abundance was increased only in compatible combinations and the species of nematode influenced the proportions of the mixed isolates. *Pochonia chlamydosporia* has complex interactions with its hosts that may influence its biological control efficacy.

468 Comparative study on the nematicidal activities of garlic and mustard oils

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The nematicidal properties of mustard and garlic oils were compared to those of allyl isothiocyanate in a

glasshouse study. Five percent (v/v) acetone solutions of allyl isothiocyanate, garlic oil and mustard oil were prepared and each delivered at rates of 0.1, 0.2, 0.4 and 0.8 ml a.i./kg soil that was naturally infested with *Rotylenchulus reniformis*. Treated soils and untreated control were placed in pots in the glasshouse. Pre-plant soil samples for nematode analysis were collected 1 week after application of the materials. The pots were planted with soybean 'Young' and grown for 8 weeks. Plants were then removed and nematodes were extracted from soil and roots. Fresh shoot weights were determined. Pre-plant sampling results indicated that all three materials were active against the nematode when applied at rates >0.2 ml/kg soil. Nematicidal activities of allyl isothiocyanate and mustard oil were much higher than that of garlic oil. In the end-of-experiment sampling, populations of reniform nematodes in soil were lowest in soils treated with the two highest rates of allyl isothiocyanate and the 0.8 ml rate of mustard oil; numbers in soil with garlic oil did not differ from the control. Nematode populations in the roots followed the same pattern as for the soil samples, except that the 0.8 ml rate of garlic oil reduced nematode numbers compared to the control. Treatments with allyl isothiocyanate and garlic oil had no effect on shoot weight; those with mustard oil at >0.1 ml/kg soil resulted in improved shoot weights. Results indicate that garlic oil does not have the nematicidal properties shown by allyl isothiocyanate and mustard oil.

469 Mechanism of action of *Corynebacterium paurometabolum* strain C-924 on nematodes

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Simultaneous production of hydrogen sulphur and chitinases by *Corynebacterium paurometabolum* strain C-924 was demonstrated. It happens in sufficient amounts to impede egg hatching and produce death in juveniles of several species of plant and animal parasitic nematodes. This result permitted further study of the mechanism

of action against other parasites (trematodes, fungi and bacteria). It was also demonstrated in several experiments that concurrent applications of chitinolytic agent or a chitinolytic activity-inducing agent, and sulphide or sulphide-producing agents from microorganisms or chemical compounds, required ranges or doses of application significantly less than any of the compounds applied individually, in order to achieve an effective control of the nematodes. This indicates that there is a synergistic effect in the combination of chitinases and sulphide for the control of nematodes and other parasites. The results are subject of a patent application on any antiparasitic composition obtained from the metabolites of *Corynebacterium paurometabolum* strain C-924, as well as some other possible antiparasitic compositions and biocontrol microorganisms producing chitinases and sulphide concurrently.

470 Population of *Corynebacterium paurometabolum* strain C-924 in soils treated with HeberNem

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A procedure to re-isolate bacterium *Corynebacterium paurometabolum* strain C-924 from ferritic soils treated with HeberNem, was developed. Strain C-924 is the active agent of HeberNem, a new bionematicide formulated as a concentrated and humectant powder. It was tested in glasshouses on several crops, such as pepper and tomato. HeberNem applications were carried out by means of the irrigation system in doses from 1 to 5 kg/ha. A selective culture medium was developed, taking into account antibiotic combinations and also based on bacteria colony chromogenesis for potassium telurite reduction, so the differentiated re-isolation from soil samples of the specific strain C-924 was performed. The results confirmed the effectiveness of the bacterium in the control of *Meloidogyne incognita*, when it is in a concentration higher than 10⁵ ufc/g of soil. It was proven that soil populations of C-924 diminished progressively

and it was not possible to detect it in treated ferritic soils between 60 and 120 days. It was concluded that for the best effectiveness of the product, a first application should be done 7 days before planting and a second one 30 days after it, using doses between 2 and 5 kg/ha.

471 Examination of rhizosphere-associated microbes for production of compounds active against plant-parasitic nematodes

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In vitro studies identified fungi and bacteria that produce compounds active against plant-parasitic nematodes. Assays of fungus culture filtrates were conducted with *Heterodera glycines* (soybean cyst nematode: SCN) and *Meloidogyne incognita* (root-knot nematode: RKN). The tested filtrates exhibited a low correlation in activity against SCN compared with activity against RKN. Only 5.1% of the tested isolates produced culture filtrates that strongly inhibited egg hatch of both SCN and RKN (*i.e.*, egg hatch was 40% or less than egg hatch in potato dextrose broth controls), 3.9% strongly inhibited SCN egg hatch only, and 13.4% inhibited RKN egg hatch (but not SCN). Percentages of filtrates that inhibited second stage juvenile mobility were 0.8% (affected both SCN and RKN), 0.4% (SCN only), and 1.2% (RKN only). Compounds with broad-spectrum activity can be identified without testing specific nematode taxa, but identification of compounds with more specific activity should be tested against the target nematode. Similar experiments with rhizosphere-inhabiting bacteria and RKN identified isolates producing compounds active against RKN. One filtrate that suppressed egg hatch was selected for further study; size-fractionation of the culture filtrate showed that the active component is larger than >3 kDa in size.

472 Morphological and morphometric characteristics of Sri Lankan populations of mature endospores *Pasteuria*

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The morphology and morphometrics of mature endospores of *Pasteuria* populations recovered from Sri Lankan nematodes belonging to several taxonomic groups were evaluated. *Pasteuria* populations originating from *Meloidogyne* and *Pratylenchus* from Australia, Japan, Kenya and UK were also included in the study for comparison. A wide variation in spore morphometrics was evident. *Meloidogyne* yielded comparatively large spores while *Pratylenchus* and *Radopholus* generated small spores; intermediate sizes were seen with *Xiphinema*. Sri Lankan endospores possessed morphological changes following attachment onto nematodes. The endospores that originated from Sri Lankan soils had the three distinct forms (I-III). PPKB exhibited the very rare Form I; PPKB attached to *M. brevicauda* retained the sporangium wall covering the surface. PPPT exhibited rough intact peripheral fibres and the central body of the endospores was attached to *Pratylenchus loosi* after the sporangium wall and the exosporium were sloughing off. PPNW endospores possessed peripheral fibres as fine, delicate, remarkably long and prominent hairy structures. Spores of PPKenya isolated from *P. goodeyi* and PPBotesdale isolated from *M. ardenensis* were found to be smallest and largest, respectively, described so far among the members of the *Pasteuria penetrans* group. PP-a also showed a higher mean endospore diameter.

473 Endophytic behaviour of egg parasitic fungi in roots

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The rhizosphere is an important zone for the activity of nematophagous fungi since plant-parasitic nematodes commonly attack plant roots. Micro-organisms are found in numbers ten to 20 times (occasionally 100 times) higher than in the root-free soil. The presence of nematode-trapping fungi in the rhizosphere of agricultural plants has been studied. The capability to colonise the roots was

not advantageous for the biocontrol potential of isolates on root-knot nematode (*Meloidogyne* spp.), in contrast to the egg-parasitic fungus *Verticillium chlamyosporium*, where root colonisation was suggested to be a prerequisite for successful biological control. We have recently shown cellular details of barley root colonisation by *V. chlamyosporium*. It seems that the fungus is not recognised by the plant, similar to mycorrhizae and non-pathogenic endophytes. We have also found signs of late induction of plant defence responses (3 weeks after inoculation) such as phenolic droplets and papillae similar to how other fungal antagonists of plant pathogens modulate plant defences. We therefore hypothesise a double mode of action for *V. chlamyosporium* and perhaps other nematophagous fungi: *i*) direct parasitism on nematode eggs; and *ii*) immunisation agent. We present our initial results on plant defence modulation by *V. chlamyosporium* and show examples of its potential to control root pathogens.

474 The potential of TerraPy® to reduce nematode damage

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TerraPy stimulates microorganism activity in the soil and promotes growth of plant-beneficial microorganisms such as mycorrhiza and plant growth promoting rhizobacteria (PGPR). As a result, damage caused by plant parasitic nematodes is reduced. In greenhouse experiments, *Meloidogyne incognita* infestation on tomato was significantly reduced following a soil drench with TerraPy at 2 kg/ha. Increasing application rates up to 200 kg/ha further reduced nematode damage. At these higher rates, plant growth was also significantly enhanced. Yield of cherry tomatoes was 50% higher when treated with 200 kg/ha TerraPy than in the control. The increase in yield was caused by more fruits per plant. However, fruit weight was not affected. Similarly, for commercial tomato production in Turkey an increase in the number of flowers is reported as major factor of yield enhancement. Due to its positive effect on beneficial microorganisms, coapplication of TerraPy with mycorrhiza or *Pseudomonas* inoculum will further reduce

nematode damage and stimulate plant growth. In 2001, TerraPy was successfully marketed in the mediterranean region as a plant growth and yield-enhancing agent in vegetables.

475 Ultrastructural study by TEM of eggs and juveniles of nematodes treated with *Corynebacterium paurometabolum* strain C-924

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Changes in the ultrastructure of *Haemonchus* spp. eggs, treated with *Corynebacterium paurometabolum* strain C-924, were observed by Transmission Electron Microscopy (TEM). Alterations in the eggs of *Haemonchus* spp. exposed to the action of the bacterium, were detected after 12 h treatment. Regions normally containing lipids were occupied by determined vacuolar spaces, which separated the lipidic and chitinous layers. Closely bound to these spaces, numerous foldings produced in the external layer of the eggs were observed as the main damage suffered in eggshell. These structures were not observed in the eggs without C-924 treatment. It was also observed that *C. paurometabolum* strain C-924, caused the death of *Meloidogyne incognita* second stage juveniles (J2). After 24 h exposure to C-924, vacuoles observed for *Haemonchus* spp. eggs were smaller than for the *M. incognita* J2. This situation could be related to greater susceptibility of *M. incognita* J2 to gases emitted by the bacterium. Studies on toxic gas production, proteolytic activity, as well as the capacity of *C. paurometabolum* strain C-924 to degrade chitin and collagen are proposed.

476 The relationship between glucosinolates in canola (*Brassica napus*) and their ability to control the plant-parasitic nematodes, *Pratylenchus neglectus* and *Meloidogyne javanica*

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The toxicity and susceptibility of canola (*Brassica napus*) plants containing different root levels of glucosinolates were tested against the root lesion (*Pratylenchus neglectus*) and root-knot (*Meloidogyne javanica*) nematodes in glasshouse trials. Plants containing higher root levels of 2-phenylethyl glucosinolate were significantly more resistant to *P. neglectus* and more toxic as they degraded in the soil. This confirms previous findings, and suggests that high root 2-PE glucosinolate canola crops should provide a disease break effect against this nematode. High 2-PE glucosinolate tissues were also more toxic to populations of *M. javanica*, but plants showed no significant advantage in terms of resistance to the nematode. In fact, a significant and positive relationship between glucosinolate levels and nematode numbers generated was observed in one variety, suggesting that the elevated presence of the glucosinolate actually gave the nematode an advantage. The disease break effect of the crop against the root-knot nematode may be undermined by its susceptibility, and so caution should be used in crop selection in areas where the root-knot nematode is a problem.

