CONTENTS

Plenary Session

Nematode anhydrobiosis and cryobiosis: insights from molecular studies

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Nematodes require a moist body surface for normal activity. Yet some nematodes survive severe desiccation (anhydrobiosis) or freezing (cryobiosis) conditions. Anhydrobiotic nematodes can survive conditions where there is no continuous aqueous phase in the cytoplasm and the hydration shell of biomolecules is lost. Life processes come to a halt during anhydrobiosis, but they resume again on rehydration.

When in an anhydrobiotic state nematodes are resistant to freezing, as they lack the water necessary to form intracellular ice crystals. A link between desiccation tolerance and cold tolerance is also evident in those nematodes that undergo cryoprotective dehydration. These nematodes dehydrate when the soil freezes because of their permeable cuticles and the vapour pressure differences between their supercooled body fluids and soil ice.

Unlike desiccation sensitive taxa, anhydrobiotes evolved mechanisms to maintain the structure and integrity of macromolecules and membranes when desiccated and during rehydration. Are these mechanisms the result of an enhanced expression of the generalised stress responses common to all organisms (e.g. molecular chaperones, antioxidants, compatible solutes) *or* do anhydrobiotes possess unique or novel molecules, or specialist adaptations?

Taxa from the main groups of animal anhydrobiotes – rotifers, tardigrades, brine shrimps and nematodes – are being investigated at the molecular level. Such comparative transcriptome and proteome data will aid in identifying core anhydrobiotic processes. I will present an overview of these studies, with particular reference to nematodes.

Global food security and the role of nematology

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Across the globe crop productivity is continuing to increase, including across the developing world, but not in sub-Saharan Africa. Similarly, while output per capita is increasing across the developing world, it is decreasing in Africa. When considering crop productivity, it is also important to understand that in addition to yield it also includes a measure of other parameters, such as food quality. Increasing food productivity therefore, is not useful if it is unsuitable for consumption or cannot be accessed. Most of the ten fastest growing cities in the world are located within developing countries, three of them in Africa. Rapidly expanding urban centres require equally increased volumes of food, including perishable vegetable products, cultivated locally under intensive conditions. In order to attain global food security significant attention needs to be paid to developing countries and especially sub-Saharan Africa. To improve outputs of high quality crop products, in Africa particularly, there is a need to access increasing areas of more marginal land and to intensify production systems. The sustainable intensification of such cropping systems will need to undergo a significant shift in production style. Access and availability to quality inputs will be necessary, as will improved pest and disease management. Nematode pests are commonly overlooked and/or misdiagnosed across the globe. Nowhere is this more marked than in developing countries under resource limited conditions. A consequent neglect of nematode management thus results. Not only are innovative IPM options for nematode management required but it is equally necessary to develop greater capacity in nematology. Attracting interest in nematology as well as other plant health aspects should be a key pillar within the overall strategy to increasing agricultural productivity, where nematode parasites are significant contributors to current poor levels of productivity. In developing countries most farmers are small scale, but will be increasingly reliant on more suitable management options, chemical or otherwise, to overcome production losses. This will require substantial investment in training and capacity building, as well as developing stronger links with the agro-input industry for synergy between the public and the private sectors.

Interactions between the entomopathogenic nematode/bacterium complex and non-host organisms

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Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae occur naturally in soil and infect a wide range of soil insects. The genera *Steinernema* and *Heterorhabditis* are symbiotically associated with bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively. To infect and reproduce successfully, the nematode–bacterium complex must overcome a number of constraints imposed by an insect host and the environment. The nematode and bacterium have a mutualistic relationship to aid each other to successfully invade and reproduce in the insect host. Thus, nematode growth and reproduction depend upon conditions established in the host cadaver by the bacterium. The symbiotic bacterium produces chemical compounds within the insect host to assist the nematode in overcoming host defences and suppresses colonisation of the cadaver by competing secondary microbial invaders. Although the soil provides a suitable habitat, a number of potential interactions can occur between the EPN—bacterium complex and non-host organisms. These potentially non-host interactions include such organisms as plant-parasitic nematodes, predacious nematodes, other EPN—bacterium complex species, entomopathogenic fungi, insect parasitoids, and scavengers (i.e., ants, crickets, wasps, cockroaches, calliphorid flies, springtails and mites). Some of these interactions have been studied in detail and will be discussed in terms of their positive and negative impacts on the EPN—bacterium complex.

S1 – Molecular basis of the compatible interaction: nematode effectors

Convenors: Sophie Mantelin & Geert Smant

S01–T1

A root-knot nematode effector injected into giant-cells and targeted to the nuclei is able to suppress plant defences

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Root-knot nematodes (RKN) are obligate endoparasites able to infect almost all cultivated plants worldwide. The banning of most chemical nematicides on environmental grounds means that alternative control methods are required. Blocking the activity of parasitism gene products involved in the success of infection would offer specific alternatives to reduce nematode populations in the field. In this regard, nematode effectors are particularly attractive targets. RKN maintain, for weeks, a biotrophic relationship with their hosts and induce the differentiation of root cells into specialised feeding cells. Nematode effectors produced in the oesophageal glands and injected into host cells rthrough the stylet play a role during infection by altering plant physiology and promoting nematode establishment. In a search for new nematode effectors, we used comparative genomics on EST datasets to identify *Meloidogyne incognita* genes encoding proteins potentially secreted into the host tissue during the early steps of infection. We identified 28 candidate parasitism genes that were specifically expressed in early parasitic stages and encoded predicted secreted proteins. We localised the expression of candidate parasitism genes in the pharynx, the intestine or specifically in the esophageal glands of parasitic juveniles. Among these new effectors identified, Mi-EFF1 was a pioneer gene with no similarity in databases. We demonstrated the secretion of Mi-EFF1 by the nematode *in planta* and localised the secreted Mi-EFF1 in the cytoplasm and in the nuclei of giant-cells. Mi-EFF1 was able to suppress the PTI defence response in *Arabidopsis thaliana*. Our results add to previous knowledge on nematode proteins secreted in the apoplasm of infected tissues and show that effectors are injected within plant cells during infection and are able to modulate plant defences.

S01–T2

Functional analysis of the feeding tube of cyst nematodes

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There are several different groups of plant-parasitic nematodes, the most economically damaging of which are the sedentary endoparasites. These nematodes are obligate biotrophs and modify host root tissue by using a suite of effector proteins to create a feeding site that is their sole source of nutrition. These nematodes feed by withdrawing assimilates from the feeding site though a structure known as the feeding tube. Although everything that the nematode ingests during its life must pass through the walls of the feeding tube, the size exclusion limit of this structure remains uncertain. Several previous studies have used molecular weight as an indicator of protein size, but have produced conflicting results. In order to resolve these issues we have developed and tested a new program designed to predict protein size based on cross sectional area calculated from protein database coordinates. We have confirmed its accuracy by testing predictions using Traveling Wave Ion Mobility spectroscopy–Mass Spectrometry (TWIMS-MS). This program has been used to help clarify the feeding-tube size exclusion and provides a basis for future experimental observations. Current work is aimed at identifying candidate genes that could encode components of the feeding tube from the genome sequence of *Globodera pallida.*

S01–T3

Functional analysis of *Globodera pallida* SPRYSEC proteins

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The potato cyst nematode, *Globodera pallida*, is an extremely destructive pathogen with a wide distribution in potato growing regions. A lack of major resistance genes and the removal of many active ingredients used to control this nematode mean that there is a need to better understand the molecular basis of the interaction between host and nematode in order to develop sustainable control methods for this pathogen.

The interaction between the plant and cyst nematode is mediated by effectors that are synthesised in the nematode oesophageal glands and injected into the host cytoplasm through the stylet. As a result of an Expressed Sequence Tag (EST) project on *G. pallida* and as part of the ongoing genome sequencing project for this nematode, a large number of effectors have been identified. Of particular note is the SPRYSEC gene family. Analysis of the current *G. pallida* genome assembly suggests that over 300 different SPRYSEC proteins are present. Previous work has shown that all SPRYSECs examined to date are expressed in the dorsal esophageal gland cell and that different members of this gene family localise to different compartments of the plant cell after being introduced into the host. In this study, the role of SPRYSEC gene family members in suppression of plant defences was investigated. A panel of interesting potential host target proteins have been found after yeast two hybrid screening against an infected potato cDNA library. Several of the interactions have been confirmed in yeast cells and the confirmation of the interaction *in planta* is now underway.

S01–T4

Members of the Gr1106 effector family of the potato cyst nematode *Globodera rostochiensis* modulate plant immunity

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Potato cyst nematode *Globodera rostochiensis* oesophageal glands produce effectors that facilitate plant parasitism. These effectors are delivered into host cells through the stylet leading to the formation of a complex multicellular feeding site. Some potato genotypes specifically recognise the effectors of an avirulent *G. rostochiensis* pathotype Ro1. Disease signalling triggered upon effector recognition results in isolation of the feeding site at an early stage by a ring of necrotic cells. Ro5, a virulent *G. rostochiensis* pathotype, can overcome this response and develop normally on resistant plants. Two lines of *G. rostochiensis* differing in their (a)virulence on *H1*-carrying resistant potato were selected from single female crossings. Proteome and transcriptome analysis of both lines indicated presence of fewer than 2% polymorphisms. We assessed the differences within the secretome of the virulent and avirulent nematode lines early in the parasitic development. We constructed a cDNA library from the avirulent line of *G. rostochiensis* isolated fromsusceptible potato roots 11 days post inoculation. Single run sequencing of 4000 clones generated a set of ESTs, which were clustered and translated into peptides *in silico*. In total 74 ESTs were predicted to begin with a signal peptide for secretion. The clones from which these ESTs originated were fully sequenced. Primers designed on the predicted open reading frames were used for PCR amplifications on genomic and cDNA of both the virulent and avirulent line. Polymorphic amplicons were explored by studying gene variation on the population level, performing nematode *in situ* hybridisation*, in planta* localisation and subcloning of underlying genes into plant expression vectors for functional studies. One of the polymorphic amplicons (*Gr1106*) showed specific expression in the dorsal oesophageal gland as well as significant sequence variation at the population level. We then tested the sequence variants using Phylogenetic Analysis by Maximum Likelihood (PAML) in order to find evidence for positive selection acting on this gene family, which. PAML performed on the set of 26 gene variants cloned from the Ro1 population showed strong positive selection acting on several sites of Gr1106. To assess the role of these effectors in nematode parasitism we overexpressed them in susceptible potato and knocked them down by RNAi in pre-parasitic second stage juveniles. Both assays proved the positive influence of Gr1106 effector expression on nematode parasitism. Moreover Gr1106-overexpressing plants showed more susceptibility to a completely unrelated fungal pathogen. We explore the role of this novel nematode effector family in modulation of plant immunity, and hypothesise that the nematodes likely use Gr1106 effectors to protect their feeding sites.

S01–T5

Deep sequencing of the *Heterodera schachtii* transcriptome and secretome

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The plant-parasitic sugar beet cyst nematode *Heterodera schachtii* is an agronomically important sedentary nematode. Successful infection processes such as invasion, migration, and induction of feeding structures (syncytia) are dependent on secretory proteins that act as effectors modulating the host plant to match the nematode´s needs. Next generation sequencing (RNAseq) is a straight-forward approach to sequence the nematode transcriptome as a basis to analyse the secretome. Our transciptome sequencing of pre-infective juveniles of *H. schachtii* using Illumina technology has produced millions of reads that were assembled into nearly 67,000 contigs. The contigs were annotated and categorised based on their relationship to all available sequences of free-living, animal-parasitic and plant-parasitic nematode species. A high level of coverage in our dataset was determined with available cyst nematode sequences. All ORFs were detected and used for translation. To determine the secretome, predicted proteins containing signal peptides and lacking transmembrane domain were selected. Nearly 40% of the identified secretome was defined as plant-parasitic nematode specific sequences according to the nembase4 database. This study provides new insights into the structure and function of the transcriptome of cyst nematodes. The isolation of the secretome will help to identify important effector proteins acting in the interaction between *H. schachtii* and its hosts such as Arabidopsis and sugar beet.

S01–P1

Functional characterisation of effectors from the potato cyst nematode *Globodera pallida*

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Globodera pallida is an economically important cyst nematode that is an increasing problem for the UK potato industry. Cyst nematodes are plant parasites that are capable of inducing extensive changes in the function and structure of their host's cells which will eventually be used as a nutrient sink for the growing nematode. The host defence system is suppressed or avoided during these changes. The effectors secreted from the nematode pharyngeal glands into an initial plant cell are likely to be responsible for this array of events. A large number of these effectors are pioneers, so their function is unknown, making the parasitism mechanism hard to decipher. We have carried out a functional characterisation of several putative effectors that were identified from the *G. pallida* genome sequence to gain a better understanding of the mechanism that defines the plant-nematode interaction. The *G. pallida* effectors studied were localised in the pharyngeal glands by *in situ* hybridisation, to confirm their involvement in the parasitism process. RNAi was employed to evaluate the impact of the effectors on the parasitic success of *G. pallida* second stage juveniles. Potential interaction partners in potato roots of the *G. pallida* effectors have been identified by using a yeast two hybrid system. The results obtained will allow a better insight into the plant—nematode interaction mechanism that could lead to the development of new control strategies.

S01–P2

Silencing of *msp18*, a dorsal oesophageal gland gene of *Meloidogyne incognita*, through host delivered dsRNA in eggplant (*Solanum melongena*)

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Oesophageal gland secretions delivered through the stylet of *Meloidogyne incognita* play a major role in plant parasitism. Even though many genes specifically expressed in the oesophageal glands have been identified, the function and role of many novel genes in parasitism needs to be established. Gene silencing by RNA interference is a powerful tool for functional genomics of genes both under *in vitro* and *in vivo* conditions. Here we report the effect of silencing by RNAi of the *msp18* gene, which encodes a novel secretory protein specifically expressed in the dorsal oesophageal glands of parasitic second-stage juveniles and the late parasitic third-stage juveniles. *In vitro* RNAi by soaking the second-stage juveniles in dsRNA solution significantly interfered with their attraction towards host roots. *In vivo* RNAi through host delivery of dsRNA of *msp18* in eggplant was found to be very effective in reducing infection including development and reproduction. An *in vivo* assay revealed that number of females produced per plant was reduced by approximately 73 per cent in transgenics over the wild type control. Likewise, the total number of egg masses and eggs per egg mass was reduced by 80 and 40%, respectively. The gene integration in these transgenics was confirmed by PCR and Southern hybridisation. Expression hybridisation and expression of the transgene was verified by qRT-PCR. The present findings demonstrate that all stages of the parasite cycle are affected due to silencing of *msp18* resulting in overall reduction of population build up at the end of the disease cycle. Disease management under infected field conditions is effective only when nematode multiplication (PF/PI) is drastically affected resulting in effective disease management. Thus, the transgenic eggplants generated are promising tools for management of root knot nematode under field conditions.

S01–P3

Characterisation of compatibility effectors secreted by *Meloidogyne incognita* in Rice (*Oryza sativa*)

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The root knot nematode*, Meloidogyne incognita,* is responsible for important rice production losses in many countries and especially in Africa. It has been shown that secretions from the nematode are crucial in invasion and establishment in the host. Identification of nematode effectors and their plant targets may provide new insights for developing control strategies towards *Meloidogyne* spp. The objective of this study is to investigate the involvement and the role of these proteins in compatible rice—nematode interactions. Candidate effectors were selected from an exhaustive mass spectrometry analysis of *M. incognita* secretions and isolated from a cDNA library made from oesophageal gland cells of the nematode. The time-course of *M. incognita* development in rice roots was assessed and expression of candidate effector genes was monitored by real-time quantitative PCR. In parallel, *in situ* hybridisation of candidate gene probes was used to verify the specificity of gene expression in the juvenile nematode gland cells. We used onion epidermal cells as a transient gene expression system for imaging Effector—GFP fusion proteins localised to different cellular compartments. Furthermore, immunolocalisation of secreted proteins will be achieved by generating polyclonal antibodies. By studying over-expression and host-mediated RNA interference (hmRNAi) of the nematode genes in rice, a functional analysis is being performed on candidate *M. incognita* effectors.

S2 – Free-living terrestrial nematodes

Convenor: Howard Ferris

S02–T1

Ecosystem services of free-living soil nematodes

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The activity and abundance of soil organisms, and the effects of environmental conditions and resource availability, determine the magnitude of their ecosystem services. Diversity of nematodes within functional guilds enhances the continuity of their services. Indices based on relative proportions of taxa are bioindicators of the framework of ecosystem structure and of its service potential. Metrics of the magnitude of services may be provided by estimates of carbon utilisation by different functional guilds of nematodes. Below ground biodiversity and food web connectance can be enhanced by resource subsidy to increase organism abundance, and by the mitigation of environmental constraints to organism survival and function. Management to ameliorate disservices of plant-feeding nematodes and other herbivore species often results in unintended, but long-lasting, collateral disruption of organisms at higher trophic levels. Effective biological regulation requires co-location of predator and prey organisms, or overlaps in their ranges. A challenge is to understand the dynamics of aggregations of nematodes and other organisms and to assess the functional connectance within and among aggregations. Advances will be enhanced by continued application of molecular tools in ecological questions and experimental validation of the evolving concepts of soil ecosystem interactions and dynamics.

S02-T2

The fascinating biology of *Halicephalobus gingivalis*, a free-living bacterivore with a deadly streak

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The genus *Halicephalobus* (Panagrolaimidae) is known for its wide environmental range, i.e. as inhabitants of compost, humus, soil, rotten wood, water enclosures in mines up to 1 km deep belowground, and in association with insects and crabs. Of special interest is *H. gingivalis*, a free-living bacterivore which is capable of facultative parasitism in horses, donkey, zebra and humans and which often causes fatal infections. The species appears to have ultra-structural adaptations facilitating its facultative parasitic lifestyle and an *in vitro* study on the anthelmintic tolerance of several isolates reveals a trend of increasing tolerance from fully free-living isolates towards horse-associated isolates. In order to understand the remarkable morphological variation observed within this one species, the progeny of a single female was cultured under varying conditions revealing that *H. gingivalis* exhibits an unprecedented degree of intra-specific variation to the extent that surpasses described inter-specific variation. Furthermore, molecular characterisation and analyses on different markers indicate the presence of cryptic species within the morphospecies *H. gingivalis*. These molecular data are complemented with biometrical and morphological data of all available isolates but also with other species of *Halicephalobus* that have a completely different biology. All available data (morphological, biological and molecular) are combined toshow the phylogenetic relationship between different isolates to clarify whether *H. gingivalis* is merely an opportunistic invader or that it is evolving towards parasitism in mammalian hosts.

S02–T3

Life history traits of a free-living nematode *Panagrolaimus* sp.

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Life cycle analysis data of a free living bacterial feeding *Panagrolaimus* sp. were assessed at different temperatures using the hanging drop method with single male and female individuals and a food density of 3 x 10^9 *Escherichia coli* cells/ml. Lifespan was 4.7 days at 21°C and 3 days at 25, 27 and 29°C. The intrinsic rate of natural increase (r_m) was 0.6 at 21°C, 0.99 at 25°C, 1.15 at 27°C and 1.0 at 29°C, corresponding to population doubling times (PDT= $\ln(2)/r_m$) of 1.2, 0.7, 0.6 and 0.7 days, respectively. Over 200 offspring per female were produced at 27°C. All other temperatures yielded fewer offspring. When females were kept without males the life span was 49 days, whereas the first reproductive female (hanging drop with male individual) died after only 16.5 days.

S02–T4

Nematodes in a rapidly changing environment: colonisation, microbial links and functional succession in compost

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Composting is the semi-artificial, heat producing process in which aerobic disintegration of organic materials is executed by several organisms in a complex and rapidly changing community. In spite of the recent growing interest in the biology of compost, the compost fauna remains largely unknown. In this study nematodes were used as a gateway to examine the composting ecosystem, making use of their excellent qualities as environmental bio-indicators.

Quantitative and taxonomical analysis of the composts showed an abundance of nematodes during the entire process (except for the compost heat peaks where nematodes were less abundant). The importance of resistant stages and insect phoresy for colonisation of the compost heap after the heat peak were experimentally explored and different possibilities of how nematodes can reach compost heaps are discussed. The taxonomic/functional structure of the nematode community clearly changes during the composting process and is linked to several abiotic variables (temperature, pH, C/N ratio and moisture). This nematode succession showed a highly informative pattern which was complemented with non-nematode biotic data, i.e. fungal and bacterial biomass and their ratio, obtained by performing PLFA (Phospholipid Fatty Acid) analyses. The relation of the nematode community with their microbial food resource is far from linear and will be further discussed. Finally, two nematode based indices (f/b ratio and Maturity Index (MI)) showed significant changes during the process and are thus evaluated as potential indicators of compost maturity.

S02–T5

First insights into the meiobenthic nematodes of the oxic–suboxic–anoxic transition zones in the Turkish Black Sea (Sinop Peninsula)

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The Phylum Nematoda is the most abundant taxon among meiobenthic organisms and has the potential to inhabit a wide range of different environments on earth. They tend to survive better under low-oxygenated, even anoxic conditions compared to other meiobenthic taxa. Extreme habitats are the focus of several recent surveys in the world oceans and also in the Black Sea. Oxygen-deprived zones are one of these areas gaining more importance due to climate changes. The Black Sea is well known with its large anoxic basin; however, a permanent suboxic zone where O_2 and H_2S are extremely low has also been described. This preliminary study represents the first findings on the contribution of free-living marine nematode densities to meiofauna of the sediments under anoxic, suboxic and oxic conditions. Faunal sampling was done aboard *E/V Nautilus* (Ocean Exploration Trust) with the video-guided ROV Hercules in August 2011 during the Black Sea Leg of the expedition at three locations representing anoxic (203m), suboxic (120.5m) and oxic (90m) zones off Sinop Peninsula. Dissolved oxygen levels were monitored *via* the optode equipped on the ROV to determine the sampling location from the suboxic zone. Sediment cores were collected by push corers (32 cm^2 surface area) with the help of the manipulator arms of ROV and fixed onboard using 75% ethanol. Samples were washed through a series of sieves and meiobenthic material retained on 63 μm mesh size was stained with Rose Bengal solution, examined and sorted to high taxa under a stereo dissecting pmicroscope. Meiobenthos was represented by 5 to 9 major taxa and total abundance was found to be highest at the oxic sample (217078 ind./m^2). Nematodes were counted using modified Bogorov chambers and all specimens were mounted. Slides were examined by DIC (Differential Interference Contrast) microscopy and identified to possible taxon level. Nematoda was numerically the most abundant meiofaunal taxon at every station making its highest contribution (90%) to the sample associated with the suboxic zone. As expected, the sample of oxic zone yielded enhanced nematode abundances (176026 ind./m^2) with a dominance of Comesomatidae (22..4%) and Linhomoeidae (15%). Suboxic zone revealed a lower nematode density (65621 ind./m^2) and characterised mostly by Trefusiidae (49%). The number of families was similar whereas the oxic zone harboured a richer nematode species diversity, as expected. Surprisingly only five nematodes were recruited from the anoxic sample although only three replicates were taken from this zone. Several taxa are new records for the Black Sea and detailed studies are in progress. Our results demonstrate that severe oxygen depletion does not strongly affect nematode abundance, probably due to their tolerance of suboxic conditions and reduction of predation. Moreover, such conditions support the abundance of several taxa. TThis preliminary study gives us the first insights into the nematode assemblages of the oxic/anoxic interface in the Turkish Black Sea coasts.

S02–P1

Nematode communities under monoculture of *Phleum pratense* on drained peat soils over a nine-year period

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In the Republic of Karelia seeded meadows formed on peat soils are used as hayfields. The object of study was a single-species 1, 3 and 9-year crop *Phleum pratense* L. on peat soils. In the year of sowing perennial grasses developed slowly, phytomass supply was low, and weed grasses built up to 50% of biomass present. *Phleum pratense* became the dominant crop 3 years after seeding causing changes in nematode communities: the total nematode abundance was the lowest under 1-year monoculture and the highest under 3-year *P. pretense*. The proportion of genus *Paratylenchus* was high under 1 and 3-year crops (32.9% and 48.2% of total fauna, respectively) and declined under 9-year-old crop (13.3%). Soil nematode communities in the soil under 1 and 3-year crops had similar values of structure index *SI* (40.0—42.9). *SI* index of nematode communities decreased at 9 years of growing (33.2) due to reduced diversity of fauna during prolonged *P. pratense* cultivation. Enrichment index *EI* had the highest values in the soil under 9-year monoculture (58 versus 26—37). While phytomass reached its maximum in the ninth year, the process of organic matter mineralisation by bacterial-feeding nematodes was intensified. The results indicate the close relationships between plants and soil nematodes inhabiting the rhizosphere.

Research was supported by Biology Department of RAS (№ 01201262102).

S02–P2

Population dynamics of *Panagrolaimus* sp. in monoxenic liquid media for use in marine aquaculture

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Free-living nematodes have the potential to be used as live food for early life stages of several species in aquaculture. *Panagrolaimus* sp. displays several favourable characteristics for this application. The aim of the present study was to demonstrate the feasibility of liquid culture production of this nematode and to provide basic information on yields. Monoxenic liquid culture was conducted on *S. cerevisiae* in a total of 10 flasks. The development of food density, nematode density and size distribution was assessed daily for 15 days. After 4 days the inoculated first stage juveniles started to grow to reproducing adults with occurrence of most F1 juveniles after day 8—9. Yields in terms of nematode density as well as biomass were highly variable. The maximum number of nematodes varied from 45,000—238,000/ml and maximum biomass from 49—143 g/l. The size spectrum of *Panagrolaimus* sp. individuals ranged of 176 × 8 µm to 1377 × 61 µm and 8.15 to 3202.39 ng wet weight. The water content of nematodes was 71.71 ± 2.54%, so dry weight/individual was 2.31 to 905.95 ng. A method previously described in literature to identify size ranges for different developmental stages was applied, but the results were not convincing.

S02–P3

Study of feeding behaviour of bacteriovorous nematodes and their propagation on WA 2%

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The order of bacteriovorous Rhabditida is found in most soils and some species can be used as model organisms for biological studies. In this study the feeding behaviour and nematode propagation were studied on various media. For feeding behaviour of nematodes, three bacterial strains, *Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli,* were used. WA 2% and bacteria were kept in the incubator in the same conditions and temperature for 24 h (T = 37°C). After 48 h, 10 individuals of each nematode species including *Acrobeloides* sp., *Panagrolaimus* cf. *trilabiatus* and *Acromoldavicus skrjabini* were inoculated onto the medium. The results of feeding behaviour demonstrated that *Acrobeloides* sp. prefers *E. coli, P. cf. trilabiatus* prefers *P. aeruginosa* and *A. skrjabini* prefers *E. coli* and *B. cereus*. Moreover, *P.* cf. *trilabiatus* was propagated on WA 2%. This experiment was performed in a completely randomised design with four replications. The data were analysed using SPSS 15. In a further study, three strains of bacteria, *P. aeruginosa, B. cereus* and *E. coli* and one fungus species, *Fusarium culmorum,* were used. After 24 h of bacterial inoculation on WA 2%, 10 nematodes were added to each medium. Total nematodes were counted after 10 and 20 days. After 10 days, the results showed that *F. culmorum* (n=148) and *B. cereus* (n=199) have the highest impact on nematode population ($P < 0.05$). After 20 days, the treatments showed no significant differences in comparison with control; however *F. culmorum* (n=1318) indicated the highest impact on nematode population. It might be assumed that Rhabditid nematodes can also reproduce in the absence of bacteria and fungi. As the Rhabditid nematodes can easily reproduce on medium, possibility of easier biological and molecular study in Nematology is obtained.

Workshop1 – EUPHRESCO *Meloidogyne* project closing meeting

Workshop 1-T1

EUPHRESCO *Meloidogyne* project: finished?

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The root-knot nematode species *Meloidogyne chitwoodi* and *M. fallax* are both quarantine organisms in Europe. Awareness of these nematode species is of utmost importance and was one of the main goals of the EUPHRESCO project. This umbrella project contained five elements: 1. ring testing of nematode extraction processes from soils, 2. ring testing molecular identification/detection methods, 3. a workshop on sampling, detection, identification and management of *Meloidogyne*, 4. gathering information on treatments of waste (e.g. waste products of potato/vegetable processing industry) contaminated by nematodes to prevent dissemination in the future and 5. designing a European *Meloidogyne* research agenda by gathering information from all joining countries, identifying gaps in the research and making proposals (calls) for filling in those gaps. In this meeting the results of all topics will be shown in short presentations and the consequences of the findings will be discussed. Evaluation of the project will take place and future plans for continuation within EUPHRESCO will be discussed.

S4 – Plant-parasitic nematodes in tropical crops with focus on *Meloidogyne* spp.

Convener: Danny Coyne

S04–T1

The effect of abamectin as a seed treatment for protection against plant-parasitic nematode damage on maize, cotton and soybean in Brazil

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The use of abamectin as a seed treatment nematicide has been shown to be an effective method for protecting crops from plant-parasitic nematode damage. Surveys of grower fields in Brazil show a widespread distribution of plant-parasitic nematode species including *Meloidogyne incognita* and *Pratylenchus brachyurus.* Field and greenhouse trials conducted in Brazil with abamectin on maize, cotton and soybean show a significant reduction in *Meloidogyne* spp. and *Pratylenchus* spp. and associated root damage and increases in grain or lint yield per hectare.

S04–T2

Identification and characterisation of *Meloidogyne* spp. from Brazil using morphological, biochemical and molecular approaches

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Root-knot nematode diseases, caused by *Meloidogyne* spp., are one of the most important diseases in Brazil in many economically important crops. The implementation of alternative measures of control, such as resistant varieties or crop rotations, are essential for maintaining or increasing agricultural production. For the adoption of these management measures, the correct identification of species and knowledge of intraspecific variability of populations are essential. There are about 90 species of *Meloidogyne* described worldwide and 15 of those species are recorded in Brazil. Some studies were conducted with foreign and Brazilian populations of *Meloidogyne* spp. and showed that different species can be split into clusters according to their phylogenetic relationship, enzymatic and SCAR profiles. The identification of species by esterase is highly correlated with the identification using molecular techniques. No separation of physiological races through molecular markers was observed. A high intraspecific variability in populations of *M. exigua* and *M. arenaria* and a low variability among populations of *M. incognita*, *M. enterolobii* and *M. ethiopica* were observed. For *M. exigua,* the populations formed a cohesive group and for *M. arenaria*, probably clusters of swarm species. In recent years, some atypical species were characterised and identified in Brazil, as in the case of *M. enterolobii*, *M. ethiopica* and *M. hispanica*. More recently, the revalidation of *M. inornata* as a valid species and its isozyme and molecular characterisations proved once again that the use of the perineal region as a parameter for *Meloidogyne* spp. identification is subjective and leads to many errors. Although some studies have been conducted using a series of complementary techniques to identify *Meloidogyne* spp., some new species were described in Brazil using only classical taxonomical approaches and sometimes overlooking the enzymatic phenotypes. This is the case of *M. brasiliensis,* presenting an enzymatic profile of *M. ethiopica* and molecularly clustered with several populations of this species. Another example is *M. phaseolus,* which showed the enzymatic profile of *M. morocciensis*, although it has been described as a new species. Thus, there is a pressing need for methodological advances in the taxonomy of species of the genus *Meloidogyne*, using all available tools together: classical taxonomy, isozymes and molecular techniques.

S04–T3

Pathotype and molecular analysis of *Meloidogyne graminicola* diversity from Vietnamese rice fields

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In many countries Root-Knot Nematodes (RKN) are pests of rice, and induce important economic losses. The most common RKN infecting rice, *Meloidogyne graminicola* (*Mg*), can cause significant yield losses in all rice ecosystems throughout Asia. This is especially the case for upland-rainfed rice or for rice agro-systems that become aerobic for a short period. These rainfed systems occupy about 32% of the rice-growing area in Asia making the discovery of new resistant to *Mg* infection a priority. Using the internally transcribed spacer (ITS) region as a DNA marker, Pokharel was able to determine a DNA signature for *M. graminicola* and build phylogenetic relationships between isolates from several countries. Unfortunately this study on *Mg* diversity excluded Vietnam, which is the second largest rice exporter in the world and a country known to be a hotspot for rice pathogen diversity and emergence. Improved knowledge of this major rice pathogen is required before planning any pest control strategy. For a better understanding of *Mg* diversity and aggressiveness in Vietnam, a nematode survey was performed in 10 representative geographical areas represented by 40 different rice fields. All these populations were characterised using pathogenicity tests, morphometric analysis and molecular markers. It was concluded that Vietnam is also a hotspot of *Mg* diversity. Environmental conditions that allow rice to be cultivated throughout the year in the South, coupled to a wide diversity of rice cultivars grown in the North may help explainthe strong *Mg* population diversity across the country.

S04–T4

Screening and characterisation of fungal endophytes with activity against root-knot nematodes on tomato in Kenya

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Tomato (*Solanum lycopersicum* L.) is one of the most important local market vegetables in Kenya. Plant-parasitic nematodes, especially root-knot nematodes (*Meloidogyne* spp.), pose a major threat to vegetable production in Kenya and sub-Saharan Africa in general. Farmers usually rely on synthetic nematicides to manage the nematodes, which are not always effective, while increasing environmental concerns dictate the need for effective, affordable and safe alternative or complementary control measures. Seed treatment with endophytic fungi has been suggested as an effective method for management of plant-parasitic nematodes. The application of endophytic fungi, especially by seed treatment, has been shown to have potential in nematode control. However, research in this field remains limited. Little is known about the efficacy of endophytic micro-organisms associated with the tomato cultivars grown in Kenya, and yet this may hold a solution to the much sought after safe alternatives. The objective of this study was to isolate, screen and characterise fungal endophytes associated with tomato along the Kenyan coast and central Kenya where tomatoes are produced on a large scale. A survey was carried out between March and December 2011. Over 100 fungal endophytes were isolated from surface-sterilised tomato root tissues and root-knot nematode females and cultured on potato dextrose agar. Morphological characterisation of the isolates revealed that most of the isolates were *Fusarium* spp., followed by *Rhizoctonia, Pythium* and *Trichoderma* spp. Twenty of these isolates are currently being screened in Kenya and Germany and characterisation undertaken based on both morphology and DNA (ITS) sequences. The results of the screening and DNA characterisation are presented.

S04–T5

Root-knot nematodes in Thailand: a current overview

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Root-knot nematodes (*Meloidogyne* spp.) are currently viewed as the most damaging plant-parasitic nematodes occuring in Thailand. A range of crops, such as vegetables, ornamentals, fruit trees and field crops have been reported as hosts for these pests. To date, nine species of root-knot nematodes have been identified in Thailand. *M. arenaria* was reported from onion in the Central region of Thailand, and *M. exigua* and *M. graminicola* from lowland rice paddy fields in the Central and the Northeastern regions of Thailand, respectively. *M. hapla* was encountered in more temperate conditions in the highlands of Northern Thailand on ornamentals and herbs. *M. incognita*, the most prominent and widespread species, is frequently recovered and associated with damage to over 60 plant species throughout the country, while *M. javanica* has been associated with tuberose and gerbera in a localised area of central Thailand. Other species identified include *M. microcephala* from tobacco in Northern Thailand, a single record of *M. nasi* from sorghum in the Northeastern region and a recent identification of *M. enterolobii* causing severe damage to guava near Bangkok. However, some identifications are based on perineal patterns alone, from before the advent of molecular techniques, while *M. enterolobii* was identified using a combination of esterase enzyme patterns, and DNA analysis. Although potato, chilli and guava are currently identified as three key crops that are heavily affected by root knot nematodes, it is suspected that much greater damage is occurring across a wide range of crops. A recent report of root-knot nematode damage on cassava is currently being assessed, for example. In Thailand, research areas requiring a stronger emphasis include techniques for more accurate diagnosis towards delivery of appropriate management options. Improving identification, and knowledge on nematode—host or nematode–pathogen interactions, is desired to help deliver improved control.