477 Examination and disclosure of the multifaceted modes of action of DiTera[®], a biological nematicide

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Research laboratories such as IACR-Rothamsted, Imperial College, UK and Auburn University, AL, were involved in the investigation into the complex activities found in DiTera[®] (a biological nematicide) on plant-parasitic nematodes, during 1997-2000. Egg hatch inhibition, muscle paralysis, feeding inhibition, depletion of lipids, and changes in sensory perception affecting activities such as host/mate-finding were affected by exposure to DiTera[®]. In most instances these activities were found to be irreversible. In addition to nematode activities, in-

creased plant health, shoot and root weights and greening were also noted in multiple field trials, prompting investigations into microbial changes in DiTera[®] treated soils in greenhouse and under field conditions. Various studies including FDA hydrolysis, enzymatic activity, pH and conductivity measurements, and determination of soil, root tissue and rhizosphere microbial populations demonstrated DiTera[®] increased gram-positive microbial populations especially in the rhizosphere and soil. These direct and indirect effects on nematodes and the soil rhizosphere contributes to the overall beneficial response of plants to DiTera[®].

478 Effect of mycorrhizal arbuscular fungus *Glomus manihotis* on micropropagated banana cultured on soil naturally infested by lesion nematode under microplot conditions

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The root lesion nematode *Pratylenchus goodeyi* Sher & Allen is considered an important nematode pest of commercial bananas in the Canary Islands. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonise the roots of most cultivated plant species. It has been demonstrated that early mycorrhizal inoculation promotes plant growth and increases the tolerance of banana to attack by nematodes. However, most interaction studies between both microorganisms have been developed during the nursery phase where most conditions are under control. In this trial we study the effect of the interaction between the AMF *Glomus manihotis* and *Pratylenchus goodeyi* on micropropagated banana Grande Naine, under microplot conditions. This experimental method is the nearest to field conditions. Several samples were taken at different times to evaluate mycorrhizal effect and nematode infection. Six months after transplanting to microplots there was still a positive effect of AMF. This effect decreased at the end of trial, after 18 months in the microplots. At final harvest, plants showed important levels of nematode infection and the consequent damage.

479 The elaboration of biological control methods of nematode-, virus-, fungi-, bacteria-complex infections on leguminous plants

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Nematodes, virus, fungi and bacteria and their associations were studied in different type phytocenoses of Moscow on leguminous plants. The complex seed infections included two to five species, mainly of fungi and bacteria. The root and aboveground plant organ-infections included, as a rule, five more pathogens, consisting mainly of nematodes, fungi, bacteria and viruses. The methods, dates and efficiency of Russian biological pesticides (planrhiz, trichodermin) and nematicidal strains of bacteria antagonists (*Bacillus*, *Pseudomonas*) and bacteria which secrete antibiotic substances (*B. polymixa*, *Enterobacter* spp.) were studied as ecologically safe methods to restrict the quantity and harmful influence of complex infections (nematodes, viruses, fungi, bacteria) on leguminous plants grown under the conditions of the Moscow region. The influence of different species of plant predecessors on number and species of nematodes of different ecological and trophical groups in the process of growing leguminous plants in seven-field crop-rotation were studied.

480 Biocontrol of plant-parasitic nematodes by *Trichoderma harzianum* – possible role of proteases

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Strains of the fungal biocontrol agent *Trichoderma harzianum* can affect the root-knot nematode *Meloidogyne javanica* by various modes: *i*) direct parasitism on

second-stage juveniles (J2) and eggs; *ii*) metabolites produced by the fungus affect the J2 viability and egg hatching. *In vitro* parasitism ability was presented, to different extents, by all the strains tested: IMI206040 (designated WT) and its transgenic strain P-2 which contains multiple copies of the proteinase gene *prb1*, strains T-203(Y), T-44, T-35 and T-315. A transgenic strain of IMI206040 carrying a fusion of the *prb1* promoter with the green fluorescent protein (GFP) gene was used to demonstrate that this gene is turned on during the interaction between the fungus and the nematode. Culture filtrates of WT and P-2 paralysed and killed the *M. javanica* J2, as well as other plant-parasitic nematodes. The activity on *M. javanica* J2 was heat sensitive and restricted to the low molecular weight fraction (less than 3 kDa). The higher molecular weight fraction, which contained the proteases, did not directly affect the J2. However, a ladder of proteases between 20-31 kDa, which presented optimal proteolytic activity at low pH range, was positively correlated to the nematicide activity of the various strains filtrates.

481 Evaluation of Polish isolates of nematophagous fungi for the control of the root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) *in vitro* and in glasshouse conditions

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The effects of different species of nematophagous fungi: *Ponchonia chlamydosporia* var. *chlamydosporia*, *Paecilomyces lilacinus* and *Cylindrocarpon destructans* on *Meloidogyne arenaria* eggs and juveniles were tested *in vitro*. All species of fungi were isolated from eggs of sugar beet nematode (*Heterodera schachtii*) and controlled *M. arenaria* population *in vitro*. Isolates Vc-1 of *P. chlamydosporia* and *P. lilacinus* parasitised 90% of eggs, while *C. destructans* parasitised only 50%. *C. destructans* infected more than 19% of juveniles at a temperature of 25°C, while *P. chlamydosporia* infected 3-10% and none was infected by *P. lilacinus*. The potential of *C. destructans* as a biological control agent against *M. arenaria* in glasshouse conditions is under investigation and results will be presented.

482 Endotoxin activity of lipopolysaccharide produced by *Moraxella osloensis*, a bacterium associated with the nematode *Phasmarhabditis hermaphrodita*, against the slug *Deroceras reticulatum*

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Moraxella osloensis is a gram-negative bacterium associated with *Phasmarhabditis hermaphrodita*, a slug-parasitic nematode that has prospects for the biological control of mollusc pests, especially the grey garden slug *Deroceras reticulatum*. This bacteria-feeding nematode acts as a vector to transport *M. osloensis* into the shell cavity of the slug where the bacteria multiply and kill the slug within 1 to 2 weeks. We discovered that *M. osloensis* produces endotoxin(s) highly toxic to the slug when injected into the shell cavity. Injection of purified lipopolysaccharide (LPS) from *M. osloensis* cultures into the shell cavity caused slug death with an estimated LD₅₀ of 48 µg per slug. No contact or oral toxicity of the LPS to the slug was detected. Isolated lipid A portion from the LPS was toxic to the slug after injection into the shell cavity, but the polysaccharide portion of the LPS was not. Further, we semi-quantitated the LPS as 6×10^7 endotoxin units per mg and detected endotoxin activity in the lipid A portion but not in the polysaccharide portion by *limulus* amoebocyte lysate assays. This appears to be the first report of an active biological toxin against molluscs.

483 Research on the influence of five plant steroid substances on plant-nematode relationships (tomato – *Meloidogyne incognita*)

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It is known that plant steroids, with various chemical structures, can influence the growth and moult of parasitic nematodes and their hatch from eggs. The estimation of five plant steroid substances (solasonine, eñdisone, solamargine, tomatosid and furostanol glycosides) on nematode and plant in the host-parasitic system (tomato – root-knot nematode *Meloidogyne incognita*) was carried

out. The data have shown that the treatment of seeds of tomatoes with water solutions (0.5 mg/ml) of the substances stimulated development of plants and suppressed nematodes. The size of galls and females of nematodes in tested plants were much less than in control plants. The treatment by eñdisone stimulated formation of males in a population. The most effective were the furostanol glycosides, solamargine and tomatosid. These reduced infestation of roots by nematodes and increased duration of development of the parasites 1.5-2 times. The combinations in the concentration used did not affect nematodes *in vitro*. It is possible that the action of some steroids (in particular eñdisone) is caused by their participation in biosynthesis and metabolism of moult and sexual hormones of nematodes, as it resulted in change of their age qualification-sexual structure.

484 Effect of cultural practices on the management of *Meloidogyne* spp. on glasshouse crops by the nematophagous fungus *Verticillium chlamydosporium*

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Several isolates of *Verticillium chlamydosporium*, an egg-parasitising fungus of *Meloidogyne* spp., were selected for application in commercial vegetable production in glasshouses. For this purpose, the fungus must be compatible with cultural practices such as fertiliser use, soil type and pesticide application. Glasshouse experiments in pots were performed to study the effects of those practices on the efficacy of the biocontrol agent. Second-stage juveniles (J2) of *Meloidogyne* spp. were used as inoculum and tomato was the host plant. Fertilisers did not have negative effects on the efficacy of *V. chlamydosporium*. Addition of the fungus together with inorganic, organic-mineral and pure organic fertiliser caused up to 75, 76 and 86% reduction in the number of J2 per 100 cm³ soil, respectively. Without fertiliser, the effect of *V. chlamydosporium* on the nematode population was not significant. Of the four soil types tested, organic heat-sterilised soil had a negative influence on the performance of *V. chlamydosporium*. In contrast, addition of *V. chlamydosporium* to organic untreated soil, mineral untreated soil and mineral heat-sterilised soil reduced between 71 and 82% of the numbers of J2

per 100 cm³ soil. The influence of pesticide use on the performance of *V. chlamydosporium* will be discussed.

485 Compatibility of *Corynebacterium paurometabolum* strain C-924 with Nematicur and other chemical pesticides

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Interactions with chemical pesticides are required during the registration process of biological products. Bacterium *Corynebacterium paurometabolum* strain C-924 is a representative case, which has been reported previously as a new nematicidal agent (patent application PCT/NL95/00271). A selection of the eleven chemical pesticides, more widely used in agriculture, were tested *in vitro*. Pot experiments to confirm *in vitro* assays are shown, and positive results were obtained specifically for Nematicur (phenamiphos). Interaction tests with chemical pesticides were developed under controlled conditions, growth inhibition zones of C-924 appear when interactions with different chemical products were performed. Three test concentrations of chemical pesticides were used for *in vitro* assays: work-field, higher and lower concentrations, so irregularities in field condition when chemicals are used were taken into account. Different results were observed when using different chemical agents, so broad sensitivity range and resistance degrees are shown. Pot results and *in vitro* tests showed the possibility of using both *C. paurometabolum* strain C-924 as a bionematicidal formulation and Nematicur in combination, for nematode control in Integrated Pest Management.