S04–T6

Research and training approaches to minimise root-knot nematode damage to staple food crops in South Africa

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Root-knot nematodes (*Meloidogyne* spp.) cause significant damage to a range of crops in local intensified cropping systems. However, producers generally do not perceive root-knot nematodes as a constraint to crop production. The current research was designed to i) assess the nematode problems associated with crops and weeds grown, ii) investigate management strategies to reduce root-knot nematode population levels, and iii) disseminate information about the importance and impact of these parasites. Nematode samples were obtained from maize, sunflower, soybean, tomato and various other crops and weed plants from fields of commercial and small-scale farmers across South Africa. On-farm trials were subsequently conducted to evaluate the effect of various environmentally-friendly management strategies against root-knot nematodes for various crops. Strategies used included seed-coat products with nematicidal properties, soil solarisation, manure and compost amendments, *Tagetes* spp. mulches and intercropping, use of *Brassica* spp., inclusion of host plant resistance and application of biological control agents. Root-knot nematodes (*M. incognita, M. javanica* and *M. hapla*) were identified as the predominant eciesspecies, infecting crops as well as weeds. Inclusion of *Brassica* spp. as well as solarisation resulted in superior control (> 90%) of root-knot nematodes. Solarisation combined with either cow manure or compost also resulted in a substantial reduction in root-knot nematode population levels. *Tagetes minuta* mulch reduced root-knot nematode population levels by 71% and intercropping with *T. erecta* by 30—90%. Cow and chicken manure amendments reduced root-knot nematodes significantly compared to the untreated control treatments in the respective trials. An increased awareness of producers regarding the importance and impact of plant-parasitic nematodes was evident as a result of these research activities.

S04–P1

Seasonal population fluctuation of *Meloidogyne* spp. on kiwi orchards in Ordu province, Turkey

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Kiwifruit (*Actinidia deliciosa*) is a crop of increasing importance in Turkey. In terms of production quantity, Turkey is ranked ninth (26,554 tonnes) among the top ten countries producing kiwifruit. Ordu is a province in the Black Sea region of Turkey and total kiwi production in the province is 5951 tonnes. Nematode damage reported by *Meloidogyne* spp. on kiwifruit is widespread. The objective of this study was to determine the seasonal fluctuations in population densities of *Meloidogyne* spp. in soil on kiwi orchards. For this purpose, soil samples were taken from March 2011 to March 2012 at fifteen day intervals from two different kiwi orchards of Kayabasi district in Ordu province in the Black Sea region of Turkey. These orchards were selected as galls of *Meloidogyne* spp. had previously been encountered at these sites. Sampling was performed from the vines that were randomly selected from each orchard. The point of depth of 0—30 cm and 30—40 cm away of the trunk of each vine was sampled by using a hand-shovel from the both sides of the vine. The vines were separated from each other of by at least 4 m × 4 m on rows for both orchards. Each soil sample (2.0 kg) was a composite of ten random subsamples. The soil temperature of each orchard was measured using a digital soil temperature meter to compare with the information provided by a weather forecast station. In the laboratory, a 100 cm^3 aliquot of the homogenised soil sample was processed by the modified Baerman funnel method. *Meloidogyne* spp. juveniles were counted on microscope slides with a cover slip. The highest soil populations were observed in March 2011 as 287 J2 /100 cm^3 of soil and 182 J2/100 cm^3 of soil in both orchards, respectively. After this month, the soil population levels decreased gradually until March 2012 to as low as 6 J2 / 100 cm^3 of soil and 7 J2/100 cm^3 of soil in the orchards, respectively. The results showed that nematode levels in the soil generally increased between August 2011 and January 2012 which were the months with highest precipitation, and after this period rainfall gradually decreasesdecreases. The first increases in populations in both orchards were observed in May 2011 possibly as a result of gradually increasing temperature between April 2011 and June 2011.

S04–P2

Effect of the *Mi* gene on infection and egression of *Meloidogyne incognita* race 2 on tomato cultivars

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Meloidogyne incognita is a damaging pathogen of vegetables and is often found infecting vegetable crops in warmer climates. Our objective was to determine the effect of the *Mi*-gene on root penetration and egression of *M. incognita* in tomato vaieties. Seeds of the tomato varieties Amelia and Crista carrying the *Mi*-gene for *Meloidogyne* resistance and the susceptible variety, Talladega, were germinated in sterile vermiculite in bathroom cups. Fifteen days after germination, all of the tomato plants were transplanted to pots filled with autoclaved construction sand. *Meloidogyne incognita* race 2 was used as the inoculum; a suspension of freshly hatched second-stage juveniles (J2) was prepared and delivered at a rate of 350/plant in a 1 ml suspension placed into three 2.5 cm wide × 9 cm deep holes around the base of plants. The plants were placed in a growth chamber for 14 days at 28°C with 14 h of light and watered daily. Afterwards, plants were removed from pots 2, 4, 6, 8, 10, 12, and 14 days after they were inoculated. Two roots from each variety were selected, washed and then stained in acid fuchsin lactophenol for 1 min. The number of J2 within the entire root system was counted. Percentages of egressed nematodes were calculated from the total number egressed and those that remained inside the roots. Up to 14 d after nematode inoculation no galls were found on roots of either Amelia or Crista varieties, but Talladega roots were found to be highly infected.

S04–P3

Efficacy of growth medium supplemented with certain amino acids or mineral salts mixed with activated charcoal plus coconut water on tomato roots suitability to *Meloidogyne incognita* infection

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The effects of certain amino acids, *e.g*. glutamic acid or proline, or mineral salts, *e.g*. ferric sulphate, or urea mixed with activated charcoal plus coconut water (AC) on *Meloidogyne incognita* were compared with oxamyl at the recommended dose. Treatments were added to MS (Murashige and Skoog) medium used for growth of tomato seedlings cv. Strain-B before transplanting into pots with sterilised mixture soil/pot (1 loam:1 sand, v:v). The effects on *Meloidogyne incognita* infection were separately evaluated under greenhouse conditions at 30±5°C. The results indicated that of the tested treatments, glutamic acid concomitant with AC gave the highest percentage increase in shoot and root lengths, whole plant fresh and shoot dry weights for both the tomato seedlings infected by *M. incognita*; and uninfected plants as well, since their values averaged 65.3, 90.6, 107.4 and 110.8%; and 130.3, 107.3, 145.5 and 124.7%, respectively. Plants receiving oxamyl plus AC treatment ranked first, with the highest percentage reduction in root-knot nematode population; however, it ranked second to glutamic acid plus AC treatment in diminishing number of galls and egg-masses without significant differences. Application of proline plus AC had the lowest percentage reduction for the same nematode criterion.

S04–P4

The relationship between root-knot nematode (*Meloidogyne incognita*) population density and plant growth and damage on tomato

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Root-knot nematodes are the most important pests for vegetables almost all over the world. The damage level depends on various factors including initial population levels of root-knot nematode. The aim of this study was to determine the effects of initial population density on growth of both susceptible and resistant tomato plants. This was determined by applying 0, 20, 40, 80 and 240 eggs/100 cc soil. Two sets of experiments were performed in controlled environment greenhouses; in the first set, plants were grown for 5 weeks and in the second set, for 12 weeks after artificial inoculations. Plant growth parameters were determined for both sets and yields were measurmeasured for the second set of experiments. As a result of the study lots of important results were found, such as that there were statistically important differences for tomato varieties and also time and initial population level. It is seen that the reproduction of *M. incognita* did not occur in resistant tomato in both short and long times. Even 20 eggs /100 cc soils were enough to cause plant growth reduction and yield loss. When initial nematode population increased, plant growth was reduced and, in the second experiment, yield was found to be decreased by 5..9 —100%.

S04–P5

Nemadicidal Activity of Extracts from *Peganum harmala* L. (Zygophyllaceae) against *Meloidogyne incognita*

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This present study was undertaken to assess the effect *in vitro* at different concentrations of (25, 50 and 100%) of hexanic, ethanolic and aqueous extracts from leaves and seeds of *Peganum harmala* L. (Zygophyllaceae) on the mortality of second stage juveniles (J2) after 24, 48 and 72 h exposure and on hatching of eggs of *Meloidogyne incognita* maintained in the same test solutions for 12 days. These effects were compared with those of control solutions and fenamiphos. DL_{50} were also calculated for both effects. The results showed that the extracts tested demonstrated a nematicidal activity on J2 and inhibited hatching of *M. incognita,* with these effects varying with the nature of the extracts, time of exposure and concentration. The study was completed by phytochemical analysis (screening) of leaves and seeds of this plant to determine the groups of secondary metabolites present. .

S04–P6

Relative susceptibility of some *Brassica napus* and *B.campestris* to the stunt nematode, *Tylenchorhynchus latus* and other tylenchids under field conditions

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Thirty eight imported oil seed-rape cultivars were evaluated under field conditions for their relative susceptibility against the stunt nematode, *Tylenchorhynchus latus* and other tylenchids. Statistical differencies ($P \leq 0.05$ and 0.01) in the tested cultivars were found in either final nematode populations or the yield components. The potential of each cultivar to support reproduction of the stunt nematode or other tylenchids was calculated in relation to that of PF 1/85 cv. or Semu DNK 86/233 cv.; respectively which were regarded as check cultivars and the host category (HC) based on the potential reproduction index of each cultivar was estimated. Accordingly, the tested cultivars could be categorised for their susceptibility against *T. latus* as follow: Sedo, Semu DNK 85/201 , Semu DNK 204/83, Semu DNK 232/84 , Semu DNK 86/233 and Semu DN 205/82 were graded as highly resistant. Candle, Hanna, Duplo, Lirasol, Loras, Semu DNK 85/202, Semu DNK 249/84, Semu DNK 232/83, PF 550/86 and PF 2886/85 were graded as resistant cultivars. Fourteen cultivars out of thirty eight graded as less susceptible. Tower, Silva, Moneta Semu 249/84, Anima Semu 204/83 and Semu DNK 235/84 were rated as moderately susceptible. On the other hand, only two cultivars, namely Gloda Semu 250/84 and PF 1/85, were categorised as highly susceptible. It was observed that reproduction of nematode was favoured on highly susceptible and susceptible cultivars but inhibited on resistant and highly resistant ones. Therefore, all tested cultivars showed great variability in their reaction to the nematode infection according to the host type. Also, different yield components of oil seed rape varieties were also discussed. Finally, the differences among the tested cultivars should serve as a good resource for plant breeders and cropping systems to limit the loss due to the nematode infection.

S04–P7

Positioning nematology research and development through NIESA (Nematology Initiative for Eastern and Southern Africa)

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Since its inception in 2005, the Nematology Initiative for Eastern and Southern Africa (NIESA) is a project that has been sponsored by the Gatsby Charitable Foundation (UK) to build capacity in the discipline of nematology, and to develop a network of expertise in Eastern and Southern Africa, originally with technical support from a UK consortium – CABI Bioscience, Rothamsted Research and the University of Reading. At present, NIESA is composed of a cadre of qualified nematologists from Kenya, Malawi, Tanzania, Uganda and Zimbabwe. The project has also started to move from its capacity building phase to sharing and transferring NIESA nematologists' expertise to ascertain the extent to which plant-parasitic nematodes act as a constraint to local and regional crop production and to create awareness among farmers and local communities about the importance of nematodes. To achieve this, NIESA, as a group, will continue carrying out scientific research and training of farmers and phytosanitation staff for the practical benefit for local communities, crop health and food security. Networking, joint research fund application, cross-learning and peer support among practising nematologists in Africa can facilitate an active and interactive support to overcome the lack of a critical mass of nematologists in any one country. It can also link the network to information services available through partner scientists, formal research and training collaborations to improve understanding and raise the profile of nematology within Africa. Further details of the NIESA partners and activities can be found at the web site www.africannematology.org

S5 – Morphology and taxonomy

Convenors: Wim Bert & Erik Ragsdale

S05–T1

Reconstruction of pharynx anatomy to test phylogenetic incongruence and hypotheses of feeding evolution in tylenchid nematodes

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Molecular phylogenetics has consistently challenged the monophyly of both Tylenchomorpha and Aphelenchoidea, groups long established on the basis of feeding morphology. To address this incongruence, we re-examined taxonomic characters circumscribing these groups by three-dimensionally reconstructing the anterior pharynx (corpus) of the tylenchid *Aphelenchus avenae*. Results were combined with a review of available data on other taxa to map the evolution of feeding morphology in Rhabditida *sensu lato*. Complete reconstruction of the corpus revealed that numbers of cell classes and the numbers of nuclei per cell class are identical between *A. avenae* and *Caenorhabditis elegans*, enabling robust homology statements. Positions of neurons are highly conserved and support homologies of other cell classes based on spatial connectivity to those cells. Arrangement of individual cells in the corpus differs among the three lineages of Tylenchomorpha, even between the grossly similar and possibly convergent pharynges of "Aphelenchoidea," precluding grouping of any two lineages. In particular, differing cellular architectures reject the homology of the "procorpus." Consequently, the position of the dorsal gland orifice in the metacorpus, the primary character defining "Aphelenchoidea," is also not homologous. Despite differences peculiar to regions within the corpus, cellular components of the corpus as a whole are strikingly conserved: by a conserved modality of evolution, the same sets of cells could successfully be co-opted for divergent feeding morphologies and lifestyles across Rhabditida.

S05–T2

Development of species-specific SCAR markers for identification of two root-knot nematodes of coffee, *Meloidogyne arabicida* and *M. izalcoensis*

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In this study we developed a PCR-based assay for specific detection of two root-knot nematodes *Meloidogyne arabicida* and *M. izalcoensis* (Tylenchida: Meloidogynidae), major pathogens of coffee crops in Central America. RAPD fragments specific for these two species were converted into sequence characterised amplified region (SCAR) markers. PCR amplification using the SCAR primers produced a specific fragment of the expected size (i.e. 300 bp and 670 bp) in *M. arabicida* and *M. izalcoensis*, respectively, in contrast with the other coffee-associated *Meloidogyne* spp. tested. SCAR primers also allowed successful amplification of DNA from single infective juveniles, males and females. In addition, these primers were able to unambiguously detect the target species in field samples, in different isolates of a same species or when used in multiplex PCR reactions containing mixtures of species. These results demonstrate the effectiveness of these SCAR markers, and their multiplex use with those previously developed for *M. exigua*, *M. incognita*, *M. paranaensis* and *M. enterolobii* may further contribute to specific diagnosis of the major root-knot nematodes infecting coffee in the Americas.

S05-T3

Molecular analysis of species belonging to *Panagrolaimus* Fuchs, 1930 from Iran based on 18S ribosomal DNA sequencing

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Bacteriovorous *Panagrolaimus* nematodes are easily grown on media and can be a suitable source of material for basic studies in biology, medicine and genetics, particularly in animals. During a study of panagrolaimids in Kerman (in the South of Iran), *Panagrolaimus* cf. *trilabiatus*, a species from the infected seeds of wheat, was isolated. After its propagation on 2% WA, DNA was extracted and 18S rDNA was amplified using specific primers and sequenced. Molecular analysis using Mega5 software and UPGMA and Maximum Parsimony methods showed that the studied population was set in the same clade as*P. subelongatus* from The Netherlands (AY284681) with 88 and 71 bootstrap values respectively. Genetic distance and nucleotide similarity of *P.* cf. *trilabiatus* (collected from Kerman) and *P. subelongatus* showed that these two populations have minimum genetic distance. The genetic distances estimated for these two species according to 18S rDNA for Dutch and Iranian populations were 0.090 and 0.087 respectively. Sequencing of the other rDNA regions can be helpful in identifying and understanding its species and phylogenetic relationships. Furthermore, the phylogenetic relationships of the species of *Panagrolaimus* is discussed.

S05–T4

Plant-parasitic nematode collections as an element of nematology research ın the Russian Federation

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Collections of plant-parasitic nematodes are a major part of helminthological collections and museums which are located at the leading biological institutes of the Russian Federation. These are the Central Helminthological Museum (K.A. Skryabin Institute of Helminthology RAAS), the Helminthological Museum of RAS (Centre Parasitology IEE RAS), the Collections of Parasitic Worms (Zoological Institute RAS) and the Helminthological Collection (Institute of Biology and Soil Science FEB RAS). The total resources within these collections include more 200,000 microscope slides. Moreover, many small (laboratory) collections are held by other research institutes or groups. These include the Russian Center of Quarantine (quarantine nematodes), Russian Research Institute of Phytopathology and Russian Research Institute of Plant Protection (plant-parasitic nematodes) and the Institute of Biology KRC RAS (free-living and plant-parasitic nematodes,). These collections are part of the global nematological resources and reflect the diversity of all trophic and biological groups of nematodes from various regions of Russia and the world. They are used in the study of fauna, morphology and systematics as well as theoretical studies on the evolution and phylogeny of nematodes.

S5–T5

Challenges in nematode taxonomy: diverse taxa from disparate habitats and regions require a diversity of integrated toolkits

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Without basic taxonomic information, biodiversity analyses at both local and global level are bound to fail. However, the frontier being pushed in taxonomy is an increase in diversity assessment from multiple and complementary perspectives. Here, we will provide an impression of our taxonomical work on free-living, plant-parasitic, virus-vector, facultatively parasitic and entomopathogenic nematodes from natural and agricultural ecosystems of several continents. Our priority of coping with the enormous nematode diversity lies in integrative work in connection with other relevant research. Hereby, we will address the following elements of our approach: ways to combine DNA sequencing data and morphology, the assessment of intraspecific versus interspecific variation, applications of species delineation that are both theoretically sound and practically feasible, encoding taxonomic descriptions for computer processing such as interactive keys, the integration of ecological and biological data, and the relevance to crop protection and biomonitoring. Finally we will discuss ways of integrating our data by the use of databases and a phylogenetic framework.

S5–P1

Morphometric variability of sting nematodes (*Belonolaimus* spp.) populations from Kansas, Texas and Florida, USA

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The sting nematode (*Belonolaimus longicaudatus*) is an important plant-parasitic nematode, affecting many plant species, including a wide range of economically important agricultural and horticultural crops. The aim of this study was to study the morphometric variability of this nematode isolated from three States of USA namely, Kansas, Texas, and Florida. Sting nematodes were extracted from 100 cm^3 subsamples using a combined sieving and sugar flotation method. Measurements were made using a drawing camera plan Axiophoto attached to a Nikon light microscope. For light microscope observations 20 individuals of different life stages (second-stage juveniles, males, females) were examined alive. Additional specimens of different stages were killed and fixed in lactophenol. The preserved nematodes were used for detailed observation of morphology or variation of some structures. The evaluated characters were body length, body width, lip length, stylet cone length, stylet length, DEGO, nerve ring, excretory pore, esophagus, median bulb length, median bulb width, anal body width, tail length, stylet/tail and V. The morphological variation among females was much greater than that of males and J2s, especially in stylet length. Females, males, and J2s of the Kansas population had the shortest body length, body width, lip length, stylet cone, and stylet length.

S5–P2

Bursaphelenchus fungivorus and *B. minutus* associated with *Pinus pinaster* bark in Portugal

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The genus *Bursaphelenchus* includes nematodes associated with insects and trees, mainly coniferous. In Portugal, a total of nine species (*B. antoniae*, *B. hellenicus*, *B. leoni*, *B. mucronatus*, *B. pinasteri*, *B. sexdentati*, *B. teratospiculari*, *B. tusciae* and *B. xylophilus*) have been described associated with *Pinus pinaster* wood and insects. In this study, the presence of *B. fungivorus* and *B. minutus* associated with *P. pinaster* bark in Portugal is reported for the first time. *Bursaphelenchus fungivorus* showed the main diagnostic characters: males with tail ventrally curved, compact spicules without cucullus and females presented a vulva without flap and a long tail ventrally bent with terminus mucronate. Morphological identification was confirmed by ITS-RFLP analysis with the 5 restriction enzymes *Afa*I, *Alu*I, *Hae*III, *Hinf*I and *Msp*I. Restriction patterns were similar to those of other isolates from other parts of the world. *Bursaphelenchus minutus* was identified by its small body size (length < 300 µm) and by the main morphological characters. Males with body curved in tail region forming almost a helical and spicules rose-thorn shaped with a small cucullus. Females with a characteristic body C-shaped in thermal death position, vulva without flap and tail ventrally arcuate with pointed terminus. Restriction patterns of ITS regions were obtained.

S5–P3

Plant-parasitic nematodes associated with Phoenician juniper in Jordan

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Phoenician juniper, *Juniperus phoenicea*, is found throughout the Mediterranean regions including the southern highlands of Jordan. However, there is a significant die-back occurring in these juniper communities at the highest elevations. It has been suggested that this die-back may be caused by a drop of the water table. However, no studies have investigated the role of biotic factors in this severe decline. We therefore conducted a survey to investigate the plant-parasitic nematodes iassociated with the roots of junipers. Results of this survey revealed the presence of two species of plant-parasitic nematodes that belonged to the two genera, *Mesocriconema* and *Geocenamus*. Higher numbers of *Geocenamus* sp.were recovered from the soil samples. Molecular and morphological characterisation of this nematode suggested that it represented a new species of *Geocenamus*. More studies will be conducted to determine the damage caused by these nematodes on the Phoenician junipers and whether they have a role in the die-back phenomenon.

S5–P4

Meloidogyne christiei infecting oaks (*Quercus* spp.) in Florida: A case study

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Meloidogyne christiei Golden and Kaplan, 1986 was reported infecting turkey oak (*Quercus laevis*) in the central region of Florida, USA. To date its distribution has been limited to this region. Recently, we discovered another oak species, laurel oak (*Q. laurifolia*), infected by a *Meloidogyne* sp. This raised the question whether this species was also *M. christiei*. Species identification was performed using morphological characters, biochemical (esterase [EST] and malate dehydrogenase [Mdh]) and molecular analyses. Ten *Meloidogyne* species found in Florida were compared in the biochemical and molecular analyses. For the PCR, DNA was extracted from multiple single females using a Qiagen DNeasy Kit (Qiagen, Valencia, CA) and mtDNA was amplified with C2F3/1108 primers set at 58ºC annealing temperature. Only those specimens extracted from turkey and laurel oak trees matched those of the original description of *M. christiei*. The phenotype N1a detected from a single young egg-laying female of *M. christiei* showed one very strong band (Rm: 28.1) of MDH activity; however, no EST activity was detected even when using up to 20 females. *Meloidogyne christiei*, *M. graminis*, *M. graminicola, M. hapla*, and *M. partityla* produced fragments of 530~540 bp, whereas *M. enterolobii* produced ca. 700 bp. The PCR products were 1.2 kb for both *M. floridensis* and *M. arenaria*, and 1.7 kb for *M. javanica* and *M. incognita*. The mtDNA was useful to detect and distinguish *M. christiei* from the three major agricultural species of *Meloidogyne* (*M. arenaria*, *M. incognita*, and *M. javanica*), as well as *M. floridensis* and *M. enterolobii.*

S5–P5

Identification and molecular characterisation of *Laimaphelenchus* spp. associated with cork oak

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Plant-parasitic nematodes have been recognised as important pathogens of trees. The *Montado*, an agro-silvo-pastoral ecosystem dominated by cork oak, *Quercus suber*, occupies a large land area in Portugal. Cork oak decline has being observed since the second half of the 20th century and the occurrence of *Bursaphelenchus* spp. in oak trees has been described in international reports. The objective of this study was to acquire knowledge on the most common nematodes associated with the cork oak crown (wood and bark). In a total of 40 samples, taken from two farms, each having two areas with different cork oak decline history (low and high mortality areas), more than 67% of the 86% of the specimens extracted from the bark belong to the genus *Laimaphelenchus*. This genus comprises several non-pathogenic species similar to *Bursaphelenchus* spp. mostly found associated with moss, algae and lichen on conifers and frequently co-habiting with other genera of economic importance. *Laimaphelenchus* cultures were established in *Botrytis cinerea* grown on Malt Extract Agar. Isolates are being studied using light microscopy, scanning electron microscopy and sequence analyses of the mtCOI, the D2/D3 expansion segments of LSU and the SSU rRNA gene.

S5–P6

Head papillae, postanal swelling and vulval patterns: diagnostically valuable characters in the family Heterorhabtidae

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All species of the family Heterorhabditidae have been characterised by the head region, which has six separate lips, each bearing the terminal labial papilla. The only exception is *H. atacamensis,* which has four papillae in the cephalic ring of hermaphrodites. However, in the current description of *H. beicherriana* the authors confirmed the second circle with six cephalic papillae. Very likely, these minute papillae were overlooked in former species descriptions. Therefore, our main goal was to study and compare the morphology of the head region in the three heterorhabditid clades *bacteriophora, megidis* and *indica*. The cephalic ring of papillae could be generally present in the family Heterorhabditidae. While in the family Steinernematidae there are many valuable taxonomical characters in infective juveniles and adults, the family Heterorhabditidae lacks most of these characters. Therefore, we also studied vulval patterns and postanal swelling of hermaphrodites and assessed their usefulness for species delimitation.

S5–P7

Morphological data on *Dorydorella bryophila* (de Man, 1880) Andrássy, 1987 and its phylogenetic relationships with other dorylaimid taxa as inferred from D2—D3 of 28S rRNA gene sequences

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A morphological and molecular study of the species *Dorydorella bryophila* (De Man, 1880) Andrássy, 1987 was performed on specimens collected from soil of mixed forest in Bryce Canyon, Utah (USA) and from the banks of the Danube River (border between R. Moldova and Ukraine). Morphologically, *Dorydorella* shows intermediate characters between *Eudorylaimus* in Qudsianematidae (general morphology) and *Longidorella* in Nordiidae (comparatively longer and more slender odontostyle). Andrássy (1987) erected the genus *Dorydorella* to transfer three species previously classified under *Eudorylaimus* Andrássy, 1959, namely *D. bryophila* (De Man, 1880), *D. pratensis* (De Man, 1880) and *D. tenuidens* (Thorne & Swanger, 1936), which are distinguishable by a more slender odontostyle (10—15 times as long as wide), straight tail and large pharyngeal dorsal nucleus. In Nordiidae, *Dorydorella* was originally compared to *Longidorella* Thorne, 1939 from which it was distinguished by its much shorter odontostyle (*vs* 3—5 times the lip region diameter). The phylogenetic relationships of *Dorydorella bryophila* with species from families Nordiidae and Qudsianematidae was analysed using new D2—D3 of 28S rRNA gene sequences and sequences deposited in GenBank. The phylogenetic trees obtained from ML, MP and BI analysis have similar topology. The sequence of *D. bryophila* clustered with two *Longidorella* sp. sequences published by Holterman *et al.* (2008) and formed a moderately to highly supported clade with other *Longidorella* sequences plus *Microdorylaimus miser*. The relationship between *D. bryophila* and *Longidorella* species deserves more detailed discussion.

S5–P8

Characterisation of *Meloidogyne enterolobii* intercepted in guava seedlings imported from Sri Lanka to Japan

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Meloidogyne enterolobii (= *M. mayaguensis*) is currently considered as one of the most important root-knot nematode species because of its high pathogenicity and wide host range including many economically important crops. In June 2009, *M. enterolobii* was discovered on roots of guava (*Psidium guajava*) seedlings imported from Sri Lanka that were intercepted by inspectors of the Plant Protection Station at Narita International Airport, Japan. In this study, the intercepted population of *M. enterolobii* from Sri Lanka was characterised by morphological, biochemical and molecular methods. Morphometrics of females, males and second-stage juveniles were generally within the expected range previously reported for *M. enterolobii*. The perineal patterns were quite variable and some were similar to *M. incognita* with a high trapezoidal dorsal arch. Isozyme analyses showed the esterase phenotype VS1-S1 with two major bands, and the malate dehydrogenase phenotype N1a with one strong band. The sequence comparisons of the mtDNA region between the *COII* and 16S rRNA genes and IGS-rDNA region confirmed the identity of the nematodes as *M. enterolobii*. This is the first report of *M. enterolobii* from Sri Lanka.

S5–P9

Correlation analysis of morphometric data in the species of the genus *Pselinchus* de Man, 1921

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The genus *Psilenchus* commonly occurs in most soils and feeds as an ectoparasite on algae, mosses, lichens and plant roots. During a survey on nematodes of the Lalezar region of the Kerman province, two species belonging to this genus including *P. hilarulus* de Man, 1921 and *P. terextremus* Hagemeyer & Allen, 1952 were isolated using a tray method and identified. In addition studying morphological and morphometric characteristics, correlations of all morphometric data were analysed by SPSS 13. Results revealed that b (r = 0.669) and c (r = 0.649)have significant correlation ($p \leq 0.01$) with body length. The indexes c (r = -0.643) and ć (r = 0.614) dhad significant correlation ($p \leq 0.01$) with tail length. Median bulb and pharynx togetherhad the highest correlation ($p \leq 0.01$; r = 0.876). In conclusion, some morphometric indexes such as b, c and ć were more effective in comparison with other morphometric characters for identifying the species. Measurements, illustrations and correlation table are provided for the species. Both species are reported for the first time from *Platanus orientalis* L. in Iran.

S5–P10

Molecular characterisation of some species of the Pratylenchidae family from Iran

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Fourteen species belonging to the genera *Pratylenchus, Zygotylenchus, Pratylenchoides* and *Hirschmanniella* of the family Pratylenchidae were preliminary-identified based on the morphological characters and then analysed using molecular methods. The samples were collected from a range of crops and plants throughout the country. After morphological identification DNAwas extracted from individuals and the D2—D3 expansion segments of the 28S rRNA gene were amplified with the forward D2A and reverse D3B primers. The purified PCR products were sequenced with the same primers. The newly obtained sequences were aligned with other sequences of Pratylenchidae published in GenBank. The sequence datasets were analysed with Bayesian inference using MrBayes 3.1.2 under the GTR + I + G model. Sequence and phylogenetic analyses confirmed the presence of fourteen Pratylenchidae species in the collected samples. Sequences of isolates of *Pratylenchus neglectus* and *P. thornei* collected rfrom cereal fields, *P. loosi* from tea plantations*, P. coffeae* from banana, *P. penetrans* from ornamental plants*, P. vulnus* from pine and *Zygotylenchus guevarai* from almond rhizosphere showed high levels of similarity and clustered with the sequences of corresponding species published in GenBank. The nucleotide differences between sequences of Iranian populations and corresponding reference species were in intraspecific ranges. *Pratylenchus delattrei* found in vegetables fields formed a highly supported clade with *P. zeae* and *Pratylenchus* sp. *Pratylenchus* sp. from the rhizosphere of palm, were morphologically very close to *P. zeae,* except for the tail shape and had a sister relationship with *P. zeae. Pratylenchus pseudopratensis* found in cereal fields clustered with *P. vulnus* with a low posterior probability value. Morphologically similar *Pratylenchoides ritteri* and *P. alkani* differ only in 5 bp of the D2—D3 expansion segments of 28S rRNA gene from each other. *Hirschmanniella* sp. from a rice field in Iran formed a highly supported clade with *H. loofi* and *H. kwazuna*. Phylogenetic relationships within *Pratylenchus* and *Hirschmanniella* species were mainly congruent to those obtained in previous published studies.

S5–P11

Integrative diagnosis of a new *Pratylenchus* species from Ghana and the *P. coffeae* species complex

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Root-lesion nematodes of the genus *Pratylenchus* have a worldwide distribution and are regarded as severe production constraints for numerous important crops. Correct and accurate identification of species within this important genus of plant-parasitic nematodes is critical but is complicated by their stenomorphic status. A polyphasic approach to diagnosis is therefore proposed as a better strategy for distinguishing species and to infer phylogenetic relationships within the genus. Recent studies on *Pratylenchus* spp. recovered from damaged plantain (*Musa* spp., genome AAB) in Ghana, West Africa, has resulted in their description as a new species, *Pratylenchus speijeri* n. sp. Morphological and molecular features of this species were compared with those of *P. coffeae*, its most closely related species, and other amphimictic species of *Pratylenchus* that have and undivided face with two lip annuli. Morphological characterisation of *P. speijeri* n. sp. did not result in an unambiguous separation from *P. coffeae*, whereas molecular analysis clearly distinguished the two species. Only a few and often variable morphological features, such as larger stylet knobs and a more frequently indented tail terminus separated these populations from *P. coffeae*. Sequences and phylogenetic analyses of D2—D3 of 28S rDNA and ITS containing regions of 60 individual nematodes from the *P. coffeae* species complex, sourced from various geographical locations, generated majority consensus BI trees with three major clades, where *P. speijeri* n. sp. formed its own separate clade from *P. coffeae*. During recent years the use of SEM, LM and molecular approaches to study *P. coffeae*, or representatives of the *P. coffeae* species complex, has resulted in the descriptions of several new cryptic species, namely *P. jaehni*, *P. floridensis*, *P. parafloridensis* and now *P. speijeri* n. sp. The morphological and molecular features of these cryptic species are compared and the practical application of such molecular data discussed.

S6 – Molecular Diagnostics

Convener: Alex Reid

S6–T1

Complexity within potato cyst nematode field populations

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Potato cyst nematodes, *Globodera pallida* and *G. rostochiensis* were introduced into Europe from South America and have been further dispersed into many potato growing regions around the world. Determining the number of initial introductions into Europe has been of interest to understand the genetic diversity of PCN in Europe and to establish relevant reference populations to use in potato breeding programmes for resistance testing.

Various biological and molecular assays have been used to assess the intraspecific diversity of PCN and these have indicated that *G. pallida* in Europe is probably more diverse than *G. rostochiensis*. Analyses using mitochondrial DNA (mtDNA) have also revealed that European populations of *G. pallida* probably originated from Southern Peru. The mtDNA genome of PCN has an unusual and complex multipartite structure. Some regions of this genome are also highly polymorphic. We have been examining these polymorphic regions to determine if they can be used to characterise field populations and to determine whether this information can be used to predict possible virulence characteristics of field populations. Analyses of mtDNA from *G. pallida* and *G. rostochiensis* populations in the JHI collection and from Scottish field populations will be presented and discussed in relation to the introductions of PCN and the use of resistance to manage PCN.

S6–T2

Q-bank – identification tool for quarantine nematodes and their close relatives linked to reference collections across Europe: I) Molecular approach

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Taxonomic expertise is considerably decreasing in the nematode scientific community. In an attempt to conserve existing knowledge but also provide modern tools for nematode identification, Q-bank was started (www.q-bank.eu/nematodes). Q-bank uses morphological, physiological and/or molecular features to compute a global similarity coefficient using different characters or criteria at the same time. Besides standard pairwise sequence alignments (Blasts), where only one sequence can be accounted at the same time, performance of a Polyphasic identification is provided. Q-bank has the option to use several features at the same time to compare an unknown profile against a list of reference specimens or populations out of reference nematode collections across Europe.

The strength of Q-bank compared with other molecular databases is that any metadata in the database has been confirmed for correctness by curators. Currently there are more than one hundred entries covering all regulated nematode species in Europe and their close relatives; however, as the database was launched recently, it is still evolving. The concept and structure of Q-bank is presented and examples of using Q-bank are given. Q-bank is freely accessible to all interested users. Q-bank is supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by QBOL, an EU-project with partners from 20 countries.

S6–T3

Looking for a needle in a hay-stack: diagnostic kits for the detection of plant-parasitic nematodes in complex DNA backgrounds

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Recently, many agricultural laboratories have invested in the set up of a molecular laboratory in the expectation that DNA-based identification and detection of plant pathogens, including nematodes, will be the golden standard in the near future. However, as molecular methods for the detection of some specific (quarantine) nematodes have the potential to fully replace the current microscopic routine screening of (soil) samples, there is a strong need to check for their accuracy and performance. Whereas a molecular method in itself may be far more precise and accurate than a microscopic method ever can be, its accuracy will largely depend on the biological information that was used to develop it. Its performance will depend on the robustness of the method and the test performed.

On the basis of a phylum-wide, small subunit ribosomal DNA (SSU rDNA)-based framework consisting of ~2,500 full-length nematode sequences, we have developed over 20 diagnostic Q-PCR tests for plant pathogenic nematodes. Some nematode species are parasites of major crops such as potato, sugar beet and soybean. Among the most notorious ones are cyst (e.g. *Globodera rostochiensis* and *G. pallida*), root knot (e.g. *Meloidogyne chitwoodi, M. fallax* and *M. minor*), stem and bulb (e.g. *Ditylenchus dipsaci* and *D. destructor*), and foliar nematodes (various *Aphelenchoides* species). By using this SSU rDNA framework, we could define unique DNA sequence signatures that enable 'blind' identification of these individual nematode species in highly complex DNA backgrounds. A number of examples will be presented during this meeting.