486 Evaluating the effect of TerraPy Ag[®] on densities of *Meloidogyne javanica* and yield of tomato in a plastic house

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TerraPy Ag[®] stimulates microbial activity in the soil and promotes growth of beneficial plant organisms. The effect of TerraPy Ag[®] on nematode densities, gall rating, and yield was evaluated in a plastic house. Tomato cv. Durinta was planted in March and harvested in July 2001. TerraPy Ag[®] was applied at a total dose of 20 ml/m² per plot, split and applied in two ways: *i*) monthly applications at a rate of 5 ml/m² for 4 months starting at planting, and *ii*) application at a rate of 10 ml/m² in the 2nd and 3rd month after planting. Untreated plots served as control. Final densities, egg production, and gall rating were lower ($P < 0.05$) in plots treated monthly with 5 ml/m² of TerraPy Ag[®] than in those left untreated. Densities in soil and root, and gall rating were unaffected by TerraPy Ag[®] in the 2nd and 3rd month after planting. The number of fruits and their average fruit weight were higher ($P < 0.05$) in plots treated monthly with 5 ml/m² of TerraPy Ag[®] than in those untreated. Percent yield increase in those plots was 30% with respect to untreated ones which provided a profit of 18 030 euros/ha.

487 Observations on the life cycle of *in vitro* cultures of *Pasteuria penetrans*

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Studies of the life cycle of *Pasteuria penetrans* in *Meloidogyne* spp. have employed light microscopy, scanning electron microscopy and transmission electron microscopy. These studies used *P. penetrans* infected juveniles that were inoculated on plants, sequentially and temporally retrieved from roots and destructively sampled. The close association between *P. penetrans* cells and nematode tissue allows for observations of only large structures, *i.e.*, mycelial balls, thalli, and endospores. Development of *in vitro* culture techniques allows detailed observations of early, small growth structures of this bacterium. Early growth stages of this bacterium were recorded using light and scanning electron microscopy. These early growth stages progressed in *in vitro* cultures to classic *P. penetrans* structures, similar in size and shape to those reported from *in vivo* studies.

488 Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory bioassays

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Isothiocyanates (ITC) are released upon enzymatic degradation of glucosinolates of Brassicaceae. Glucosinolate profiles differ among plant species and the associated ITC differ in toxicity to organisms. Control of plant-parasitic nematodes in soil by ITC from incorporated plant material has been unpredictable but might be improved by using species of Brassicaceae based upon chemical composition. Laboratory assays were conducted to determine LC₅₀ values in sand of seven commercially-available ITC

against second stage juveniles of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. The relative toxicity of ITC (in ascending order) was: phenyl < ethyl < butyl < allyl < 4-(methylsulfinyl)butyl < benzyl < phenylethyl for *T. semipenetrans*, and butyl < phenyl < ethyl < 4-(methylsulfinyl)butyl < allyl < benzyl < phenylethyl for *M. javanica*. The LC₅₀ values ranged from 3.0 and 13.0 nmole/ml for phenylethyl ITC to 174.0 and 149.0 nmole/ml for phenyl ITC for *T. semipenetrans* and *M. javanica*, respectively. The broader context of this research is the development of approaches for consistent and reliable use of plant-derived chemicals for nematode management. The strategy is to select plants in the Brassicaceae based upon their glucosinolate profiles and the sensitivity of the target plant-parasitic nematode species to the associated ITC.

489 Effect of Oxycom on growth of tomato and reproduction of *Meloidogyne incognita*

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Single or multiple applications of Oxycom, peroxyacetic acid plus various biocontrol agents, were compared on 15-day-old susceptible tomato inoculated with 1500 second stage juveniles (J2) *M. incognita*. Forty pots were dipped into 2500 ppm (v/v) Oxycom, and 20 in water. Twenty of the 40 pots received four additional Oxycom applications at 10-day intervals. Plants were harvested at 60 days after inoculation to assess the effect of treatments on plant growth, nematode development and reproduction. A single treatment of Oxycom just prior to nematode inoculation significantly increased tomato top weight but not other growth parameters. Associated with plant growth benefit was a significant increase in the number of root-knot females. Multiple treatments of Oxycom significantly reduced leaf area, top weight and root weight while significantly increasing the number of galls, females and J2 per plant or per g of root. This study demonstrates that Oxycom stimulates plant growth and earlier fruiting while hastening nematode development. Proper timing and frequency of applications is important. No treatment reduced nematode population levels in this 60-day trial.

490 Dichloropropene with chloropicrin soil disinfection for nematode control in sweet pepper crops

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In pepper glasshouses of the Southeast of Spain, *Meloidogyne incognita* and *Phytophthora capsici* are the main soil pathogens. Methyl bromide (MB 98:2) has been used for soil-borne pathogens in the last 17 years. The mixture 1,3-dichloropropene (60.5%) and chloropicrin (33.3%) (Telopic EC) applied with PE plastic at 50 g/m² by drip irrigation was assayed as an alternative to methyl

bromide nematode control. In nine of the assays carried out since 1998 no significant differences were found Telopic EC and methyl bromide in *M. incognita* average root-knot index (Telopic: root-knot index = 0.5. MB: root-knot index = 0.5), marketable yield (Telopic: 9.4 kg/m². MB = 9.3 kg/m²) or plant growth (Telopic = 137.3 cm. MB = 143.5 cm). However, the proportion of *M. incognita* infested plants (21.1%) on the mixture treatments was significantly higher than that of MB (5.1%). When application was repeated, marketable yield and plant growth at the 3rd year were similar but root-knot index was significantly higher on Telopic (21.1%) than on MB treatments (0.6).

491 Chemical options for the management of *Heterodera schachtii* in sugar beet under field conditions

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A field experiment was conducted at the Parma Research and Extension Center, Parma, Idaho, to evaluate the efficacy of Temik at different rates and timing on the sugar beet cyst nematode management in sugar beet. The experiment was laid out in a randomised block design with seven treatments each of five replications. Temik was applied at planting on 31 May and Counter was applied at planting to individual plots (6 rows × 50 ft). Sugar beet seeds were planted on 3 March in rows 22" apart. Application of Temik at planting of sugar beet or 3 months after planting significantly increased the beet yield as compared to the untreated control. In all treatments, application of Temik @ 33 lb/acre at planting increased the beet yield and beet vigour. Percent sugar yield was maximum at the rate of Temik 14 lb/acre at planting or the split application (at planting 20 lb/acre + post application 13 lb/acre). Temik performed better than Counter in terms of beet yield, percent sugar and beet vigour.

492 Evaluation of nematicides for the management of *Meloidogyne chitwoodi* on potato in Idaho

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A field experiment was conducted at the Parma Research and Extension Center, Parma, Idaho, to study the efficacy of Mocap and Temik in combination with Vapam and fosthiazate or Vydate for control of Columbia root-knot nematode in potato. Fosthiazate and Mocap treatments were surface broadcast on 4 May 2001 and Telone II and Vapam were applied on 27 March and 2 April, respectively. Potato cv. Russet Burbank seed pieces were planted on 18 April in rows 3 ft apart. Five months after planting, the tubers were hand-harvested from 15 ft of the middle two rows of each plot and weighed. Yield of tubers under different treatments indicated that there is an increase in market yield and total yield in different combinations of Temik and Mocap as compared to control plot. Percent of nematode infected tubers in treated plots ranged from 3.9 to 13.4. Lowest level of nematode infection was observed in the treatment of 37.5 gal Vapam (3 tier shanks) + 2 gal Mocap.

493 Comparative efficacy of Fosthiazate formulations for the management of *Meloidogyne chitwoodi*

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Two field experiments were conducted to study the efficacy of Fosthiazate formulations along with Vapam on the control of Columbia root knot nematode (*Meloidogyne chitwoodi*) in a potato field. Fosthiazate was applied at the rate of 8.6 or 11.5 pt/acre in the autumn of 1999 or spring of 2000 alone or along with Vapam (37.5 gal). Potato cv. Russet Burbank was planted on 21 March 2000. In the first experiment, maximum marketable yield was recorded in the plots where Vapam was applied in autumn 1999 and fosthiazate in the spring 2000. In the second experiment, maximum marketable and total yield was observed in the plot treated with Vapam and Fosthiazate in spring 2000 before planting. Among all treatments, Fosthiazate with Vapam increased the marketable yield and total yield with the lowest nematode infection. Percent of nematode infection ranged from 0-1.9 and 0-17.4 in the first and second experiments, respectively.

494 Efficacy of dichloropropene with chloropicrin to control *Meloidogyne incognita* on pepper crops: rate of application and effect of plastic setting

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The efficacy of 1, 3 dichloropropene (60.5%) plus chloropicrin (33.3%) (Telopic EC) applied by drip irrigation has been evaluated in commercial pepper glasshouses in the southeast of Spain as an alternative to methyl bromide (BM 98:2 at 60 g/m²) *Meloidogyne incognita* control. The following treatments were assayed: 500, 400 and 300 l/ha with polyethylene plastic (PE); 300 and 200 l/ha with VIF plastic (Virtually Impermeable Film). Plastic set transversally and perpendicularly to the drip lines were assayed at 500 and 400 l/ha rates. All treatments were compared to MB 98:2 at 60 g/m² applied with PE and a non-treated control. The best results were obtained with 400 and 500 l/ha applied with PE. No significant differences were found in the percentage of infested plant, *M. incognita* root-knot average index, plant height and commercial yield. The use of VIF enhanced disinfection efficacy and allowed rate reductions. No significant differences were found between transversal and longitudinal settings. Longitudinal plastic setting makes easier commercial Telopic EC application and reduces cost.

495 Effect of nematicides on *Pratylenchus zaei* Graham 1951 in sugar cane in Goias, Brazil

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Nematicides were applied in the fields pertaining to Jalles Machado S/A, municipality of Goianésia-GO, infested with *Pratylenchus zeae* Graham, 1951. The experiment was conducted during the period 03/1999 to 09/2000, utilising the randomised complete block design with four repetitions. The treatments utilised were as follows: *i*) Check; *ii*) Terbufos 50G 60 kg/ha; *iii*) Terbufos 150G 20 kg/ha; *iv*) Aldicarb 150G 10 kg/ha; *v*) Carbofuran 50G 60 kg/ha; *vi*) Thiamethoxam 250WG 20 kg/ha. The parameters of evaluation were the population density of nematodes (specimen/10 g of roots and specimen/100 g of soil) determined at 3, 6 and 9 months after planting and yield data of the variety RB72454, determined after 18 months. The nematicides showed efficiency in reducing population level of plant nematode up to 6 months after their application and differed from the check and thiamethoxam treatment. The grain yield increase was significant as a result of plant nematode population reduction. The highest yield gains of 8.72 and 8.58 t/ha were obtained for Terbufos 150G and Carbofuran 50G treatments, respectively.

496 Evaluation of Messenger in combination with aldicarb or thiamethoxam for management of *Rotylenchulus reniformis* on cotton

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Messenger, a hairpin protein, was examined in Alabama and Mississippi for its effect on *R. reniformis* population development and cotton yields. Tests were established in locations which were in cotton production and had a history of reniform nematode infestation. Messenger was applied with a CO₂ charged backpack field plot spray system as a broadcast spray using a 6 ft boom with 8002E flat fan tip nozzles calibrated to deliver 95 l/ha. In Test 1, Messenger was applied at the two leaf stage (2LF), pin head square (PHS), full bloom (FB), and/or 3 weeks after full bloom (FB + 3) in combination with aldicarb (0.85 kg a.i./ha). In Test 2, Messenger was applied at 2LF, PHS, FB, and/or FB + 3 in combination with thiamethoxam (300 g a.i./100 kg seed) treated seed or aldicarb (0.59 or 1.2 kg a.i./ha) applied in-furrow at planting. In Test 1, in Mississippi, seed cotton yields

were increased an average of 227 kg/ha in all Messenger and aldicarb combinations compared to aldicarb alone. However, in Alabama, Messenger did not increase cotton yields over aldicarb. In Test 2 in Mississippi and Alabama, the applications of Messenger + aldicarb (0.59 kg a.i./ha) averaged an increased seed cotton yield of 69.9 kg/ha over aldicarb (0.59 kg/ha) alone. Messenger did not increase cotton yields in either location when applied in combination with aldicarb at 1.2 kg a.i./ha. Messenger in combination with thiamethoxam treated seed increased yield in Alabama but not in Mississippi.

497 Abamectin effect on root-knot nematode population in melon hybrids

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The efficiency of abamectin on root-knot nematode populations was evaluated in three melon hybrids, Rochedo, Gold Mine and AF 646. The experiment was carried out in glasshouse conditions with two completely randomised factors and two independent treatments, with four replicates, each replicate comprising a plastic pot with two plants. Three levels of abamectin, equivalent to 27, 36 and 45 l of a.i./ha, were used. Naturally nematode infested soil, with initial populations determined as 30 second stage juveniles (J2)/cm³, was used. Variables root-knot index (0-4) and nematode reproductive factor ($Rf = Pf/Pi$), in the soil were evaluated. Root-knot index was significantly lower in chemical treatments, with highest reductions in population development of J2 being obtained with 45 l/ha. The highest nematode population incidence was observed in Gold Mine hybrid.

498 Controlling parasitic nematodes in an established vineyard in Cyprus

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Xiphinema index, *Mesocriconema xenoplax* and *Paratylenchus hamatus* are all pathogenic to grapevine (*Vitis vinifera* L.). A trial in an established vineyard (cv. Sultanina) during 1999-2001 has shown that the organophosphorus contact nematicide cadusafos (as Rugby[®]) can effec-

tively control these nematodes whereas yields were also increased. It was applied as granules and in liquid formulation (microcapsules) in spring 1999 and 2000, at the rate of 3.5 g a.i. per sq. m. of actually treated area. Granules were applied in 8 cm wide bands, near and at both sides of the plants (incorporated) while microcapsules were applied through irrigation in small basins around each plant. Carbofuran (as Furadan®) at the rate of 5.5 g a.i. per sq. m. was also applied using the same method. There were four treatments, including the control, replicated three times. The soil type was clay loam. Total number of plants was 144. Nematode control performance of cadusafos, on an overall mean of 3 years, was high, whereas yields were significantly increased between 20.7-21.9%. The profit:treatment cost ratio for this nematicide reached four while no chemical residues were found in the fruit. Carbofuran was less effective than cadusafos in controlling the nematodes or increasing yields, under the conditions of this trial.

499 Economic impact of resistant tomato cultivars as an alternative to methyl bromide to control *Meloidogyne javanica*

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The economic impact of resistant tomato was evaluated in a plastic house infested with the nematode for three consecutive growing seasons starting in 1999. Treatments included susceptible (cv. Durinta) and resistant (cv. Monika) tomato cultivated in methyl bromide fumigated soil or in soil infested with *Meloidogyne javanica*. Methyl bromide was applied at a rate of 75 g/m² in October 1998 at a cost of 2.44 euros/m². Tomatoes were cultivated from March to July, and there were 2.9 plants/m². Each treatment was replicated four times. Nematode densities were determined at the beginning and end of each crop. Yield was assessed in eight plants/plot weekly for 6 weeks. Initial densities were 480 and 660 second stage juveniles (J2)/250 cm³ soil, and changed to 10 356 and 190 J2/250 cm³ soil after growing susceptible and resistant tomato, respectively, for three consecutive seasons. The average net profit of cropping resistant tomato in infested plots was 30 000 euros/ha per season with respect to the susceptible cultivar in nematode infested plots, and 10 600 euros/ha per season with respect to the resistant cultivar in fumigated plots. The average net loss by cropping susceptible tomato in infested plots was 21,200 euros/ha with respect to those cropped in fumigated plots.

500 The effect of some legumes on stimulating egg-hatch to reduce densities of soybean cyst nematode

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Hatching of soybean cyst nematode, *Heterodera glycines*, is stimulated by legume root-exudates. Non-host legumes also secrete these substances and can be used to decrease soil egg-densities. Root exudates of soybean, azuki bean, kidney bean, peas, two red clovers, white clover, alfalfa, sun hemp, Chinese milk vetch, sweet pea, lupine and mimosa were tested for stimulating egg-hatch. Kidney bean was the most effective legume and stimulated hatching of 66% of eggs assayed. The nematode reproduced on soybean, azuki bean, kidney bean and Chinese milk vetch. The non-host sun hemp stimulated 58.8% egg-hatch and was the most effective in reducing soil egg-densities. Alfalfa, mimosa and pea did not stimulate hatching. In field plots the cultivation of sun hemp (*Crotalaria juncea* and *C. spectabilis*) or red clover decreased soil egg-densities by 57-64% compared to fallow control plots. Parasitic fungi attacked 14.9-18.1% of eggs present in susceptible or resistant soybean field plots and 30.6-43.6% in egg-hatch stimulating legume field plots.

501 Comparison of physical and chemical methods of controlling *Ditylenchus dipsaci* in garlic crops

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The effectiveness of soil solarisation and oxamyl applications against *Ditylenchus dipsaci* on garlic crops were evaluated under field conditions in Central Spain. Oxamyl treatments were: dipping garlic cloves in water solution of Vydate at 3 ml/kg and at 1.5 ml/kg doses, and Vydate added in granule formulation (0.8 g/m) to the furrows at planting time. All treatments achieved a significant reduction in the rate of disease progress and the final incidence of plant death by *D. dipsaci*, which

resulted in a significant improvement of garlic yields. Soil solarisation for 8 weeks resulted in the reduction to undetectable levels of the nematode in the upper 20 cm layer of soil, even in heavily infested soils. Rates of disease progress were greatly reduced in solarised plots, with yield increments of 50-140% over the unsolarised control plots. Soil solarisation was also highly effective in a second consecutive crop of garlic, with significant improvements in yield and garlic quality.

502 Hot water drench treatments for the control of *Radopholus similis* in *Rhapis* and Fishtail palms

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Exporters of potted nursery stock face strict quarantine regulations against *Radopholus similis*, the burrowing nematode (BN). Currently no quarantine treatments to disinfest plants of BN are approved. Therefore, hot water drench treatments were investigated for possible quarantine utility. *Rhapis excelsa* and *Caryota mitis* palms were inoculated with 5000 mixed life stages of BN 14 weeks prior to treatment. Hot water drench treatments were applied at 50°C for 0-16 min. In *Rhapis* palms, a moderately good host, a 16 min hot water drench achieved 99.6% mortality of BN. In Fishtail palms, a poor host, all treatments longer than 10 min at 50°C achieved 100% nematode mortality. Probit regression estimates were used to estimate LT₉₉, resulting in 16.9 and 10.3 min for *Rhapis* and Fishtail, respectively. However χ^2 goodness-of-fit tests for deviation from observed data was significant ($\chi^2 = 21.136$, df = 3, $P < .0001$) for *Rhapis*. The high efficacy of hot water drenches for the control of BN is approaching the Probit 9 standard of 99.9968% mortality required for United States Department of Agriculture approval as a quarantine treatment.

503 Forage pearl millet for the control of *Pratylenchus penetrans* and potato early dying disease

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Root-lesion nematode *Pratylenchus penetrans* causes yield losses in potato fields in Quebec. In some areas, it has been found associated with *Verticillium dahliae* and the potato early dying disease (PED). Under field conditions, we evaluated forage pearl millet (FPM) as a rotation crop for controlling nematode populations, reducing PED symptoms and potato yield losses. In 1999, a 1-year crop rotation experiment was established on three commercial fields and where potato cv. Superior was grown the following year. FPM significantly reduced by 89% the number of *P. penetrans* in the soil and increased by 11% the subsequent potato yield when compared to oats. In 2000, again on a commercial potato field, we assessed the usefulness of combining FPM rotation with preplant soil fumigation (fall in-furrow application of metham sodium 148 l/ha). The three following treatments were made: 1/rye + fumigation, 2/FPM alone, 3/FPM + fumigation. FPM alone performed as good as the rye + fumigation treatment. FPM + fumigation treatment reduced by 98% *P. penetrans* soil populations, reduced by 69% PED symptoms and increased by 31% the subsequent potato yields when compared to rye + fumigation.

504 Integrated and ecological nematode management in Canary Island crops

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The Canary Islands are characterised by Mediterranean and subtropical climatic conditions, its seasonal nature with hot summers and mild winters represents an ecotone between temperate and tropical regions. Integrated and ecological nematode management are analysed. For the most part, Mediterranean agrosystems are represented by potato, vegetables, citrus and fruit trees and vineyards. Coastal environments are largely represented by bananas

(‘plátanos’) and horticultural crops under cover. The main nematological problems in order of their economical importance are: root-knot (*Meloidogyne incognita* and *M. javanica*), cyst (*Globodera pallida* and *G. rostochiensis*), virus vector (*Longidorus*, *Paratrichodorus*, *Trichodorus* and *Xiphinema*), endoparasitic (*Pratylenchus goodeyi*), citrus (*Tylenchulus semipenetrans*) and ectoparasitic nematodes (Criconeematidae, Hoplolaimidae and Tylenchidae). It is also indicated that the environmental characteristics of the Canary Islands climate and soil conditions permit the design of integrated and ecological production systems based fundamentally on the use of non-chemical alternatives such as crop rotation and fallow, soil amendments, biofumigation, plantation date and other cultural practices, sanitation, plants grown on natural substrates, the use of resistant varieties, grafting, biological control agents and solarisation. These alternatives prevent pest and diseases from becoming serious problems and obtain high quality agricultural products.

505 Non-chemical alternatives for the control of *Meloidogyne incognita* in Uruguay

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Among other problems caused by soil-borne pathogens, losses are greatest from the root-knot nematode *Meloidogyne incognita* in Uruguay intensive horticulture. Several non-chemical alternative control methods have been tested: biofumigation, using various organic amendments and green manures, resistant varieties, plant covers, trap crops and other crop techniques, all within a programme of integrated production. Resistant varieties are used in tomato crops; however, we must take into account that resistance can be inefficient when soil temperature is higher than 27°C and particularly when virulent nematode populations exist. These alternatives are being applied to several crops and production zones in Uruguay, generally with similar, and sometimes even better, effectiveness than conventional phytosanitary products. Moreover, farmers find no difficulty in applying these alternatives since they have been developed through a participative research process.