S6–T4

Molecular approaches to diagnostics for plant-parasitic nematodes of biosecurity concern

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The focus of this work was to assess existing methods and to develop new molecular approaches to detect and characterise plant parasitic nematodes of quarantine concern.

ITS-based PCR diagnostics were used to identify three *Pratylenchus* (RLN) species. In a protein-based approach nematode proteins were analysed using MALDI-ToF MS to generate distinct species-specific protein profiles for eight species. Two dimensional protein gel electrophoresis was also undertaken to develop diagnostic biomarkers for nematode identification. Of 58 distinct protein spots for *Pratylenchus* spp. and 89 spots for *Heterodera* spp., 13 and 9 spots respectively were further analysed as potential diagnostic biomarkers. Individual proteins were excised and sequenced after trypsin digestion and the identities of 16 proteins were confirmed.

An additional diagnostic method was developed – 'Multiplex anti-primer denaturation PCR' (MAD PCR). It combines 'anti-primer' technology with 'auto-sticky' PCR, qPCR and fluorescent labels. Three RLN species were identified in a multiplex system. Rapid nematode DNA extraction from soil samples was combined with molecular identification in a method termed 'DNA Isolation Rapid Technique from soil' – 'DIRTs'. DNA is extracted from soil samples in 2 min using a blender followed by qPCR. The complete procedure takes 4 hr. Combining the DIRTs extraction and 'anti-primer' technology, three different RLN species were identified successfully in multiplex reactions.

Both protein and PCR-based procedures can provide robust approaches to detect and characterise plant-parasitic nematodes.

S6–T5

Determining the diversity of European *Globodera pallida* populations.

Reid, A.

Potato cyst nematodes (PCN) are responsible for loses in potato production totalling millions of Euros every year in the EC (over €50 million in UK alone). Of the two species, *Globodera pallida* and *G. rostochiensis,* a single major resistance gene *H1* has been bred into many commercial potato varieties which gives good resistance to *G. rostochiensis*. To date there is no significant resistance for *G. pallida* and as a result of this, and the widespread cultivation of varieties containing the *H1* gene, there has been a steady rise in the occurrence of *G. pallida* in land used for growing potatoes. There are two main pathotypes of *G. pallida* (Pa1 and Pa2/3), a differentiation based on lengthy bioassays to asses their reproduction on a set of different *Solanum* genotypes. However, both biological and molecular assays suggest that additional populations, such as the Luffness population from Eastern Scotland, display high levels of virulence even to potato genotypes containing partial resistance to *G. pallida*. The spread of such populations would create new management problems for the potato industry and there is therefore a need to be able to identify the level of interspecific variability within PCN species in European soils and then to determine the virulence of these populations on a variety of potato genotypes.

S6–P1

A geographic information system for statutory (and generic) soil sampling of (quarantine) nematodes

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A geographic information system was developed, based on freely available background maps and dedicated software, to improve the way soil sampling is requested, fields are split into sampling units, and to disseminate the resulting information to farmers, extension services and legislation. Soil sampling agencies provide farmers with information about the presence/absence of nematodes and the number of nematodes found by collecting and analysing soil samples from agricultural fields. The system can be used to:

- generate maps for soil samplers providing information on the location of the field, the field sections and the different sampling units.
- report to the farmers visualised sampling results.
- report to government the infested area (q-nematodes) and the calculated official buffer zone.

The system consists of three main parts:

- a web service for on line requests for a soil sampling effort. The field polygon can be either drawn by hand, downloaded from the government or uploaded from any file containing geographic information, e.g. a shape file.
- software to divide farmer fields into the appropriate number of areas to be sampled based on the constraints of the utilised method.
- Storage and retrieval of the data, visualisation of the results, presentation of overviews of the whole farm and the development of infestations in time.

The system can be used as input for decision support systems like NemaDecide. It can be used to generate task maps for the application of granular nematicides, or any other application helping the farmer to manage his nematode problem. It functions as a repository of farm data, normally only on paper and easily lost.

S6–P2

Q-bank – identification tool for quarantine nematodes and their close relatives linked to reference collections across Europe: II) Morphological approach

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With taxonomic expertise dramatically declining in the scientific community, fast and accurate identification of regulated plant-parasitic nematodes has become more and more difficult. In an attempt to conserve existing knowledge but also provide modern tools for nematode identification, Q-bank was started (www.q-bank.eu/nematodes). Q-bank comprises ecological, morphological, physiological and sequence data of properly documented populations of plant-parasitic nematodes providing plant protection organisations, inspection bodies and private laboratories the required information for accurate identification. To clearly distinguish regulated from non-regulated plant-parasitic nematodes, information of both groups of nematodes are equally important. Classical identification, based on morphological features, requires expert taxonomic skills. Pictures and morphometrics of important morphological features are included in Q-bank. The concept and structure of Q-bank is presented and discussed. Q-bank is freely accessible to all interested users and provides metadata which have been confirmed for correctness by curators and are linked to specimens in relevant physical, phytosanitary reference collections (e.g. JKI, ACW and PPS Wageningen), of which items as sequences, permanent slides and populations can be obtained. Q-bank is supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by QBOL, an EU-project with partners from 20 countries.

S6–P3

The impact of the new EU Potato Cyst Nematodes Directive in Scotland

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Since 1 July 2010 when Directive 2007/33/EC on the control of PCN came into force, implementation of the new statutory measures by the Scottish Government has resulted in an increase in the rate of pre-crop soil sampling of 120%. SASA has increased its capacity for soil testing by introducing new methodologies: automated cyst extraction, PCR diagnostics and a bespoke data management system. Growers now receive improved service and increases in the costs of soil testing have been minimised. The incidence of PCN detected by traditional methods based on visual examination and the new PCR diagnostic are compared and the consequences regarding the amount of land recorded as infested in future years is discussed.

S7 – Entomopathogenic nematodes: EPN biodiversity and genetics

Conveners: Christine Griffin & Ralf-Udo Ehlers

S07–T1

A novel EPN strain from Turkey with misleading signs of infection

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A new entomopathogenic nematode isolate was recovered from Turkey. It readily infects and kills larvae of the waxworm, *Galleria mellonella*, which turns greenish-silver. The tissue consistency of the cadaver is similar to waxworm larvae killed by a *Steinernema* species. That is, the tissues are not as gummy as observed in a *Heterorhabditis*-killed insect. Morphological methods and DNA sequence analysis were used to identify this new nematode isolate. The data showed that the nematode belongs to genus *Heterorhabditis*. Molecular and biochemical techniques were also used to identify the symbiotic bacterium. Results showed the bacterium belongs to the genus *Photorhabdus.* This interesting new nematode isolate and its mutualistic bacterium differ markedly from the usual signs associated with a typical *Heterorhabditis*-killed insect which normally turns the cadaver red and the tissues gummy. We hypothesise that the *Photorhabdus* bacterium is a new strain and/or mutant associated with this new Turkish *Heterorhabditis* isolate.

S07–T2

Regional entomopathogenic community structure and biological control in Florida citrus orchards

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The weevil *Diaprepes abbreviatus* is a serious pest of citrus in Florida's flatwoods eco-region where populations are larger and tree damage greater than on the central ridge. Regionality of damage by the insect might be due to natural enemies because sentinel weevil larvae were killed by native entomopathogenic nematodes (EPNs) at higher rates when buried in orchards on the central ridge than in those in flatwoods. However, EPN numbers in soil samples from 53 orchards estimated by qPCR did not differ between regions and tend to be highest in flatwoods. Among seven EPN species detected, population dominance by the 4 most frequently encountered species differed between regions (P=0.05). We are investigating effects of EPN species diversity (highest on central ridge), species dominance and behavior in different soils to explain the observed spatial patterns of *D. abbreviatus*. In greenhouse studies, weevil larvae were killed at significantly higher rates (P=0.006) by *Steinernema diaprepesi* (dominance highest on central ridge) than by *Heterorhabditis indica* (dominance highest in flatwoods). Both species killed weevils more effectively (P=0.001) in a central ridge sandy soil than in a flatwoods sandy soil. The effects of species and soils on weevil mortality measured after 7 days are small relative to weevil demographics in the two regions. However, over longer intervals (weevils reside in soil for several months) the rates we measured would result in large regional differences in weevil survivorship.

S07–T3

Desiccation tolerance of *Steinernema* spp. and its genetic improvement in *S. feltiae*

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Dauer juveniles (DJs) of *Steinernema* spp. were screened for desiccation tolerance. Desiccation tolerance was assessed in different concentrations of the non-ionic polyethylene glycol (PEG_{600}). The dehydrating conditions were measured as water activity (a_w-value). The lower the a_w-value is, the higher the desiccation stress. Adaptation to desiccation stress enhances tolerance and tolerance was therefore assessed before and after adaptation. The top three species with the greatest desiccation tolerance of all were *S. carpocapsae, S. abbasi and S. arenarium* and the species with poorest desiccation tolerance were *S. glaseri and S. ethiopiense*. The lowest a_w-value tolerated by only 10% (WA_{10}) of the population for non-adapted nematodes ranged from 0.62 to 0.93 and from 0.38 to 0.86 for adapted nematode populations. We also investigated the desiccation tolerance of 24 different strains of *S. feltiae*. The lowest WA_{10} of non-adapted nematode populations ranged from 0.78 to 0.93 and from 0.66 to 0.88 for adapted nematode population. There was no correlation found between non-adapted and adapted nematode populations. Six most tolerant strains (non-adapted and adapted) were crossed and the WA_{10} of the hybrid HYB7 tolerated an a_w-value of 0.43. HYB7 was further subjected to genetic selection steps. After six selection steps the WA_{10} of HYB13 was 0.83 for non-adapted and 0.64 for adapted populations. The LD_{50} value tested with cocooned larvae of *Cydia pomonella* was 19.6 DJs/insect for HYB13 and 37.9 for the commercial strain. The most productive strain was HYB13 with 23,450 DJs/insect.

S07–T4

Effect of in- and outbreeding on desiccation and heat tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora*

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Desiccation and heat tolerance of *Heterorhabditis bacteriophora* have been genetically improved through genetic selection and hybridisation. However, the improvements in these traits were rapidly lost when nematodes were sub-cultured in insects. Therefore a method to stabilise success of breeding for desiccation and heat tolerance was developed. Propagation in liquid culture prevents copulation of male and female and offspring e only from self-fertilising hermaphrodites. As a result highly homozygous inbred lines are obtained. Nematodes were propagated *in vivo* in *Galleria mellonella* or in *in vitro* liquid culture. When exposed to selection pressure for desiccation and heat between each reproduction cycle, the tolerance increased in *in vivo* and *in vitro* batches. But when selection pressure was released, the gained tolerance was lost again during *in vivo* production, whereas the tolerance was maintained at a high level when nematodes were cultured in liquid culture. During *in vivo* propagation without selection pressure the breeding progress was lost. Transfer of homozygous inbred lines from liquid culture to *in vivo* conditions, allowing crossing of amphimictic adults, increased desiccation tolerance of the heterozygous progeny (heterosis effect). In contrast, heat tolerance of the heterozygous offspring was lower than that of the homozygous population (out-crossing depression or trait deterioration).

S07–T5

Transcriptome analysis of desiccation and heat tolerance of entomopathogenic nematodes

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Entomopathogenic nematodes (EPNs) in nature are exposed to extreme environmental stresses such as desiccation and heat. A substantial increase in survival at high temperature was achieved when EPNs were pre-exposed to high relative humidity or mild heat. An adaptation period is also needed for acquisition of the anhydrobiotic state, which allows survival under desiccation. We focused our studies on the adaptation period of these two important stresses affecting nematode survival in *Steinernema* species, using whole transcriptome expression analysis of anhydrobiotic and heat stress related genes. For this purpose we used 454 pyrosequencing on three different nematode strains, *Steinernema feltiae* strain IS-6 (SFG), *S. feltiae* Carmiel strain (SFCar), and *S. riobrave* (SR). The 454 sequencing run obtained 26 to 50 million sequences per sample, 67,000—123,000 passed filter, averaging 374±12.6 bp. There were *ca* 370,000 reads that were used for the assembly in all samples. We obtained 9274 uniqe transcripts that were functionally classified using Gene Ontology (GO) hierarchy. Transcripts showed highest similarity (BLAST top-hit score) to the parasitic nematodes *Loa loa* (23.6%) and *Brugia malayi* (20.5%), *Caenorhabditis elegans* (14.2%), *Caenorhabditis briggsae* (12.6%), *Caenorhabditis remanei* (11.6%) and other non-nematode species (17.4%). Analysing gene expression patterns revealed an inverse correlation between gene expression and the phenotype of desiccation tolerance. SFCar was the most susceptible to desiccation and heat and had higher percentage of upregulated genes, while the stress-tolerant SR and SFG had higher percentage of downregulated genes. At the moment bioinformatics on the gene expression is still in process, and further analysis will be presented in the future. This is the first report of next generation sequencing of EPNs.

S7–T6

Genetic selection for low temperature activity in *Steinernema feltiae*

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Steinernema feltiae are used to control overwintering larvae of the codling moth *Cydia pomonella*. Application is in autumn and efficacy can often be limited due to low temperature. Twenty-two strains of *S. feltiae* were screened for their low temperature activity. Dauer juveniles (DJs) were pre-adapted to low temperature at 4°C for 24 h and then exposed to 2, 5 and 8°C. The temperature at which 50% (AT_{50}) and 10% (AT_{10}) of the DJ population were moving ranged between 2.5 and 6.3°C and 0.98 and 3.5°C, respectively. The most active strain at low temperature with an AT_{50} of 2.5°C was a strain from Finland. The least active strains was from Israel (AT_{50} = 6.3°C). The AT_{10} ranged from 0.98°C for the Finnish strain to 3.5°C for the Israeli strain. The AT_{50} and AT_{10} of the commercial strain were 3.2°C (rank number 5) and 1.5°C (rank number 5), respectively. The most active strains are currently being crossed, and selective breeding is being used to further enhance activity at lower temperature.

S8 – Biodiversity and evolution

Convener: Hans Helder

S8–T1

Genetic mechanisms for developmental plasticity and evolution of teeth in Diplogastridae

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Teeth are an evolutionary novelty of Diplogastridae and have enabled predation of other nematodes. In many species, including the model *Pristionchus pacificus*, the teeth are part of a stomatal dimorphism, of which the more complex (eurystomatous, "Eu") form shows a claw-like dorsal tooth and an opposing subventral tooth. To study developmental mechanisms driving the evolution of these structures, we integrate natural history, phylogenetics, and genetic analyses. We surveyed 27 new and described *Pristionchus* species to find and map changes of the dimorphism phenotype in macroevolution. We screened for natural variation (microevolution) among the hundreds of worldwide isolates of *P. pacificus* and found multiple strains highly biased toward one or other form. Genome-wide association will correlate mutations with phenotypic differences. Finally, we used forward genetics to isolate mutants that are Eu-form-defective (*eud*). A dominant mutant with several alleles, *eud--1*, was rescued by genetic transformation with a wild-type allele, suggesting haploinsufficiency. We tested if hemizygosity for this X-linked gene could explain the low-Eu phenotype of wild-type males: extra copies of *eud-1* could indeed induce Eu males, indicating a role for the gene in sexual dimorphism. Experiments show that over-expression of *eud-1* is sufficient for a higher frequency of the Eu form in both sexes. Findings are the first to articulate a genetic basis for a morphological dimorphism. Because the dimorphism is correlated with a structural innovation, study of the system can test the role of developmental plasticity in the evolution of novelty.

S8–T2

Revisited chromadorean phylogeny based on complete mitochondrial genome sequences

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The clade Chromadorea is an ecologically diverse and economically important nematode class. This monophyletic group includes a diverse array of free-living forms in soil, freshwater, and marine environments, plus many plant and animal parasites. Recent phylogenetic hypotheses for chromadorean nematodes have almost exclusively been based on nuclear ribosomal DNA genes, mainly SSU rRNA. However, relationships among major chromadorean lineages defined in the earlier SSU trees have been challenged by an alternative source of sequence data from complete mitochondrial genomes. The diversity of currently sampled mtDNA for chromadorean nematode species permits comparison with phylogenies based on nuclear SSU sequences. With respect to parasites of vertebrates, differences include the lack of monophyly for representatives of clade III (e.g., Ascaridida, Oxyurida, Spirurida) in mtDNA trees. Evaluations of alternative trees (representing the conflicting gene tree) are often significantly worse interpretations of these datasets. This indicates that there are strongly supported, but different relationships supported by these different loci. Such differences are not unexpected because these results are based on only two independent loci and each has known shortcomings for tree inference. In this presentation, some updated mitochondrial genome phylogeny will be discussed in more detail.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0021692).

S8–T3

Insight into phylogenetic relationships among nematodes based on a phylum-wide molecular framework of ~ 2500 full-length small subunit ribosomal DNA sequences

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For decades nematodes systematics has been unstable. This can in part be attributed to the limited number of informative morphological characters. Another major factor underlying the volatility of nematode systematics is the extensiveness of convergent evolution; it is hard to find any characteristic that has not arisen at least twice during evolution. These issues can be solved in part when a switch is made towards molecular data as this gives access to inexhaustible numbers of characters. Most likely, nematodes arose during the Cambrian explosion about 530 MYA. Keeping the ancient nature of the nematodes as a group in mind, a relatively conserved gene should be selected for phylogenetic reconstruction. Two of the ribosomal DNA genes, the small and large subunit (SSU and LSU) rDNA, could be considered for phylum-wide analysis. However, the LSU rDNA (D1—D3) was shown to be too diverse for this purpose; it was almost impossible to align partial LSU rDNA sequences from Tylenchida (Clade 12) and Dorylaimida (Clade 2) (Helder, unpublished results). So far, the SSU rDNA gene is the only target gene that can be amplified easily with standard universal and nematode specific primers and properly aligned. Over the last few years, we hahave collected, identified and sequenced a large number of mainly terrestrial and freshwater nematode species. This data set was supplemented with all publicly available animal-parasitic and marine nematode sequences, and the results and implications of an overall phylogenetic analysis will be presented. Special attention will be paid to Clade 1, the most basal nematode clade harboring mainly representatives of the orders Enoplida and Triplonchida, and the most distal Clade 12, a clade that includes the most of the economically relevant plant-parasitic nematode species.