506 Reaction of bacterial-feeding nematodes to pig manure with antibiotic and manure without antibiotic

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The study was carried out in three experimental plots in grassland (10 m² each) treated in three different ways: *i*) 100 l of water, *ii*) 100 l of manure from pigs fed on forage with antibiotic ZnBacitracin, *iii*) 100 l of manure from pigs fed on forage without antibiotic. Nematode communities were studied 6 and 12 weeks, and 10 months after the treatment. In our study, bacterial-feeding nematodes belonged to five orders: Areolaimida, Dorylaimida, Monhysterida, Rhabditida and Teratocephalida. In every treatment, on all sampling occasions nematodes belonging to the order Rhabditida visibly dominated. The following parameters were used for evaluation of the effect of two kinds of manure on bacteriovores: *i*) total abundance of bacterial-feeding nematodes, *ii*) Diversity index (H'gen), *iii*) Maturity index (MI), *iv*) number of genera in order Rhabditida. Abundance and dominance of particular genera belonging to Rhabditida in three plots were also analysed. Obtained results showed that the values of analysed parameters as well as the response of particular genera of Rhabditida differed in three plots: untreated, and treated with two kinds of manure, from pigs fed on forage with and without antibiotic.

507 *Heterodera glycines* distribution within a field in no-till production over time

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Heterodera glycines (SCN) is a major pest of soybean causing significant yield losses when present and when management techniques to reduce egg population density are not used. A study investigated the distribution of SCN at planting and harvest in a claypan soil field in central Missouri under no-till soybean production. Comparisons were made of the nematode distribution in 1999 and 2001, years of soybean production. Nematode distribution in

the 2 years was compared to yield, topsoil thickness, elevation, and soil nutrient maps. Nematode distribution was most closely correlated with topsoil thickness (*i.e.*, depth to the claypan). Lower *H. glycines* population density occurred in areas with shallow topsoil thickness.

508 Computer modeling of integrated control of potato cyst nematodes

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A computer program modelling the integrated management of PCN has been developed based on equations calibrated and validated with field trial data. Population trends and damage are presented for up to six rotations, and the control measures can be varied for each potato crop. Currently, it is an effective educational tool for demonstrating the effects of soil type and of varying rotation length, nematicide effectiveness and the resistance and/or tolerance of the cultivar being grown. To develop its predictive potential, the relationship between PCN population density, damage and multiplication rates are being calibrated for specific fields. Nematicide effectiveness and rates of PCN population decline between potato crops are also being determined. Slider controls are used to vary the parameter estimates to produce a least squares 'best fit' line and the new parameters are inserted into the main management program. The advantage of this approach is that relatively crude/variable data, based on a small number of samples, can be used to calibrate the models and avoid 'convergence' problems.

509 The interaction between an arbuscular mycorrhizal fungus and migratory endoparasitic nematodes in *Musa* genotypes differing in host plant response

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Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of plants that biotrophically colonise the root cortex and develop an extramatrical mycelium which helps the plant acquire water and mineral nutrients from the soil. AMF also may protect plants against soil-borne pathogens, including nematodes. Two *Musa* genotypes, *i.e.*, Grande Naine as susceptible genotype and Pisang Jari Buaya as resistant genotype, were selected for their known host plant response to two nematode species. The migratory endoparasites, *Radopholus similis* and *Pratylenchus coffeae*, are important pests in *Musa*. The AMF can protect both Grande Naine and Pisang Jari Buaya against these nematodes, since nematode reproduction was significantly suppressed by the AMF. In the case of *P. coffeae* the AMF was able to reduce the damage in the roots, caused by the nematodes. For *R. similis*, no reduction of damage was observed. It appears that the nematodes reduced the frequency of mycorrhization without reducing the intensity of the mycorrhizal association. Mycorrhization resulted in a significant better plant growth, even in the presence of *R. similis* and *P. coffeae*.

510 The challenge towards integrated control of root-knot nematodes for developing agriculture in South Africa

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Root-knot nematodes are omnipresent in most resource-poor areas of South Africa. A survey conducted in five provinces confirmed the extent of this problem, while demonstrations of significant crop yield losses also accentuated an urgent need for development and application of integrated, effective nematode management strategies to enable sustainable food production. On-farm trials, where alternative, low-cost nematode control strategies were evaluated, revealed treatments with a fair potential for implementation by resource-poor farmers to reduce root-knot nematode numbers significantly. A significant reduction of root-knot nematodes by means of crop rotation, using crops popular to small-scale farmers in a particular cropping sequence was established in a multi-seasonal microplot trial. Resistance screening of dry

bean and cowpea genotypes supplied additional, valuable information on the application of increased resistance for the prevention of root-knot nematode population build-up in cropping systems. Finally, use of a cover crop such as Vetiver grass can contribute significantly to root-knot nematode control, particularly where livestock forms an integral part of a farming system. Integration of some or all of these control strategies that are currently available can already contribute significantly to household food security of developing farmers with minimal additional input costs and effort.

511 Grapevine management, virus vector nematode populations and GFLV: an approach to disease epidemiology

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Factors affecting GFLV transmission in vineyards from the Canary Islands have been studied for more than 10 years. The special agricultural characteristic of grape crops in Lanzarote, La Palma, El Hierro and southern Tenerife, which use traditional methods of grapevine selection, have prevented the dispersal of the virus by nematodes. Moreover, no problems occur with nematodes in those vineyards growing on volcanic substrates except in Gran Canaria, because there vineyards are localised in the Laurisilva where high humidity exists. However, new crop techniques could contribute to an increase in infection, especially irrigation and non-controlled plants introduced in new plantations. *Xiphinema index* and *X. italiae* populations are increasing. Moisture in the soil and the steady year-round temperature on the island increase the viral infection. Besides, film water is a good vehicle for nematode and viral dispersal, especially on slope farms with irrigation. Both vectors and GFLV are widespread in the whole archipelago, disseminated mainly in new plantations through infected rootstocks, as a consequence of the introduction of non-certified plants. The greatest focal points for both pathogens appeared in irrigated vineyards or areas with the highest soil moisture, where the viral infection is maintained over the years.

512 *Meloidogyne incognita* control by biofumigation plus solarisation on glasshouse pepper crops in the southeast of Spain

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Meloidogyne incognita is one of the main soil borne pathogens in pepper glasshouses of the southeast of Spain. In this area pepper may be considered a monoculture grown over more than 90% of the 1800 ha total crop surface. Timing of the biofumigation-solarisation application as a nematode control method was assayed in commercial and experimental greenhouses. Efficacy was evaluated by measuring the percentage of infected plants, average root-knot index and marketable yield in relation to methyl bromide (MB). When a mixture of fresh sheep manure (FSM) at 7 kg/m² and chicken manure at 3 kg/m² was used as an organic amendment applied from the end of August to the beginning of September, marketable yield (9.4 kg/m² in August and 12.0 kg/m² in September) was similar to that of MB (9.9 kg/m² in August and 12.2 kg/m² in September). However, the percentage of *M. incognita* infected plant was lower in biofumigation treatments. When applications were initiated at the beginning of October and November, biofumigation treatments differed significantly from MB treatments in all the parameters measured.

513 Changes in nematode faunas under different short- and long-term management regimes

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Different trophic groups of soil nematodes were affected by both short-term and long-term agronomic practices in

replicated trials at several sites in eastern Australia. In the short-term (2-3 years), tillage and residue management affected plant-parasitic nematodes (*Pratylenchus*, *Tylenchorhynchus*, *Paratrichodorus*, *Paratylenchus*). Microbial feeding, substrate ingesting and certain predatory nematodes were more abundant and diverse in less cultivated soils, while fungivorous nematodes were more abundant and diverse in cultivated soils. Omnivorous nematodes showed few differences with cultivation but were less abundant where more organic residues were retained. There were more microbivores and they were concentrated closer to the surface where residues were retained; fungivores, by contrast, were deeper in the soil. There were no differences in the Maturity Index. Over longer-terms (5-10 years), rotation from wheat to pasture decreased the abundance of most plant-parasitic nematodes (*Pratylenchus*, *Tylenchorhynchus*, *Criconematidae*, *Tylenchidae*), but not *Ditylenchus* or *Dorylaimoides*, which increased. *Helicotylenchus* populations were unchanged. Fungivores, predators, omnivores and substrate ingesters were more abundant in continuous cropping; microbivores and algal feeders more abundant after rotation. Short-term management practices such as cultivation and residue retention affect soil nematodes, and by implication the energy pathways in the soil, in different ways from longer term practices such as rotation.

514 Effect of hot-water treatment on nematode species composition and densities in plantain roots in short and long fallow (Central Province, Cameroun)

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Root nematodes were identified as a major cause of yield loss in plantains. The objective of this study was to determine the effects of plantain variety, fertiliser application, hot-water treatment (20 min at 52°C) of suckers before planting and fallow length, on the root damage parameters and nematode numbers in plantain roots. The experiment was set up on-farm as a randomised complete block design with 64 plots, each of 25 plants. Roots of five plants per plot were sampled at 15 months

after planting. Preliminary results indicate that variety and fertiliser application had no significant effect on nematode numbers or root damage parameters. Hot-water treatment significantly reduced total nematode numbers in both fallow types, although fallow length had no significant effect on nematode numbers. Significantly higher root necrosis indices (RNI) were observed in plants in long fallow plots compared to plants in the short fallow plots. However, the total number of functional roots was significantly more in the long fallow plots, giving an overall significantly better health of the root system, as indicated by the non-damaged root index (NDRI = number of functional roots \times (100-RNI)). Hot-water treatment did not significantly reduce the RNI in the short fallow, although it did significantly increase the NDRI in both fallow types.

515 Sanitation efficacy of a combined thermal filter press and vacuum dryer on potato cyst nematode and wild oat in the production of VEAS-Biosolids

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The soil conditioner, VEAS-Biosolids, is produced through compression, heating and vacuum drying of sewage sludge at the VEAS wastewater treatment plant in Slemmestad, Norway. The sanitation efficacy for potato cyst nematode (PCN), *Globodera rostochiensis* and wild oat, *Avena fatua*, was evaluated for the final production step, the combined thermal filter press and vacuum dryer. Germ carriers, each carrying three nylon bags with 20 nematode cysts and one bag with 100 seeds of wild oat, were placed in three filter presses. Germ carriers were either exposed to sludge for 4 days, or kept dry before the test. The test pathogens were exposed to pressures of up to seven bars at 35°C, followed by heating to 80°C and vacuum at -0.92 bar during continued heating. Nematode vitality was studied by recording juvenile hatch, by vitality staining with New Blue R, and by recording nematode development on susceptible potato. Viability of wild oat was examined by tetrazolium test. There was a 100% efficacy in the sanitation of PCN and wild oat. For both organisms pre-exposure to sludge seemed to reduce vitality and viability. The mechanism of sanitation may

rely on an interaction between sludge toxicity, and the changes in temperature and pressure.