S8–T4

Soil nematode communities as influenced by some agronomic techniques to control sugar beet cyst nematode in long-term experimental fields

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The effects of long-term fertilisation regimes such as the combined application of manure with nitrogen fertilisers on plant-parasitic and free-living soil nematode communities were evaluated on continuous culture of sugar beet and crop rotation (maize, wheat and sugar beet) of experimental fields of RIFC "Selectia" of ASM. Significance of comparative treatment effects were observed on the base of abundance, species diversity of nematode communities and functional guilds combining feeding groups and life strategy. Forty four species of plant-parasitic and free-living nematodes were encountered with different population densities and the number of eggs and second-stage juveniles of *Heterodera schachtii* depending on the different agronomic treatments. In the continuous plots the number of *H. schachtii* eggs and second-stage juveniles exceeded the threshold of economic damage and endoparasitic species *Pratylenchus pratensis, P. subpenetrans* and ectoparasitic species *Paratylenchus nanus* were also numerous, causing losses of sugar beet crop yield and effects on quality. Long-term crop rotations including wheat and maize in combination with organic manure and nitrogen fertilisers effectively reduced the population of *H. schachtii* below the damage threshold. In the experimental plots with fertilisers the nematode bacterivores *Cephalobidae, Plectidae* (guild Ba2) and fungivores *Aphelenchus*, *Aphelenchoides* (Fu2) were numerous, thereby suppressing plant-parasitic species *Hoplolaimidae* (*Heteroderinae*), *Pratylenchidae* (PP3) and partly *Anguinidae,* and *Dolichodoridae* (PP2). The long-term application of fertilisers and crop rotation changed the species structure of nematode communities and decomposition pathway.

S8–T5

Ditylenchus dipsaci races: variable populations or on-going speciation?

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Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936) causes considerable economic losses to, for example, flower bulb crops and onion even at low numbers. Their quarantine status is partly due to their ability to survive in soil for many years. Within this nematode species biological "races" are distinguished that are defined on the basis of their host ranges. These host ranges can be relatively broad such as the "tulip race", or narrow such as the "hyacinth race". To some extent these host plant preferences are reflected in ribosomal DNA sequence data. For the *D. dipsaci* hyacinth race there are indications for a genetic basis for its host preferences (unpublished results). No recent data are available concerning morphological differences between individuals of races in flower bulbs and other host plants. At the moment, differentiation of races of *D. dipsaci* in flower bulbs relies on host plant tests which take many months to perform. In a search for race-specific characteristics, about 44 different morphological parameters of 10—15 adult males and females, each sampled from populations *D. dipsaci* from infected Phlox, tulip and onion, were measured. Using four different morphometric parameters from male and female individuals the Phlox race could be distinguished from the tulip and onion races. Differences in AFLP patterns were found within 30 different *D. dipsaci* populations with two main groups: the tulip/daffodil populations on one hand and the hyacinth populations on the other. However, as new populations of *D. dipsaci* were assessed, no significant morphometric differences between individual *D. dipasaci* nematodes of both sexes were found. These conflicting results will be discussed in relation to the ongoing speciation in the *D. dipsaci* species complex.

S8–P1

Response of plant-parasitic nematode communities to land-use changes: The case of a heath—forest—crop succession

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Plant-parasitic nematodes (PPN) are major pests on carrot in the Landes region (South-West France). This region has been subjected to successive land-use changes: the pine *Pinus pinaster* was introduced 300 years ago in a heathland; maize has been established for 60 years in some places after deforestation; rotations with carrot were introduced 15 years ago. Surveys led to the detection of 55 PPN species. Although dominated by *Rotylenchus* species, communities observed in the natural ecosystems show a high regional diversity, mainly due to plant and soil diversity in forests. In less than 7 years-old maize crops, community patterns are close to those observed in forests.However, they are extremely homogeneous (no regional diversity) in older maize crops and dominated by *Pratylenchus* and *Paratrichodorus* species. In maize—carrot rotations, carrot leads to richness erosion and increases *Pratylenchus* spp. populations. Laboratory diachronic analyses in non-disturbed soil columns with native communities confirmed that *Paratrichodorus* populations proliferate under maize cultured in forest or maize soils,while carrot cultured in maize or carrot soils enhances *Pratylenchus* populations. Such land-use changes lead to new PPN community structures that induce production damage; consequently, management strategies must be designed to pilot structures to less pathogenic communities (resilience).

S8–P2

Diversity of nematodes in arid biotope Valley of Oued Righ of Algeria

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This study of diversity and community assemblages of nematodes was conducted in a vegetable oasis of Oued Righ Valley. The results revealed a diversity of 24 taxa whose mean abundances vary with study sites.

The distribution of trophic groups in the valley showed a highly significant difference (P = 0.000, P <0.05). Bacterivores are the most abundant followed by phytophagous while predators, omnivores and fungivores are poorly represented. In the bacterivores group, *Rhabdidtis* is the most represented genus, whereas the phytophagous group is dominated by the root knot nematode *Meloidogyne*. The study of ecological indices Shannon Index (H'), richness, Wasilewska (IW) and Maturity (IM) shows that they vary according to station.

S8–P3

Plant-parasitic nematode communities in the Moroccan Argan biosphere and response to cropping anthropisation

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The Argan (*Argania spinosa* L.) forest is an 870 Kha relictual patchy forest localised mainly in the Centre-West of Morocco, enclosed between the Atlantic Ocean and the High and Anti-Atlas mountains (Souss-Massa region). The erosion of the Argan forest, especially due to anthropomorphic factors, is about 600 ha per year. The most important crops in the lowlands are vegetables and citrus orchards, which induce the development of plant-parasitic nematodes (PPN), especially *Meloidogyne* spp. and *Pratylenchus* spp. respectively. Their management commands more knowledge on structure alteration in PPN communities due to land-use changes from forest to crops. A survey conducted in spring 2011 revealed that the PPN communities analysed in the natural Argan forest are structured according i) to a longitudinal gradient depending on climate and on soil texture and ii) to the phylogeographical typology of *A. spinosa*. *Pratylenchus* and *Meloidogyne* species are present in the natural Argan forest. The land-use changes had significant effects on community patterns, leading to high amounts of these two damaging species. We can hypothesise that these PPN species occurred in the Argan forest before anthropic disturbances and that the land-use changes induced new community structures with high pathogenicity.

S8–P4

Anhydrobiosis and cryobiosis in the nematode *Panagrolaimus superbus*

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The genus *Panagrolaimus* contains many species that occupy diverse niches ranging from polar and subpolar regions, temperate and semi-arid soils to terrestrial mosses. We show that Arctic and Antarctic species of *Panagrolaimus* are members of a cryobiotic clade that also contains strains from temperate and continental regions. Members of this clade are capable of surviving freezing to -80°C when fully hydrated in water and they also have strongly anhydrobiotic phenotypes. Outside of this cryobiotic clade the other *Panagrolaimus* taxa in our dataset are either freezing-sensitive or weakly freezing-tolerant, although many of these latter strains are anhydrobiotic.Tissue extracts from all strains were tested for ice binding activity and we found that extracts from the cryotolerant clade generated hexagonal faceted ice crystals whereas the extracts from the other panagrolaimids generated circular ice crystals – evidence that the cryotolerant strains may contain ice binding proteins to control the growth of ice at temperatures below freezing, as has been previously shown for *P. davidi* from Antarctica (*Cryobiology* 51, 198—207).Using a Bayesian relaxed-clock molecular dating method (PhyloBayes 3.0), we estimate that the mean divergence times for the Antarctic *P. davidi* and the sub-Arctic *P. superbus* from their closest temperate congeners are 17 Mya and 10 Mya respectively.

S9 – Quarantine nematology: PWN

Convenors: Manuel Mota & Christer Magnusson

S9–T1

Construction of recombinant inbred lines of the pine wood nematode, *Bursaphelenchus xylophilus*, and evaluation of their key phenotypes

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Pine wood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease. This nematode exhibits a wide range of intraspecific variation in several biological traits. Previous studies have provided meaningful observations on such variation, but nevertheless, key factors relevant to each of these traits are not clearly determined. For further detailed research, using genetically homogeneous lines of PWN in multifaceted approaches would be valuable.

In this study, as a new effective material, recombinant inbred lines (RIL) of PWN were constructed and some important phenotypes of traits were characterised. Two inbred lines, F7 and P9, which originated from conventional strains named OKD-1 (avirulent) and S10 (virulent), respectively, were used as parental lines. F7 and P9 have phenotypes extremely different from each other and their genetic homozygosity was confirmed using AFLP marker. By consecutive full-sib mating of these two lines until the F_{22} generation, a set of RIL consisting of 17 lines was established. In addition, we conducted bioassays to estimate key traits such as virulence, reproduction and transmission to the vector beetle of the newly obtained RIL. Based on this information, the inheritance pattern of each trait and correlative relationship between traits will be discussed.

S9–T2

Bacterial flora and its association with the pinewood nematode (*Bursaphelenchus xylophilus*)

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Pine wilt disease is caused by the pathogen, pinewood nematode (PWN) *Bursaphelenchus xylophilus*. Recently, it has been suggested that the PWN needs bacteria inhabiting its body surface for to infect and cause disease, although this hypothesis still remains a matter of debate. In this study, and in order to clarify the significance of such associated bacteria in pine wilt disease, we describe the bacterial flora on the nematode body surface and determine the potential pathogenicity of isolated bacteria to host pine trees. Studies on the bacterial flora were conducted using samples of PWN individuals taken from two differently affected forests in Japan, and from two different seasons. In each study, although the described bacterial flora was mainly composed of Enterobacteriaceae, the similarity of flora among samples was relatively low, consistent with preceding studies. In a pathogenicity assay, axenically grown pine seedlings were challenged with axenically grown PWN displaying relatively high virulence, and each of the associated bacteria obtained as described above, or both of them. As a result, inoculation of only axenic PWN caused host death as well as the combination of PWN and bacteria. Considering these results, the relationships of bacteria and PWN will be discussed.

S9–T3

On "non-vector transmission" of the pinewood nematode (*Bursaphelenchus xylophilus*)

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It has been argued that wood infested with the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, but in the absence of *Monochamus* spp., poses no threat to forests due to the lack of an abiological transmission system. However, this does not acknowledge the occurrence of "non-vector transmission", a phenomenon which has received little attention. In the literature, PWN has been reported to disperse readily through temporary stem grafts of *Pinus sylvestris*. Infection from soil to roots has occasionally been observed in small plants. In Japan, PWN was reported to infect *P. thunbergii* from nematode-infected wood discs buried in the soil. Another study has demonstrated an infection route from infested wood chips to wounded roots of *P. strobus*, *P. resinosa* and *P. sylvestris*, resulting in high mortality in the latter species. In *P. resinosa*, mortality was independent of root wounding, indicating a capability of PWN to infect non-wounded roots. In *P. sylvestris*, killed by pine wilt disease, PWN is abundant in roots. Root grafts occur in many species of pine. Through root grafts the PWN could spread on a local scale, since transmission of the nematode between pieces of wood has been demonstrated to occur within the first week of contact. The fact that the PWN may survive for 3 years in dead trees and for 1 month in soil, means that nematode-infested wood in forests could represent a risk of transmission over time.

S9–T4

Development of *Bursaphelenchus xylophilus*-specific microsatellite markers to assess the genetic diversity of populations from European forests

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934), Nickle (Nematoda: Aphelenchoididae) is the causal agent of pine wilt disease (PWD) and is currently considered as one of the most important plant pests and pathogens in the world. Its introduction and spread into new forest ecosystems has considerable economic and environmental consequences. Therefore, it is of crucial importance to identify its invasion routes, to determine the origin of new outbreaks and to understand the invasion process of this species to prevent further dissemination of the disease in Europe. In order to address these questions using population genetic approaches, we have been developing a set of PWN-specific microsatellite markers, usable in routine conditions at the individual level, thanks to multiplex PCR coupled with a fast DNA extraction method. Microsatellites were isolated from a genomic library using a procedure combining DNA enrichment and high throughput pyrosequencing as recently described by Malausa *et al*. (2011). Primers were designed for 71 and 23 perfect and compound microsatellites, respectively, 26 of which have been experimentally validated to date. Among them, 18 markers exhibited polymorphism after several rounds of amplification tests. Preliminary results on a set of 190 nematodes from 13 populations indicated a very low level of polymorphism in PWN populations from Portugal including Madeira Island, compared to populations from the native area in North America. The genotyping of a wide collection of samples from Europe, Asia and North America is currently underway in the laboratory. Assessing the genetic diversity of populations will determine whether the European invasive PWN populations are the result of a single or several independent events of introduction.

S9–T5

Morphological variation among new species of *Bursaphelenchus* in the '*xylophilus*' group

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The economic significance of the pinewood nematode, *Bursaphelenchus xylophilus*, as a devastating pest of pine in Far East Asia and its recent spread to new localities in Portugal and Spain together create a serious threat to coniferous forests in Europe. Precise identification of this nematode is essential for effective prevention and implementation of quarantine procedures. However, with the growing number of described *Bursaphelenchus* species, the morphological characters widely used at the first steps of wood sample examination and nematode identification have become ambiguous. This could be particularly important to packaging materials, for which the wood derived from a number of different tree species is frequently used. Here, not only the nematode species associated with conifers, but also those invading broadleaf trees should be precisely separated from the quarantine *B. xylophilus*. Our wide-scale screening of forest trees and comparative morphological and molecular study revealed a series of new, non-pathogenic *Bursaphelenchus* species in Europe and North America, which are associated with broadleaf trees and belong to the '*xylophilus*' group or are closely related to it. The ranges of their essential morphological and morphometric characters, such as shape of the female tail, vulval region, male tail and spicules, as well as position of the excretory pore overlap those reported for *B. xylophilus*. Thus, species identification based exclusively on morphological characters of nematodes extracted from potentially unidentified species of package wood could be difficult for a non-specialist. The ranges of variation of selected morphological characters examined in recently identified species of *Bursaphelenchus* from European aspen, trembling aspen, lime, and beech are discussed and compared with those of the pinewood nematode, *B. xylophilus*. This should alert the quarantine services of potential sources of *B. xylophilus* misidentification in packaging wood and encourage a wider use of molecular identification methods.

S9–T6

High-throughput RNA-sequencing of *Bursaphelenchus xylophilus*

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of the pine wilt disease, a condition responsible for devastation of pine forests in Portugal. Aiming to discover genes/pathways involved in the molecular mechanism of the disease, we pyrosequenced the transcriptome of seven PWN isolates from distinct locations: Portugal (4); China (1); Japan (1); and USA (1) and generated a digital transcriptome of *B. xylophilus*. The transcriptome assembly generated 16,297 transcripts, 10,776 with known protein domains and 7969 with Gene Ontology terms. The transcriptome information was organised in a database, where targeted searches can be conducted on transcripts, encoded amino acid sequences and gene annotations. Genes characteristic of PWN pathogenicity were identified in the transcriptome, namely cellulases, chitinases, expansins or venom allergen proteins and additional searches are undergoing to identify new genes. Validation of transcripts was done by RT-PCR of 7 genes involved in nematode development and reproduction, *lag-1, cdk-9, vit-5, gon-1, fbf-1, pha-4* and *ama-1*. Furthermore, the same genes were tested for differential expression in a Portuguese isolate grown in fungi and in pine, and between a Portuguese and USA isolates grown in fungi. We observed differential gene expression related to growth conditions, fungus *vs* pine, and to distinct geographic origins, probably reflecting different genetic backgrounds. Statistical analysis evidenced the upregulation of a transcription factor involved in metalloproteinases regulation, pinpointing this gene as target for future studies.

S9–P1

Reproductive competitiveness of the pine wood nematode *Bursaphelenchus xylophilus* and Polish isolates of *B. mucronatus in vitro*

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Bursaphelenchus mucronatus is morphologically and genetically closely related to *B. xylophilus*– the causal agent of the pine wilt disease. These two nematodes live in pine wood and have a close phoretic association with the longhorn beetle *Monochamus* spp. Moreover, they both occupy the same niche in the ecosystem. They have rarely been found on the same host, so there is a little information on a possible interaction between the two species. However, the probability of such interactions exists, particularly in regions newly colonised by *B. xylophilus*, where native populations of *B. mucronatus* are already present.

The main objective of this study was to examine the reproductive competitiveness of the pine wood nematode *B. xylophilus* and a series of Polish isolates of *B. mucronatus* (Mdz-01, NTo-01 and Wro-01) developing together in a common environment. The study was conducted *in vitro* on PDA–*Botrytis cinerea* cultures for 3 consecutive rearing cycles (10 days each). In the first cycle, for each *B. mucronatus* isolate experimental plates were inoculated with 100 adult nematodes (50 females and 50 males) in three combinations: (a) 50 individuals each of *B. mucronatus* and *B. xylophilus*, (b) 100 individuals of *B. mucronatus* alone and (c) 100 individuals of *B. xylophilus* alone. In the subsequent cycles 100 adult individuals (50 males and 50 females) were randomly withdrawn from the offspring of the previous cycle and transferred to fresh PDA–*B. cinerea* plates. Each expermental variant was performed in 10 replicates (plates), incubated at 24°C. Proportions of phenotypes of *B. xylophilus* (rounded female tail) and *B. mucronatus* (mucronated female tail) were evaluated in the offspring of the three successive rearing cycles. The experiments did not show significant superiority of any of the examined nematode populations. Both *B. xylophilus* and *B. mucronatus* were able to dominate the second species and partially eliminate it from the substrate in individual plates. However, numerically differences were observed among competitiveness of the examined populations of *B. mucronatus*. Interestingly, it was also found that the numbers of offspring nematodes obtained from cultures of *B. xylophilus* and *B. mucronatus* reared together were significantly greater than those from cultures where each of theses species was reared separately. However, the mechanisms underlying this phenomenon still remain unclear.