516 Nematode management in subsistence farming in Mexico

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Factors such as geography, climate, vegetation, biogeography and human settlement have contributed in defining Mexican rural and socio-economic scenarios. Mexican agriculture has two main approaches: the campesino and the agro-industrial, both with contrasting characteristics related to production and management strategies against pests and diseases. In campesino and subsistence systems, low external-input strategies that have proved effective in the management of the most common and important plant-pathogenic species of nematodes in Mexico (*i.e.*, *Meloidogyne* spp., *Nacobbus aberrans*, *Pratylenchus* spp. and *Punctodera chalconensis*), include production of plant and tree seedlings free of nematodes, physical control methods, rotations, organic amendments, antagonistic plants, suppressive soils and maintenance of biodiversity. Most of these practices are considered to be environmentally friendly but, although subsistence agriculture can produce enough food to feed families or small communities, it may be adversely affected by socio-economic factors such as availability of good land, adequate water, migration, pressures on land use, shortened rotations, crop value and changes in cultural patterns, thus compromising sustainability.

517 Interactions between vinyl house tomato IPM measures for control of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *lycopersici*

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Hot water (HW), *Pasteuria penetrans* (PP), *Glomus* sp. R10 (Arbuscular mycorrhiza, AM) and an avirulent *Fusarium oxysporum* (AF) were applied to 2 m² vinyl house plots where *Meloidogyne incognita* and *Fusarium*

oxysporum f. sp. *lycopersici* were present. The experiment used a 2³ factorial design with three replicates to identify interactions between IPM measures. Untreated plots and fumigated (D-D 52% + chloropicrin 40%) plots were the controls. Tomato seedlings were planted and grown from March to July 2001 (15 weeks). HW completely suppressed *Fusarium* wilt of tomato. HW and PP-AM suppressed root galling and juvenile population ($P < 0.01$), with synergistic interactions between HW and PP-AM ($P < 0.05$). When applied together HW × PP-AM root galling was similar to that of the fumigated plots. AF applied with HW was associated with increased galling; however, this effect diminished when AF, HW, and PP-AM were applied together. HW and PP-AM yielded the most fruit ($P < 0.01$) without an interaction between the two measures. When applied together, HW × PP-AM and HW × AF × PP-AM had yields 28 and 39% greater than the untreated controls, respectively. The interaction between HW and PP-AM, as well as increased galling associated with AF and HW, will be discussed.

518 Mechanisation has added benefits in nematode management in tea

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Replanting has paramount importance in sustaining the productivity of tea. This involves uprooting old tea followed by rehabilitation of soils using grasses. Mechanical uprooting of old tea bushes has recently been introduced to the industry owing to shortage of workers, cost factor and timing of replanting operation *etc.*, over conventional manual uprooting. The present exercise compared the effect of mechanical and manual uprooting on management of populations of the root lesion nematode, *Pratylenchus loosi* in old tea fields. Mechanical uprooting left significantly low amounts of root fragments and the soils possessed low residual populations of *P. loosi* compared to that in manually uprooted soils. The growth of rehabilitation grasses was superior in mechanically uprooted field. It resulted in a greater proportion of organic matter addition and significant elevation of densities, activation and establishment of nematode biocontrol agents, especially free-living nematodes, in the soil. Improvements in

soil biological and chemical properties were also seen. The newly planted tea in mechanically uprooted fields exhibited significantly greater root biomass.

519 The effect of crop rotation and tillage systems on soil nematode trophic structure

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The effect of the use of crop – pasture rotation and tillage systems (conventional tillage – CT and no-tillage – NT) on soil nematode communities was studied during 1 year in a long-term crop rotation and tillage system field experiment on a Typic Argiudoll at Balcarce, Argentina. Four seasonal samplings were done and nematodes were extracted from soil using the centrifugation-flotation technique. The trophic structure was dominated by bacteria feeder and plant feeder nematodes and the highest nematode abundance was found in winter. The highest plant feeder nematode abundance was shown in pasture in autumn ($P < 0.05$), followed by crop – pasture rotation. Tillage system did not significantly affect the abundance of such trophic group. The fungivores abundance was lowest in crop rotation under NT in autumn. The bacterivores were lowest in the pasture in spring and were not affected by the tillage system. The maturity index (MI) was affected by treatments in spring ($P < 0.05$). The pasture and crop – pasture rotation under CT showed the highest MI value. The diversity index (H') was affected by the treatments in winter and spring ($P < 0.05$). The H' was lowest for crop – pasture rotation under CT in winter. The H' was lowest under pasture in spring. A higher nematode diversity richness was detected in comparison to previous reports from this region.

520 The harmful influence of associations of nematodes and mono- and complex virus infections on potato plants and the effectiveness of ecological safe control methods of these pathogens

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The harmful influence of associations of longidorid and trichodorid nematodes and four viruses (TRV, X, S, M) and associations from two to six virus and nematode components exercised considerable and reliable influence on the dynamics of growth (height and number of shoots) and yield of tubers of potato plants. The largest synergetic influence of complex trichodorid and longidorid nematodes and virus infections was found in cases of four, five and six virus and nematode components. The antiviral action of two biopesticides (planrhiz and trichodermin) and six physiological active agents (narciss, immunocytophyt, F-760, F-1153, immunal, and nikfan) by spraying above-ground organs of potato plants in field conditions in comparison with untreated control were studied. Different nematicidal and antiviral actions were found in most bioagents that exercised positive influence on yield of potato tubers and dynamic growth of potato plants.

521 Population growth rates of two fungal-feeding nematodes, *Filenchus misellus* (Tylenchidae) and *Aphelenchus avenae* (Aphelenchidae), on ten fungal isolates

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To clarify differences in reproduction characteristics between *Filenchus misellus* (family Tylenchidae) and *Aphelenchus avenae* (Aphelenchidae), their population growth rates were measured after 40 days incubation at 25°C on ten isolates of fungi representing nine species. *Filenchus misellus* growth rates were large on *Agaricus bisporus*, *Chaetomium cochlioides*, *Chaetomium globosum*, *Chaetomium funicola*, *Coprinus cinereus*, *Pleurotus ostreatus* and *Rhizoctonia solani*, and small on *Pythium ultimum*, *Fusarium oxysporum* f. sp. *conglutinans* and *Fusarium oxysporum* f. sp. *cucumerinum*. In contrast, *A. avenae* growth rates were large on *P. ultimum*, *R. solani*, *F. oxysporum* f. sp. *conglutinans*, *F. oxysporum* f. sp. *cucumerinum* and *A. bisporus*, and small on *C. cochlioides*, *C. globosum*, *C. funicola*, *C. cinereus* and *P. ostreatus*. These results suggest that *F. misellus*, which reproduces well on saprophytic fungi and mushrooms, may occupy an ecological niche different from that of *A. avenae*, which

reproduces well on plant-pathogenic fungi, although both nematode species did well on *R. solani* and *A. bisporus*.

522 Soil solarisation, nematicide and chicken compost for root-knot nematode control in carrot in Brazil

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A field experiment was performed in sandy soil, naturally infested with *Meloidogyne incognita* and *M. javanica*, in Piracicaba, SP, Brazil, to assess the effectiveness of soil solarisation, chicken compost (20 t/ha), carbofuran 100G (40 kg/ha), and combinations of these treatments for the control of root-knot nematodes in carrot cv. Aline. Soil solarisation was performed over 69 days during the warmest months of summer of 2000/2001, using a 100 µm polyethylene plastic film as soil covering. The soil temperature in solarised plots was 8-12°C higher than in the nonsolarised. Maximum soil temperatures were recorded in solarised soil combined with chicken compost and reached 77, 56 and 46°C, at depths of 5, 10 and 20 cm. Population densities of *M. incognita* and *M. javanica* were controlled by both soil solarisation alone and combined with chicken compost or carbofuran. Carrot cv. Aline yield was significantly increased by combination of solarisation and chicken compost plus nematicide application before planting.

523 Current management strategies employed against the reniform nematode (*Rotylenchulus reniformis*) in cotton production in Louisiana, USA

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Reniform nematode has become a major pest of cotton in Louisiana during the past decade. Since 1990, 40% of the 18 477 samples processed by the Nematode Advisory Service revealed detectable levels of reniform nematode. Nematicides and crop rotation are the primary methods of management. In-furrow application of aldicarb at 0.59 kg/ha has been a producer standard since the 1980s. Based on 35 field trials, plots receiving an in-furrow application of aldicarb (0.59 kg/ha) produced an average 262 kg/ha more seed cotton than non-treated plots. The addition of 1,3 dichloropropene at 28 l/ha to the producer standard rate of aldicarb increased yields over the grower standard by 326 kg/ha in six field trials. Approximately 25-30% of the cotton crop is rotated to a non-host or resistant crop such as corn, grain sorghum, or soybean. Only five of the 69 soybean varieties recommended for Louisiana have been reported as resistant against this nematode. Populations of reniform nematode averaged 2682 per 500 cm³ of soil in 217 fields in Rapides Parish after cotton followed by 1 year of corn. Populations of reniform nematode averaged 9558 per 500 cm³ in 158 fields after corn followed by 1 year of cotton.

524 Biological and chemical control of *Meloidogyne incognita* and its effect on soil microflora and growth of *Psidium guajava* seedlings in Zulia State, Venezuela

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The effects of *Trichoderma harzianum*, *Paecilomyces lilacinus* and carbofuran on *Meloidogyne incognita* and fungal and bacterial populations in the rhizosphere, rhizoplane, roots and soil were studied on *Psidium guajava* seedlings. The effects of the nematode on fresh root weight (FRW), fresh aerial weight (FAW) and dried aerial weight (DAW) were also evaluated at the Centro Frutícola del Zulia-CORPOZULIA. Sixty-day-old

seedlings were transplanted to 2.5 dm³ bags of sterilised sandy loam inoculated with two eggs + second stage juveniles/cm³ soil. Samples were collected at 30 and 60 days. We found statistically significant differences between treatments in all variables studied except for fungi in root samples and bacteria in rhizosphere and roots. The lowest final population level and multiplication rates were recorded from the carbofuran treatment, as well as the highest values for FRW, FAW and DAW. However, this treatment also caused the least development of fungal and bacterial colonies in the rhizosphere, rhizoplane and roots. The treatments studied showed a low capacity for controlling *M. incognita* on guava seedlings.

525 Distribution of *Globodera rostochiensis* in the central region of Russia and new control methods

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Considerable infection of potato plantation by *Globodera rostochiensis* was found in industrial and scientific fields in different regions of Russia (Karelia, Moscow, Vladimir and Voronezh regions). Cysts of *G. rostochiensis* were found at seven of 20 investigated potato crops. The influence of biological agents and bioactive substances (*i.e.*, biological pesticides, planrhiz and trichodermin; new biologically active strains of *Bacillus*, *Pseudomonas* and others; chitin containing substances narciss and agrochyt; and new Russian physiologically active substances, oligophurostanozid, immunocytophyt, compactin and lovastatin) on number and life activity of *G. rostochiensis* cysts in soil and on potato plants growth and yield, after treating tubers and plants with these biologically active agents, was studied. Nematicidal activity of three physiologically active substances (two inhibitors, compactin and lovastatin, and one resistance stimulator, immunocytophyt) was studied in experiments *in vitro*. Inhibitors of plant growth (compactin and lovastatin) demonstrated stimulating influence on the release of the infective juveniles from *G. rostochiensis* cysts.

526 Development and transfer of technology for bio-intensive nematode management in Southern India

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Increased demand in the domestic and international market for the horticultural produce has led to intensive cultivation of horticultural crops in various states of India. This has resulted in the increase of root-knot nematodes (*Meloidogyne* spp.) to an alarming extent affecting adversely the crop growth and productivity. No single method of control of nematodes and no single component of management of nematodes was effective in the management of nematodes on any horticultural crop on a sustainable basis. Biocontrol agents such as *Paecilomyces lilacinus*, *Trichoderma harzianum*, *Verticillium chlamydosporium* and *Pasteuria penetrans* are not effective individually in the management of nematodes under field conditions. Similarly, endomycorrhiza (*Glomus mosseae* or *G. fasciculatum*), botanicals (neem or castor or pongamia cakes) or any trap crop, antagonistic plant or any cultural practice is not effective individually in the field. Hence, we investigated the possibilities of integration of these components for the management of the nematodes on horticultural crops on a sustainable basis. The investigations have resulted in the development of integrated nematode management strategies of economically important nematodes of this region by integrating biocontrol agents, botanicals and endomycorrhizae under field conditions by exploiting the synergistic interaction between *P. lilacinus* + neem cake + *G. mosseae*; *P. lilacinus* + castor cake + *G. fasciculatum*; *T. harzianum* + neem cake + *G. mosseae*; *V. chlamydosporium* + neem cake + *G. mosseae* combinations. The data on the adoption of the technology, percent increase in the yield and the reduction in the use of chemicals is presented.

527 Influence of tillage on soybean pathogens in Illinois

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Fusarium solani f. sp. *glycines* (FSG), the causal agent of soybean sudden death syndrome (SDS), and *Heterodera glycines* are the two most important soybean pathogens in Illinois. Sudden death syndrome is generally more severe in areas with greater soil compaction and/or waterlogged conditions. At two locations, the effects of three tillage practices on pathogen populations and foliar severity of

SDS were evaluated. Treatments were fall subsoiling (depth of 46 cm), convention tillage, and no tillage. In each treatment, 16 varieties were planted in 2000 and 32 varieties were planted in 2001. The varieties varied with regards to SCN and SDS resistance. In both years, SCN reproduction was higher on SCN-susceptible varieties. Egg densities of SCN were higher in plots receiving subsoiling treatments at one site in 2000 and at both sites in 2001. Across varieties, subsoiling reduced the severity of SDS at both locations in 2000 and 2001. Root colonisation by FSG differed among varieties but was not affected by the tillage practices. In 2000 and 2001, soybean yield was higher in plots receiving the subsoiling treatment.

528 Crop rotation options for management of *Pratylenchus neglectus* in cereal-based production systems in Western Australia

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Pratylenchus neglectus is a key nematode parasite of cereals in Western Australia. To identify crop rotation options for nematode management, cultivars of barley (Stirling), oat (Dalyup), wheat (Nyabing), canola (Dunkeld and Karoo), chickpea (Heera), faba bean (Fiord), field pea (Dundale) and lupin (Merrit) were sown in 1999 in 40× 1.8 m plots in a randomised block design with six replications in a nematode-infested field. Nematode numbers in soil before planting and in roots after 10 weeks were assessed. Wheat cv. Machete was the highly susceptible check. All the plots in 2000 were sown with wheat cv. Cunderdin, and in 2001 with the same crop as in 1999. Nematode data were log-transformed for analysis. Field pea and faba bean supported significantly fewer nematodes than other crops. Previous year crops significantly influenced nematode infestation and the fewest were extracted from plants in plots previously planted to faba bean and most in plots previously planted to wheat. In 2001, infestations were least in faba bean and field pea and greatest in chickpea, oats and canola cv. Karoo. Faba bean and field pea were identified as suitable crop options to aid in the rotational management of *P. neglectus*.

529 Trap cropping of potato cyst nematodes using resistant Solanaceae potato clones

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In recent years the levels of *Globodera pallida* in potato land has been steadily increasing, such that some land is now considered unproductive for continued commercial potato production. The concept of using Solanaceae potato clones as biological control agents for PCN was investigated. The criteria for such clones were that they have full resistance to all known PCN pathotypes (both *G. rostochiensis* and *G. pallida*), have the ability to stimulate high levels of PCN hatch, and be tolerant to high levels of PCN invasion. A 3-year field trial was undertaken that evaluated clones that fulfilled these criteria. These clones included wild potato species from South America, advanced breeders lines and potato cultivars. Overall, the test clones significantly enhanced the annual natural decline rate of ca 19% under Northern Ireland conditions, with a range from 26 to 54%. Comparison of yield, when nematicides were applied, confirmed that several clones could tolerate extremely high levels of PCN (up to 1000 eggs g⁻¹ soil). These trials have confirmed that the basic principle of using such material is a sound way of enhancing PCN decline, and may be an attractive alternative for cleaning up land under the 'set aside' scheme, or for organic potato production.

530 Effect of applied phosphorus on population density of root lesion nematode (*Pratylenchus neglectus*)

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Trials at two sites on the Eyre Peninsula of South Australia were sampled in 1999 and in 2000 to determine effect of phosphorus application on density of *Pratylenchus neglectus* in trial plots of the susceptible wheat cv. Frame.

Initial nematode density (P_i) immediately after sowing in 1999 was 5-11 *P. neglectus*/g dry soil at Miltaburra and 1-3/g at Yandra. In 2000, P_i at Miltaburra was 8-25/g and at Yandra 1-3/g. Final nematode numbers (P_f) were determined during grain development in spring (September-October). In both years at both sites, P_f decreased as rate of applied phosphorus increased. In 1999, phosphorus rates of 20-30 kg P/ha reduced *P. neglectus* density by 30% compared to plots with no added phosphorus. Similarly, in 2000, nematode levels at Miltaburra were 37% lower and at Yandra 32% lower with 20 kg P/ha compared to control plots. The cropping soils of the Eyre Peninsula are inherently nutrient deficient, and the rainfall low (average annual rainfall 275 mm at Miltaburra and 400 mm at Yandra, with 200 mm and 320 mm, respectively, falling during the growing season). *Pratylenchus neglectus* in this region is a significant impediment to cereal production, and nutrition can play a role in the management of these nematodes.

531 Nematodes on strawberries in Southern Spain: effects on yield and control of *Meloidogyne hapla*

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The province of Huelva, with more than 7500 Has, is the strawberry largest producer area of Spain and one of the largest of Europe. The Laboratorio de Sanidad Vegetal of Seville has been receiving disease strawberry plants from this region during the last fifteen years, and the parasitic nematode species detected were *Meloidogyne incognita*, *M. arenaria*, *M. hapla*, *Pratylenchus penetrans*, *Ditylenchus dipsaci* and *Aphelenchoides fragariae*. Until now a systematic survey on incidence and severity of nematode problem was not made in this area, but during a four years (1998-2001) assays made on two localities (Cartaya and Moguer) on chemicals and non chemicals alternatives to Methyl Bromide, *M. hapla* was the most important phytopathological problem. Its presence was explained by the use of plantlets from high elevation nurseries located on Castile-Leon (Central Spain). The incidence and severity of root knotted plants and the

number of nematodes present were negatively correlated with some agronomic traits and effect of *M. hapla* on crop production and fruit quality was studied. Although Solarization, helped with some chemicals or associated to Biofumigation, exerted some nematode population control, only Chloropicrin alone or with DD proved similar efficiency to Methyl Bromide.

532 Effect of inorganic phosphate fertilisers on the efficacy of an arbuscular mycorrhiza fungus against a root-knot nematode on pyrethrum

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Effects of triple super phosphate (TSP) and single super phosphate (SSP) fertilisers on efficacy of a *Glomus* sp. (isolate KS 14) against *Meloidogyne hapla* were determined. The fertilisers were applied at the rate of 1 and 2 µg/g soil at the time of fungus inoculation. Two months later, plants were inoculated with the nematodes. Plant growth and nematode disease parameters were determined 2 months after nematode inoculation. The fertilisers at both levels improved plant growth in all treatments. The fungus, in general, improved plant growth on its own or in the presence of nematodes but not in the presence of fertilisers. Both fertilisers at both levels were more effective in improving plant growth than the fungus. The fungus suppressed nematode disease severity unlike the fertilisers. The suppressive effects of the fungus on the nematodes were, in most cases, reduced by the fertilisers. In addition, the fertilisers significantly reduced root colonisation of pyrethrum by the fungus. The nematodes, on the other hand, did not have any significant effects on root colonisation by the fungus or on its ability to improve pyrethrum growth. The presence of nematodes in fertiliser or fertiliser-fungus-treated plants, however, significantly reduced pyrethrum growth.

533 To assess the long-term effects of selected cropping sequences to control *Meloidogyne incognita* and increase the yield of cotton

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A 3-year field study was initiated in central Alabama in a cotton field infested with *Meloidogyne incognita* to assess the long-term effects of selected cropping sequences on nematode populations and yields of cotton. In the first year, six blocks were set up, each containing 24 rows on 4" centers. The six blocks were planted with cotton (CT), soybean (SB), pearl millet (PM), corn + velvetbean (CR+VB) and pearl millet + velvetbean (PM+VB). In the 2nd year the first two blocks were maintained with cotton and soybean monoculture. The remaining four blocks were split with 12 rows of cotton and 12 rows of soybean. In the 3rd year, each block was planted with three cotton cultivars (SG125, SG747, DP458) both non-treated and treated with 2.0 lb a.i./A of aldicarb in two row plots. This setup allowed for the following cropping sequences: (CT-CT-CT), (SB-SB-CT), (PM-SB-CT), (PM-CT-CT), (CR+VB-SB-CT), (CR+VB-CT-CT), (SG+VB-SB-CT), (SG+VB-CT-CT), (PM+VB-SB-CT), and (PM+VB-CT-CT). Treatment with aldicarb across all cropping sequences resulted in a 34% reduction in *M. incognita* and a 64% increase in yield compared to non-treated cotton monoculture. Most non-treated rotation sequences did not show a reduction in *M. incognita* compared to non-treated cotton monoculture except for (SB-SB-CT) and (SG+VB-SB-CT) which showed 30 and 41% reductions, respectively. All non-treated rotation sequences averaged a 20% increase in yield compared to non-treated cotton monoculture. The best performing treated rotation sequences were (PM-SB-CT), (PM-CT-CT), (PM+VB-SB-CT) and (PM+VB-CT-CT) resulting in 26, 21, 26 and 21% increases in yield, respectively, over and above what was obtained with treated cotton monoculture.