S9–P2

Survey of the pine wood nematode *Bursaphelenchus xylophilus* in France: synthesis of ten years data

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Bursaphelenchus xylophilus is a nematode of worldwide concern and a major threat for pine stands. In many countries it is listed as a quarantine pest. In the European Union, it has been considered as such in the directive 2000/29/EC. The non-European populations of its vector *Monochamus* spp. have also been regulated since 1990 (directive 90/490/EEC). In 1999, the detection of *B. xylophilus* in Portugal resulted in a supplementation of the EU legislation: in particular, since 2000, official annual surveys are required within the EU member states to allow early detection in the event of introduction of this nematode. In France, forests represent around 16..1 million hectares (29..4% of the territory) including around 4 million hectares of conifers. A current objective of 650 samples, to be increased to 1200 next year, is taken each year in declining pine stands or risk areas (locations around points of import, wood processing industries). They are analysed according to EU and EPPO recommendations (sampling regimes, handling, extraction and identification). Different pine tree species are monitored: *Pinus sylvestris, Pinus pinaster, Pinus nigra, Pinus halepensis.* Among the samples collected from 2002 to 2011, *B. xylophilus* has never been identified, although some other non-pathogenic and endemic *Bursaphelenchus* species (*B. mucronatus, B. tusciae,*…) were sometimes detected in the pine samples.

S9–P3

The effect of water stress and temperature on the pathogenicity of the pinewood nematode to *Pinus* spp.

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In order to understand the effect of water stress and temperature on the pathogenicity of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, to *Pinus pinaster*, *P. pinea* and *P. radiata*, PWN population densities were evaluated under high and low water availability conditions, at 25 and 30°C. A total of 120 five-year-old trees were grown in a greenhouse, under natural photoperiod and solar radiation. Trees were inoculated with 6000 nematodes and trees inoculated with sterilised water were used as control. The symptoms were followed for 50 days and the final PWN population was estimated in each tree at the end of the experiment, in the branches, trunk, roots and soil. Nematodes were detected in higher numbers in *P. pinaster* followed by *P. radiata* and *P. pinea*. In *P. pinaster* and *P. radiata*, nematodes were detected in all PWN inoculated trees, at the branches, trunk and roots while in *P. pinea* they were detected only in four trees, at the branches and trunk. PWN reproduction was higher under conditions of low water availability in conjunction with high temperatures, which suggest that water and temperature play an important role in the spread of PWN. This may have implications on the prediction of future enlargement of the infected area and on the shortening period of disease development under climate change scenarios.

S9-P4

Gene expression profiles of Japanese black pine after infection with the pine wood nematode, *Bursaphelenchus xylophilus*

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Pine wilt is one of the most serious worldwide forest diseases and is caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Although physiological and chemical changes following symptom development in host pine have been described in detail, the processes underlying development of pine wilt disease are not well known, especially at the initial stage of the disease. The present study compared the patterns of gene expression in host trees after infection by PWN with different virulences. For inoculation of susceptible Japanese black pine (*Pinus thunbergii*) seedlings a virulent isolate (S10) and an avirulent isolate (OKD-1) of PWN were used. 28 hours after inoculation, mRNA was extracted from the seedlings andused to generate a subtracted library enriched for genes differentially expressed in the seedlings exposed to virulent nematodes. Sequences were subsequently classified by Gene Ontology analysis. Genes which were expressed more in the S10-inoculated seedlings included those associated with anti-oxidant activity. In combination with the results of quantitative real-time PCR, it is suggested that virulent PWN may have a kind of system to evade and/or inhibit host defence responses at the initial stage of infection.

S10 – Entomopathogenic nematodes: research and use

Convenors: Itmar Glazer & Nick Berkvens

S10–T1

Parasitism of wheat stink bug, *Aelia rostrata* Boh. (Heteroptera: Pentatomidae) by *Hexamermis* sp. (Nematoda: Mermithidae)

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Wheat stink bug (WSB), *Aelia rostrata* Boh. (Heteroptera: Pentatomidae) is one of the most important pests of wheat and other Graminae in Central Anatolia. This pest overwinters as an adult under the plant leaves and debris or a few cm deep in the soil on the mountains or hills around wheat fields. Adults become inactive in aestivation and diapauses for about nine months in overwintering areas. During this period various natural enemies and entomopathogenic diseases could play an important role in reducing populations. A nematode species parasitic on *A. rostrata* belonging to *Hexamermis* genus has been reported for the first time in Turkey.The aim of this study was to determine the infection rate of *A. rostrata* with *Hexamermis* sp. (Nematoda: Mermithidae) in 2010 and 2011. For this purpose, adults of *A. rostrata* were collected from overwintering areas in Ankara, Turkey. Samples were brought to the laboratory and sexed. They were dissected in distilled water to check for the presence or absence of mermithids. Thus, the rates of parasitism were calculated individually for females and males of *A. rostrata*. Parasitism rates were 24.7 and 21.4% for females and 33.6 and 36.1% for males in 2010 and 2011, respectively. Parasitised WSB contained an average of 2.6 ± 0.72 nematodes. In a sample of 30 parasitised WSB, 63.3% contained a single worm, 16.7% contained two worms and 20.0% contained three or more worms. The mean body length of juveniles was 7.6 ± 0.24 cm for females and 3.0 ± 0.15 cm for males. The results suggest that *Hexamermis* sp. is the most important natural enemy of *A. rostrata* in overwintering areas and has potential as a biological control agent for WSB management in the future.

S10–T2

Enhanced efficiency of earthenware cups as compared to plastic containers in isolating infective juveniles of entomopathogenic nematodes from soil

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By convention plastic or glass containers are used to bait infective juveniles (IJs) of entomopathogenic nematode (EPNs) using trap insects. However, in tropical countries, with summer temperatures rising to 40°C and upwards, moisture levels in these containers are often seen to drop to levels that jeopardise the survival of IJs. To get around this problem, this study delved into tradition and discovered an eco-friendly alternative in the *kullarh* or earthenware cup commonly used in India to hold liquids: the *kullarh* could equally well serve as an efficient receptacle for holding soil in baiting experiments. Due to its porous nature earthenware maintains stable levels of moisture and temperature. To compare their ability to sustain moisture and their baiting potential, sterilised soil with 15.3% moisture, infected with either *Heterorhabditis indica* or *Steinernema glaseri* at 0.5, 1 and 5 IJs/soil, was taken along with the trap insect *Galleria* in both plastic and earthenware cups. The earthenware cups were placed in a tray filled with water just sufficient to submerge their bases. The porosity of the cups induced a capillary action wherein water rose up the sides to ensure a uniformly moist environment for the duration of the experiment. However, the moisture level in plastic containers gradually declined to 3.4% by the fourth day. This variation in moisture content in both types of cups was reflected in the mortality statistics of the trap insects, in terms of the numbers killed and the time taken to inflict mortality: earthenware cups exhibited a clear advantage over plastic cups, especially in soils with low and medium densities of EPNs. This can perhaps be attributed to the fact that earthenware closely replicates the nematodes ecological niche.

S10–T3

Movement of entomopathogenic nematodes in soil moisture gradients

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The natural habitat for entomopathogenic nematodes (EPN), the soil, is a difficult environment for persistence considering its complexity of physical, chemical and biological components. These beneficial organisms are especially vulnerable to dehydration. Some EPN species are able to survive dry conditions in the soil by entering into a dormant state known as "anhydrobiosis". However, *Heterorhabditis bacteriophora*, which is a poor anhydrobiont, was detected in soil a year after application in regions characterised by a dry season. In the present work we verified the possibility that dauer juveniles (DJs) of this species move through the soil along the moisture gradient and establish at deeper layers which contain sufficient moisture to allow nematode survival throughout the year. This was done by placing DJ of three EPN species on top of moist soil (12% w/w) and following their distribution as the soil dried in 25 cm sand columns. Samples were taken every 5 cm depth 2, 14 and 28 days after nematode application. The results suggest that, EPN species with poor anhydrobiotic capability such as *H. bacteriophora* moved toward deeper and moistened soil layers while better anhydrobionts such as *Steinernema carpocapsae* and *S. feltiae* remained at higher proportion (>70%) in the upper layers of the soil. The implications of the results will be discussed.

S10–T4

Efficacy and persistence of entomopathogenic nematodes for controlling key pests in Israel

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The use of entomopathogenic nematodes (EPN) against important pests in Israel has been evaluated, over the past three years, in field trails. This presentation describes the results of using EPN for control of *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) in peanuts and the buprestid beetle *Capnodis tenebrionis* L. in stone fruit orchards.

White grubs of *M. matrida* are major soil pests of agricultural crops causing substantial damage to ornamentals, peanuts and sweet potatoes. Three trials were conducted in peanut fields during the growing season (April to October) of 2009—2011 in the north-western "Negev" region of Israel. We evaluated the efficacy and persistence of commercial EPN products by Koppert Co., Holland. The nematodes were applied using different application methods: spray, irrigation and soil injection. The presence of nematodes in the soil was evaluated using '*Galleria* traps'. The effects of the various treatments on yield and damage to the peanuts were determined at harvest time. Application of *Heterorhabditis bacteriophora,* resulted in 80% reduction in damage to the peanuts with no effect on the yield.

Larvae of *C. tenebrionis* invade and cause damage to the roots. Trees can be rapidly killed by this destructive pest. The objective of the current study was to test the efficacy of the EPN in control of *C. tenebrionis* larvae inside and outside the tree root system. The experiments were conducted during 2011—2012 in a commercial plantation covered with insect proof netting that was deliberately infested with fertile adult beetles. Nematodes (*Steinernema carpocapsae, S. feltiae* and *H. bacteriophora*) were applied at rates of $3\times\times10^6$ or $1\times\times10^6$ infective juveniles per tree in a drench around the trunk of trees. Throughout the study, nematode survival in the soil was estimated using nematode traps in soil samples collected around the trunk. The initial results indicate substantial reduction of insect infestation by 70––80%.

In all trials, nematodes appeared to be active throughout the entire growing season. Towards the end of the season, nematode activity was also detected in the un-treated control plots.

S10–T5

Biological control of the woolly apple aphid (*Eriosoma lanigerum*) using entomopathogenic nematodes

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The woolly apple aphid (WAA), *Eriosoma lanigerum,* has become a major pest in apple orchards in Western Europe after restriction of effective insecticides. We evaluated the efficacy of commercially available species of entomopathogenic nematodes (EPN) (*Heterorhabditis bacteriophora*, *H. megidis*, *Steinernema carpocapsae, S. feltiae*, *S. glaseri* and *S. kraussei*) to control WAA. Only *S. carpocapsae* caused significant mortality in screening experiments in multiwell plates (between 20% and 40% mortality). Adding surfactants to the nematode suspension, to reduce the water-repellent properties of the cuticle and woolly structures of the aphids, did not improve control by EPN. In laboratory and semi-field experiments, parasitisation rates of WAA by EPN were frequently higher than mortality rates, as insects were often parasitised but still alive. Parasitisation rates of the aphids even increased 3 days after treatment, while mortality remained the same. Presence of EPN had no effect on aphid reproduction as numbers of embryos in parasitised and non-parasitised females were similar. Treating the aphids with the plant-parasitic nematode *Pratylenchus thornei* in the multiwell plates led to a similar mortality as applying *S. carpocapsae*. These observations indicate that mortality caused by *S. carpocapsae* to WAA throughout the study was probably caused by a factor non-specific to EPN application, e.g. excess stress. This finding warrants careful interpretation of mortality observed in artificial conditions. Hypotheses for the lack of immediate death following successful parasitisation of WAA by *S. carpocapsae* will be discussed.

S10–T6

Control of the Western Corn Rootworm with *Heterorhabditis bacteriophora*

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The entomopathogenic nematode *Heterorhabditis bacteriophora* has been tested successfully against larvae of the Corn Rootworm (*Diabrotica virgifera virgifera*) for the last 5 years in Hungary, Austria and Italy. When applied at a dose of 1×10^9 nematodes per ha the results have been comparable to those obtained with chemical seed dressing with neonicotinoids or application of granular insecticides containing the pyrethroid Tefluthrin. At the higher dose of 2×10^9 the results were more stable at control between 70 and 90%. Although the differences are minor, in comparison to chemical insecticides the nematodes usually provided higher reduction of adults whereas less root damage was recorded for chemical insecticides. The effect of nematodes is equally high whether applied during sowing of the maize or at occurrence of the larvae approximately 6 weeks later. Different application techniques have been tried. Seed dressing and granular application often caused problems under commercial conditions. Liquid applications into the drill with 200—400 litre water have provided optimal conditions for nematode establishment and persistence until the occurrence of the larvae. Article 55 of the new EU regulation 1107/2009 on the placement of plant protection products on the market explicitly implies the promotion of the use of non-chemical and natural alternatives. Directive 2009/128/EC aims to achieve the sustainable use of pesticides. Article 14 lines out that "the Member States shall take all necessary measures to promote low pesticide-input pest management, giving wherever possible priority to non-chemical methods, so that professional users of pesticides switch to practices and products with the lowest risk to human health and the environment". The biological control industry is preparing to supply the markets with the necessary amounts of the entomopathogenic nematode *H. bacteriophora*. In 2012 the first product (Dianem®) based on this nematode was introduced.

S10–P1

Occurence of Entomopathogenic Nematodes in Turkey

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This study was conducted during 2006 and 2010, with the aims of surveying and determining entomopathogenic nematode fauna and investigating the phylogenetic relationships based on ITS of rDNA and sequence analyses of D2—D3 regions. The study areas consisted of all 81 cities in Turkey. Approximately 10—20 soil samples were taken from each city between May and September. In total, 1552 soil samples were collected in two years. By using the common suitable host, *Galleria mellonella,* 67 entomopathogenic nematodes were isolated from the soil samples. The ratio of entomopathogenic nematode recovery from soil samples was found to be 4.3%. We determined that 33 (49.2%) of the positive isolates belonged to the genus *Heterorhabditis* and 34 (50.8 %) isolates belonged to the genus *Steinernema*. Recovered isolates were identified as *Heterorhabditis bacteriophora*, *H. megidis*, *Heterorhabditis* sp., *Steinernema affine*, *S. carpocapsae*, *S. feltiae* and *Steinernema* sp.

S10–P2

Morphological and molecular study on nematodes associated with insects in the Mashhad area of Iran

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The entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae are insect biocontrol agents. The identification of this group is an important issue for their use in pest management programs. Here, we examined entomopathogenic nematodes and free-living species from the Order Rhabditida associated with insect species in the Mashhad region, during 2009—2011. The samples were taken from different habitats. Among 100 soil samples, seven samples were positive for above mentioned species detected using a *Galleria* trap. Four out of seven samples belonged to the genus *Steinernema*. Morphological and morphometric characters of adults and infective juveniles of first and second generations showed that those four isolates are members of the "*feltiae*" species group. Analysis of rDNA sequences of ITS and 28S genes, as well as phylogenetic analysis on these isolates, confirmed that these four populations are isolates of *Steinernema feltiae,* although the morphological features are quite consistent with the original description provided for the species. The remaining three isolates were characterised using morphological and morphometric data as *Acrobeloides* sp., *Cruznema tripartitum* and *Diploscapter coronatus*, which are free-living and bacteriophagous species. Phylogenetic position, measurement and illustration are provided for the species.

S10–P3

Specificity of five qPCR-assays for the detection of different species of entomopathogenic nematodes

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Entomopathogenic nematodes (EPN) are applied as biocontrol agents for several insect pests worldwide. Knowing the exact identity of EPN can be important in a risk assessment context when new strains are introduced in the environment or when their persistence needs to be investigated. The exact species identity of the applied (or retrieved) EPN or the purity of commercial nematode cultures can only be verified by trained nematologists. They rely on microscopic observations which are often supplemented by DNA-sequencing, as morphological identification is not always straightforward. This combination of techniques is only applicable on a small-scale basis as it requires skilled people and is time-consuming. However, several qPCR assays for detection and quantification of EPN have been published recently. This technique can be used in high-throughput analyses. We testedfive published qPCR-assays for *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *S. feltiae*, *S. glaseri* and *S. kraussei* for their specificity in detecting and identifying several strains of EPN. DNA was extracted from nematodes belonging to ten Belgian commercial strains and nine reference EPN populations belonging to six species. The qPCR method was performed as described in the publications. Results confirmed that each assay detects the EPN-species for which it was designed. However, some false positive results were recorded. This observation could be due to several reasons which will be discussed. It also demonstrates that qPCR assays need careful preliminary evaluation before being implemented.

S10–P4

Studies on co-existence of *Steinernema siamkayai* and *Pseudomonas aeruginosa* for sustainable crop improvement

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In this study an attempt was made to evaluate the plant growth-promoting rhizobacteria (PGPR) (*Pseudomonas aeruginosa*) and the entomopathogenic nematode (EPN) (*Steinernema siamkayai*) for coexistence under laboratory conditions. *Pseudomonas aeruginosa* was determined as a potential PGPR by plant growth promoting activities — NH_3 production, seed germination assay, indole acetic acid production (IAA), HCN production, antibiotic production, antifungal activity, protease activity, chitinase activity, ACC deaminase activity, siderophores production, and phosphate solubilisation. *Steinernema siamkayai* was also assessed for its infectivity with ecological characterisation using *Galleria* as model. In addition, survival ability was assessed with *P. aeruginosa* culture medium, and *S. siamkayai* associated bacteriawere assessed for antimicrobial activity. In our observations, *S. siamkayai* was not affected by PGPR but symbiotic bacteria have a negative impact on PGPR. In the seed germination assay *S. siamkayai* acted as a vector for transporting *P. aeruginosa* under culture conditions. In potting medium using a combination of *P. aeruginosa* and *S. siamkayai*, enhanced seedling vigor was associated with increase in overall biochemical parameters. Hence, integration of selective PGPR and EPN may improve crop production, protection, soil biological, chemical and physical processes and also preserve the pristine soil fertility.