534 Induced resistance of plants to root-knot nematode

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In current investigations the mechanisms of induced plant resistance to parasitic nematode were studied. The activation of protective mechanisms in plants, in-

hibiting the growth of parasitic nematodes, was studied in the system tomato – *Meloidogyne incognita*. The biogenic elicitors (arachidonic acid and water-soluble chitosan) stimulated the development of invaded tomato plants. Arachidonic acid and chitosan induced the accumulation of phytoalexins in the tissues of host plants; decreased the total content of free sterols and changed their composition, producing adverse effects on infesters; activated the chitinase, -glucanase, lipoxigenase, peroxidase, phenylalanine ammonia lyase;

and stimulated the generation of reactive oxygen species. The mixture of elicitors with signal molecules (salicylic acid and methyl ether of jasmonic acid) caused more significant immunostimulation effect in tomato. The treatment of tomato seeds by elicitors and mixture of elicitors and signal molecules significantly suppressed the number of galls and eggs produced and increased duration of nematode development. The data obtained suggest that the natural mechanisms and those induced by biogenic elicitors in tomato to the nematode have the same origin.

535 Nematodes parasites of some freshwater fishes in Zimbabwe

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The objective of this study was to survey and document the various nematode parasites that infect some freshwater fish species of economic importance in Zimbabwe. Various species of fresh water fish Tilapia (*Oreochromis macrochir*, *O. mossambicus*, *O. niloticus*), Trout (*Salvelinus fontinalis*), Green Happy (*Sargochromis codringtonii*) and African catfish (*Clarius gariapinus*) were among some fish specimens collected from the lakes (Chivero, Manyame and Kariba). Fish collections were made during the 12 months of January to December 2001. Freshly collected fish specimens were taken to the laboratory and all gastrointestinal tracts, liver, vascular system and fish gill contents were removed and examined for the presence of nematode parasites. Excised nematode specimens were first preserved and sent to South Africa marine laboratories for expert taxonomic examination and identification. Preliminary results indicate that the most common nematode parasites associated with the gastrointestinal tracts and liver from almost all fish species collected are *Capillaria* spp., *Camallanus* spp., *Contracaecum* spp. and *Ascaris* spp. The Acanthocephalus group of parasites were also encountered and identified. It was also noted that in some fish species, nematode parasite counts correlated with the level of pollution in the respective water bodies.

536 Comparative study of *Haemonchus* species of different ruminants of Iran

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Haemonchus species is reported from sheep, goats, cattle, camel and wild sheep, as well as man in Iran. Identification of *Haemonchus* species in domestic ruminants might be very useful in the study of natural population, especially when two (or three) species are sympatric. In the recent investigation, ten males and ten females of *Haemonchus* spp. collected from sheep, goat, cattle and camel were examined for total worm length,

character of vulval flap in female, length of spicules, and distance from their tip to the tip of the barbs in males. Data were subjected to statistical analysis. Our findings revealed the presence of three species: *Haemonchus longistipes* in camel, *H. contortus* in sheep and *H. placei* in goats and cattle. Statistical findings based on one way ANOVA and Tukey *post hoc* showed that the length of spicules of worms collected from sheep is significantly different with those of goats and cattle ($P < 0.05$). Meanwhile, as far as the left and right hook distance from the tip of the spicule is concerned, there was also a significant difference between *H. placei* of goats and cattle with that of sheep ($P < 0.05$). The discriminant function analysis demonstrated further that the three groups of worm could be considered as three different strains.

537 Nematodes of wild cavicorns in the fauna of Uzbekistan

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Helminths of the class Nematoda are widespread in the biogeocenoses of Uzbekistan, parasitising all vertebrate species inhabiting aquatic and terrestrial ecosystems. Wild cavicorns in the fauna of Uzbekistan comprise six species, namely, *Gazella subgutturosa* Guldenstaedt, 1780, *Saiga tatarica* Linnaeus 1766, *Capra falconeri* Wagner, 1839, *C. ibex* Linnaeus, 1758, *Ovis ammon karelini* Severtzov, 1873 and *O. orientalis* Gmelin, 1774. The above species inhabit deserts, semi-deserts and are important components of the corresponding biogeocenoses. As a result of a study on parasitic worms of wild cavicorns, 54 nematodes were recorded, of which six are first records in Uzbekistan. Diversity of helminth fauna in wild cavicorns is as follows: *O. ammon*, 43; *O. orientalis*, 41; *G. subgutturoza*, 35; *C. sibirica*, 33; *C. falconeri*, 29; and *S. tatarica*, 22. Under natural conditions, cavicorn species show, between themselves and domestic animals, close biocenotic links. This promotes circulation of helminths in wild animals. Wild animals play a certain role in epidemiology of a number of helminthoses and take part in distribution of diseases among domestic animals and humans.

538 Population ecology of the family Protostrongylidae, Leiper 1926, parasites of animals

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Nematodes of the family Protostrongylids are adapted to the respiratory system of animals inhabiting mountain ecosystems. They are widely represented in ruminants of the Palaearctic. Fifteen species of Protostrongylids inhabit mountain ecosystems of Uzbekistan, which are the habitats of cavicorns. Dominant species of protostrongylids of cavicorns are representatives of the genera *Protostrongylus*, *Cyctocaulus* and *Muellerius*. The ecological niche for the recorded species of nematodes is the respiratory system, which witnesses high adaptive potential of the helminths. Terrestrial molluscs of genera *Xeropicta*, *Pseudonapaeus*, *Bradybaena*, *Macrochlamys*, *Pupilla*, *Deroceras* etc., participate in life cycles of protostrongylids, in which the 2-3 stage larvae populations develop. Helminths parasitise animals within the system parasite-host. Availability of certain helminth complexes is mainly predetermined by the availability of corresponding host groups and microclimatic conditions in the given biogeocenosis which provide the development of all the phases of the parasitic worm ontogeny.

539 Population dynamics of sheep digestive tract strongyloses in Estonian climate conditions

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The objective of this study was to investigate population dynamics and viability of strongyle larvae on pastures in natural conditions. According to our data, strongyloses of sheep digestive tract are the most widespread parasitic diseases in Estonia. Our observations have shown that pastures contaminated with parasites the previous year are the main sources of invasion. In order to estimate the viability of strongyle larvae on sheep pastures, five trials with lambs were carried out in different pasture regimes. All lambs were submitted to faecal examinations for numbers of parasite eggs once a week. Our observations have shown that although most strongyle larvae did not survive during the winter period, some of them remained

viable up to next grazing period. According to our results, strongyle larvae can be transferred to deep soil layers by earthworms and can remain viable during the whole winter period. To avoid sheep infections, the main prophylactic principle is separate grazing of lambs from ewes because they are the main infection sources in spring.

540 Histology of the anterior intestine of *Chabertia ovina*

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Histological studies of *Chabertia ovina* widespread in sheep have never been conducted in Armenia. *Chabertia ovina* were obtained from large intestines of freshly slaughtered sheep and fixed in Barbagallo solution before histological preparation. After azan staining the cuticle of the buccal capsule (BC) and pharyngeal funnel (PF) turn bright red while other tissues in the anterior end turn blue. The pharyngeal gland duct is clearly visible on transverse sections. Haematoxylin-eosin stains all tissues into various shades of red: the lightest are BC and PF cuticles. Clearly outlined are the structure and tissue borders of the pharyngeal musculature and dorsal pharyngeal gland. After ferrous hematoxylin staining the cuticle of the BC and PF turn black, while other tissues in the apical part take various shades of brown. Longitudinal sections clearly show the lamellar structure of the BC cuticle, which is not mentioned in the drawings or textual description of *Chabertia ovina*. Thus methods used provided new histological results for Strongylidae and complemented each other.

541 Changes in prepatent periods and species composition of *Oesophagostomum* spp. isolates over time

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Four field isolates of *Oesophagostomum* spp. were collected from Estonian pig farms in 1997 and used for inoculation of eight piglets (2000 L₃ larvae per pig). Four

new pigs were inoculated with the second generation larvae of one isolate kept in the fridge for 3 years. One extra pig was inoculated with 5000 L₃ larvae from the primary field isolate to follow the egg excretion of the two species by PCR analysis. All four Estonian field isolates contained *Oesophagostomum dentatum* and *O. quadrispinulatum*. Prepatent periods of both nodular worm species varied from 18 to 43 days. Fairly constant egg excretion of both species was demonstrated from day 19 to day 82 post inoculation by PCR analysis. Second generation worms showed shortened prepatent periods and shift towards predomination of *O. dentatum* (80-85% vs 38% in the first experiment). It is unclear whether this phenomenon is the consequence of the long storage of larvae in the fridge or some kind of host response.

542 The behaviour of the infective third-stage larvae of the animal-parasitic nematode, *Strongyloides ratti*

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We are analysing the behaviour of *Strongyloides ratti* infective larvae (L₃), a parasite of a rodent, for studying the mechanism of the amphid functions using *in vitro* agarose tracking assay method. In the present study, we show the behaviours of *S. ratti* L₃, both on a sodium chloride (NaCl) gradient and on a temperature gradient. On a NaCl gradient, two types of chemokinetic behavior were seen: a unidirectional avoidance movement in unfavourable conditions (*ca* 80 mM NaCl) and a random dispersal movement in favourable conditions. Track patterns were straight in the avoidance movement but included multiple changes of direction and loops in the dispersal movement. On a temperature gradient, the

direction of thermokinetic behaviour depended on prior experience of environmental temperature. L₃ cultured at 25°C tended to move towards higher temperatures from releasing points between 22°C and 29°C. At releasing points of 30°C or above, most L₃ moved little and showed no directional response. At 20°C or below, few or no L₃ moved towards the zone of higher temperature. L₃ cultured at 20°C tended to migrate to a high temperature area regardless of temperature, and those cultured at 30°C did not respond to the temperature gradient.

543 Mono- and mixed infection of rats with larvae of *Trichinella spiralis* Owen, 1835 and *T. pseudospiralis* Garkavi, 1972

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The experiment was undertaken to test male rats of Vistar line. Animals were split up into three groups of five animals each. Groups I and II were treated as control and infected with larvae of *Trichinella spiralis* and *T. pseudospiralis* in a dose of 20 larvae/g body weight of the rat. Group III was treated as trial group and infected with a combination of two *Trichinella* species in a dose of ten larvae from each species per g body weight. The autopsy was done on day 35 post infection. It was estimated that the formation of larvae in control animals was complete. *Trichinella spiralis* larvae were encapsulated and those of *T. pseudospiralis* had their ends twisted or curved. The stage of infection in control animals was 15-20 larvae/g body weight and eight larvae/g body weight in trial animals. These findings suggest that mixed infection of rats holds back the development of *Trichinella* and lowers their survival rates. The reason is obviously an interspecific competition of *Trichinella* species.