S11 – Plant-parasitic nematodes in subtropical crops: CCN

Convenors: Bjorn Niere & Julie Nicol

S11–T1

Occurrence of nematodes of the *Heterodera avenae* group and *Pratylenchus* spp. on wheat and barley in Morocco

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The occurrence of cereal cyst nematodes (CCN) and root lesion nematodes (RLN) in various locations in major cereal cultivating areas in Morocco was investigated. A total of 75 soil and root samples were collected from fields in Gharb, Sais, Zaers and Chaouia before the wheat and barley harvest (May to June) in 2011. Cysts were extracted from soil using flotation and decanting through 200 µm sieves. Vermiform stages were extracted from roots and soil using an automated zonal centrifuge. Nematodes were identified up to species level using morphological and molecular methods. The survey revealed that 66%, 68%, 80% and 65% of wheat and barley samples in the Gharb, Sais, Chaouia and Zaers regions, respectively, were infested with *Pratylenchus thornei, P. penetrans* and/or *P. pseudocoffeae.* Densities of mobile stages of RLN in wheat fields ranged from 32 to 123/100 g soil and from 76 to 102/10 g of root. In barley fields densities of mobile stages were similar, ranging from 6 to 112 /100 g soil and 67 to 102 /10 g of root. Cereal cyst nematodes were found in 16% of the soil samples. CCN were identified as *Heterodera avenae* in all provinces, except for one province (Sais), where *H. latipons* was also found.

S11–T2

Variation in reproduction and damage potential of Egyptian populations of *Heterodera avenae* on different wheat varieties

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The cereal cyst nematode *Heterodera avenae* causes significant economic losses in wheat worldwide. Wheat cultivars with resistance to *H. avenae* exist and may be used to control cereal cyst nematodes. Several pathotypes have been reported for *H. avenae,* while the use and effectiveness of resistant wheat cultivars varied according to the virulence phenotype of the nematode population. Recently, *H. avenae* has been reported infecting wheat fields in Egypt, while no information is available on virulence and reproduction potential of the populations present. There is also no information on the resistance of Egyptian wheat genotypes to *H. avenae* populations. This study was carried out to characterise the virulence of six *H. avenae* populations (five Egyptian populations and one German population) based on their reproduction on different wheat cultivars and to assess the resistance levels in nine Egyptian wheat cultivars compared with five wheat genotypes from the International Test Assortment (NordGen, Alnarp, Sweden). In addition, the relation between increasing initial population densities (*Pi*: 0, 500, 1000 and 2000 second-stage juveniles/100 ml soil) of *H. avenae* and the responses of different wheat cultivars were studied. Data on final population densities, reproduction factor and the damage potential of *H. avenae* on several wheat cultivars growth parameters (shoot dry weight, root dry weight, spike weight and grain yield) are presented. Regression analyses were performed on the data to describe the relation between the nematode reproduction factor and different plant growth parameters. Populations from El Shark (west Sinai) and El Kasasen showed the highest reproduction factors (*Rf*) on wheat cultivars while the lowest were recorded for the populations from Abu Suwayr and Abu Khalifah. The lowest reproduction of nematode populations were reported on the wheat varieties LOROS X KOGA and AUS 10894, while all tested wheat cultivars from Egypt were found to be susceptible. Several plant growth parameters were suppressed by *H. avenae* populations; the highest reduction in grain yield was recorded by the population from El Shark on the Egyptian wheat varieties SAKHA 8 and SAKHA 61 with 33 and 32%, respectively.

S11–T3

Development of two species-specific primer sets to detect the cereal cyst nematodes *Heterodera avenae* and *Heterodera filipjevi*

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Twelve *Heterodera* species are reported to be of major significance in wheat and barley. Of these, *H. avenae*, *H. filipjevi* and *H. latipons* are among the economically most important ones. The identification of *Heterodera* species using morphological characteristics is time consuming, requires specialised skill and can be imprecise, especially when they occur mixed in field populations. Molecular techniques can provide a more accurate way for nematode identification. This study reports the results from targeting the mitochondrial cytochrome oxidase 1 (COI) gene to identify species-specific primers that could be used for the identification of *H. avenae* and *H. filipjevi*. The COI gene ofnine *Heterodera* species was partially sequenced and the resultant sequences were aligned to find unique sites suitable for the design of primers. The alignment showed variability between *H. avenae, H. filipjevi* and other *Heterodera* species. Two sets of species-specific primers were designed, followed by optimisation of PCR conditions for identification of both species. The specificity of the designed primers was checked on 13 *Heterodera* species, nine populations of *H. avenae* and ten populations of *H. filipjevi* originating from different countries. To test the sensitivity, the PCR was run with DNA extracted from five second-stage juveniles (J2) of *H. avenae* or five J2 of *H. filipjevi* mixed with DNA extracted from varying amounts of J2 of *H. latipons*. It was possible to detect as few as five J2 of *H. avenae* or *H. filipjevi* among 100 J2 of *H. latipons*. The two primer sets allow detection of *H. avenae* and *H. filipjevi* where they occur in mixed populations with other *Heterodera* spp.

S11–T4

Studies on the response of wheat lines (*Triticum aestivum*) to the cereal cyst nematode *Heterodera filipjevi*

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This study was carried out to analyse the response of diverse wheat populations to the cereal cyst nematode *Heterodera filipjevi*. Two hundred and eighty nine wheat lines were screened in controlled growth room trials at CIMMYT for their resistance potential to be included in an international wheat breeding programme. Two different susceptible wheat cvs, Bezostaya and Kutluk, and moderately resistant cvs, Katea and Sönmez, were used as controls. Various responses were detected and categorised intofive different groups according to the number of females and cysts developing per plant with the following results: in three lines (1%) there were very low numbers, in 49 lines (17%) there were low numbers, 109 lines (37%) there were medium numbers, in 99 lines (34%) there were high numbers, and in 31 lines (11%) there were very high numbers. The results also showed highly significant differences among the cyst size and number of eggs and second-stage juveniles (J2). Large cysts had higher number of eggs and J2 as compared to medium and small cysts. In wheat lines with high number of females the proportion of large females was high, whereas in lines with a low number of females most of the females were small. More details about the host—parasite interaction in selected wheat lines will be presented and discussed. The collection is currently genotyped with the Illumina chip technology and will be analysed for genetic markers with association mapping.

S11–T5

Wheat reaction to various population levels of lesion nematodes *Pratylenchus thornei* and *P. neglectus* in Iran

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A two-year experiment was established to determine the effect of various population levels (0, 10, 20, 40, 80, 160, 320, 640, 1280 and 2560/100 g soil) of *Pratylenchus thornei* and *P. neglectus* on wheat growth parameters in 2011 and 2012 under microplot conditions in Iran. Populations of *P. thornei* and *P. neglectus* were obtained from carrot disc cultures. Wheat seeds cv. Sardari were planted in clay pots containing 3 kg pasteurised soil and inoculated with different population levels after 7 days. Pots were placed in field conditions and maintained during the wheat growing season. Experiments were arranged in a completely randomised design with 10 treatments and replicated four times. After formation of spikes before and after full maturity growth parameters of plants including: fresh and dry weight of root and aerial parts, plant height, number of spikes and tillers, spike weights and nematode population in root and soil were recorded. Data were analysed and the means were compared with Duncan's multiple range tests. *Pratylenchus negle*ctus populations levels did not have any significant effect on wheat yield parameters compared with the control; however, high nematode population were counted in treatments 640, 1280 and 2560 in both soil and root samples ofof *P. thornei* treatments. *Pratylenchus thornei* significantly decreased wheat yield parameters, including number and weight of spikes, by 28.2% and 39.6% respectively.

S11–P1

Using SCAR-PCR techniques to identify root-knot nematode infecting Ismailia orchards, Egypt

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The root-knot nematode is a real threat and causes considerable losses to fruit orchards especially in sandy soils in Ismailia governorate, Egypt. Accurate identification of *Meloidogyne* species is essential for the development of suitable management strategies. Therefore, samples of banana and grape roots infected with root-knot nematode were collected from three different regions (Abu Khalifah, Abu Suwayr and Faculty of Agriculture Experimental Farm) in Ismailia governorate. Extracted females were used to identify detected root-knot nematode populations by using perineal patterns and SCAR-PCR techniques. Examination of the perineal patterns revealed the presence of three different species of *Meloidogyne*. Four root-knot nematode populations were identified as *M. incognita* from banana roots in Abu Khalifah region, from grape roots in Abu Suwayr region and from banana and grape roots in Faculty of Agriculture Experimental Farm. One root-knot nematode population was identified as *M. arenaria* from grape roots in Abu Khalifah region, while one root-knot nematode population was identified as *M. javanica* from grape roots in Abu Suwayr region. Sequence Characterised Amplified Region (SCAR) based on PCR assays and DNA Gel Documentation System (D.G.D.S) program analysis confirmed the previous results and easily differentiated the species *M. incognita, M. arenaria* and *M. javanica*. A 1200 bp fragmentwas detected in four root-knot nematode populations and identified as *M. incognita*. A 420 bp fragment was detected in one root-knot nematode population and identified as *M. arenaria*. In addition, a 670 bp fragment was detected in one root-knot nematode population and identified as *M. javanica*.

S11–P2

Determination of some *Cre* genes efficiency against the cereal cyst nematodes, *Heterodera avenae, H. filipjevi* and *H. latipons*

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The cereal cyst nematodes, *Heterodera avenae* group, are known as parasites of cereals and grasses. Surveys of cereal fields in Turkey have revealed that nematodes belonging to the *H. avenae* group occur throughout the country, and that *H. filipjevi* is the most common species, followed by *H. avenae* and *H. latipons*. The use of host-plant resistance is one of the most effective methods for controlling cereal cyst nematode species. Several wheat germplasm were obtained containing one of the resistance genes (*Cre* gene). In this study, the efficiency of some sources of resistance (*Cre* R, *Cre* 1, *Cre* 2, *Cre* 3, *Cre* 5, *Cre* 7 and *Cre* 8) in wheat against several *H. avenae*, *H. filipjevi* and *H. latipons* population was investigated *in vitro* in climate conditions resembling those in Turkey. Plants with *Cre* 1, *Cre* 3 and *Cre* 7 genes showed resistance against *H. avenae* populations. Plants with *Cre* R and *Cre* 8 genes and plants with *Cre* 1, *Cre* 3 and *Cre* 5 genes revealed resistance against *H. filipjevi* and *H. latipons* respectively. To conclude, *Cre* 1 and *Cre* 3 genes induce resistance against both *H. avenae* and *H. latipons* populations but no gene was found to show resistance against all three nematode species together. Some highlights of this work will be presented, showing genetic resources of value in cereal crop breeding programmes in Turkey.

S11–P3

Investigation of resistance against the cereal cyst Nematode, *Heterodera avenae,* in wheat germplasm

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The cereal cyst nematode, *Heterodera avenae*, is one of the most important pests of wheat and other cereals in many countries in the world with different climatic types. It is commonly distributed in the Eastern Mediterranean region in Turkey. Pathotyping experiments showed that the *H. avenae* populations found all belong to the Ha1 group, Ha21 pathotype. This study was carried out with Karlık–Adana populations (Ha21 pathotype) from Eastern Mediterranean region. They were used to determine resistance against the national wheat varieties, wheat wild genotypes and international wheat variety and lines carrying a resistance gene (*Cre* 1). According to our results, four national wheat varieties (one bread wheat and three durum wheat), seventeen wheat wild genotypes collected from the origin of wheat (Karacadağ, South-East Anatolia), twenty-four international wheat varieties and lines contained the *Cre*1 genewere found to be resistant against *H. avenae.* However*,* some international wheat varieties with the *Cre*1 gene were not resistant against *H. avenae* Ha21 pathotype. The national bread wheat variety, Adana 99 (PFAU/SERI82//BOG"S"), can be used as a resistant variety against *H. avenae* in integrated crop management programmes. Additionally, national wheat wild genotypes need further study to determine their resistance sources.

S11–P4

Investigating of the durability of some citrus rootstocks against citrus nematode in Eastern Mediterranean Region under natural conditions

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Citrus nematode, *Tylenchulus semipenetrans* Cobb, is widespread in the Eastern Mediterranean Region where 70% of the total citrus fruit is produced in Turkey. One of the most effective and economical methods of controlling the citrus nematode is the use of resistant rootstocks. Due to the suitability to the soil conditions found in this region, sour orange (*Citrus aurantium* L.) is commonly used. To determine the resistance of citrus rootstocks to *T. semipenetrans* under natural conditions, different rootstocks were investigated in two different citrus collection orchards that contain naturally high background level of citrus nematode. In order to determine the existence of the citrus nematode, root and soil samples were taken as three replicates from 34 selected sour orange clones (Tuzcu clones), *Citrus obovoidea, C. ampullaceae, C. sulcata, C. taiwanica, C. volkameriana*; *Poncirus trifoliata,* Troyer citrange, Carriza citrange, Kleopatra mandarin and Yuzu rootstocks from collection 1. InIn addition, root and soil samples were taken as three replicates from Nasnaran, Cleopatra Ant, Cloex swingle, Citru melo 4475, Carrizo, C-35, Gou tou, Trifoliate, Sunki, (Tuzcu Clones) Tuzcu 31-31, Tuzcu 891 in the collection 2. The collection 2 has a few stress factors such as Fe deficiency and drought. Citrus nematode in different densities was found in those samples taken from roots and rhizosphere of the different rootstocks in the collections 1 and 2. According to this study, citrus nematode was also found in the roots of *Poncirus trifoliata,* Carrizo citrange and Troyer citrange which are known as resistant or tolerant, from previous studies in icollection 1. On the other hand, samples that were collected incollection 2 were resistant. In order to reach more definitive conclusions on this subject, we will be evaluating seedlings from the seeds from all of these rootstocks, under controlled conditions.

S11–P5

Plant-parasitic nematode species found on important plants grown in agricultural lands in Adana, Turkey

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Adana is one of the most important agricultural provinces of Turkey. A preliminary survey on the occurrence of plant-parasitic nematodes in this province was carried out *ca* 20 years ago. The objective of this study was to investigate the change of plant-parasitic nematode fauna in the province of Adana since the previous survey of species in plant cultivars of agricultural crops. Population densities of some nematode species are so low that they cannot be distinguished by known methods. Therefore, faunistic studies must be repeated at regular intervals to detect different nematode species. According to this study, soil samples were taken from 140 different locations of Adana province between May and August of 2010. Plant-parasitic nematodes found in this study were identified with classical methods. As a result of the study 23 plant-parasitic nematode species belonging to the orders Tylenchida, Aphelenchida, Dorylaimida and 8 families and 12 genera were recorded; five species, *Pratylenchus zeae* Graham, 1951*, P. loosi* Loof, 1960, *P. delattrei* Luc, 1958, *Helicotylenchus digonicus* Perry in Perry, Darling and Thorne*,* 1959*,* and *Scutylenchus cylindricaudatus* Ivanova, 1968*,* are new records for the Eastern Mediterranean Region. *Pratylenchus delattrei* is recorded for the first time in the Nematoda fauna of Turkey.

S11–P6

Taxonomic studies on the plant-parasitic nematode species in wheat cultivated areas in Mardin Province

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This study was carried out to determine the occurrence of plant-parasitic nematodes and to examine their faunistic and taxonomic characteristics in wheat areas in Mardin province between 2009—2010. In order to determine cereal cyst nematodes, soil samples were taken before and after harvesting stage but for the other plant-parasitic nematodes samples were taken between tilling and milky ripening stages. In this study a total of 272 nematodes were obtained from collected samples and preparation and identification of these nematodes according to their species were made. Nematodes were extracted by using two different methods including "Petri dish" method (Hooper, 1986), and Fenwick method (Fenwick, 1940). The density and distribution of plant-parasitic nematodes were determined. At the end of this study 15 genera from Tylenchina and Aphelenchina, which are the sub-orders of Tylenchida– *Coslencus* spp*., Filenchus* spp*., Scutylenchus* spp*., Ditylenchus* spp*., Tylenchus* spp*., Helicotylenchus* spp*., Pratylenchoides* spp*., Paratylenchus* spp*., Rotylenchulus* spp*., Aphelenchoides* spp*., Paratrophurus* spp*., Aphelenchus* spp*., Tylenchorhynchus* spp*., Merlinius* spp*., Heterodera* spp. – and ten species from these genera – *Merlinius brevidens,* Allen 1955*, Merlinius microdorus* Geraert, 1966*, Aphelenchoides bicaudatus,* Imamura, 1931*, Paratrophurus acristylus* Siddiqi & Siddiqui, 1983*, Aphelenchus avenae* Bastian, 1865*, Pratylenchoides alkani* Yüksel 1977*, Pratylenchus thornei* Sher & Allen, 1953*, Rotylenchulus macrosomus* Dasgupta, Raski & Sher 1968*, Heterodera avenae* Wollenweber, 1924 and *Heterodera latipons,* Franklin, 1969 were found. The density and distribution of these genera were determined.

S11–P7

Plant-parasitic nematode species associated with different cultivations in Adıyaman Province

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In order to identify the nematode species in Adıyaman province of Turkey, a total of 410 soil samples were taken between 2010—2011 from different crop plants. The total numbers of samples taken from each plant were: pistachio (*Pistacia vera*) 29, tomato (*Lycopersicon esculentum*) 16, barley (*Hordeum vulgare*) 69, wheat (*Triticum* spp. L.) 152, vineyards (*Vitis* sp. L.) 45, watermelon (*Citrullus lanatus*) 23, melon (*Cucumis melo*) 21, cotton (*Gossypium* spp.) 23, tobacco (*Nicotiana* spp.) 32. A total of 33 species were determined inin 18genera belonging to 13 subfamilies within 11 families of Tylenchoidea, Anguinoidea, Hoplolaimoidea, Dolichodoridea, Longidoridea and Aphelenchoidea, superfamilies of Tylenchina, Hoplolaimina, Dorylaimina and Aphelenchina suborders of Tylenchida, Aphelenchida and Dorylaimida orders. The most abundant plant-parasitic nematodes detected were *Merlinius brevidens*, *Pratylenchoides alkani*, *Scutylenchus quadrifer*, *M. microdorus*, *Paratrophurus acristylus*, *Xiphinema pachtaicum* and *Aphelenchus avenae*.

S11–P8

Penetration and development of *Heterodera filipjevi* on susceptible and moderately resistant cultivars of winter wheat

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The objective of this study was to evaluate the penetration, development and reproduction of *Heterodera filipjevi* in the root systems of wheat germplasm with different levels of genetic resistance. Four moderately resistant (Katea, Sönmez, Milan and Silverstar) and two susceptible (Bezostaya and Kutluk) cultivars of winter wheat were used. One single wheat seed was sown per tube (13 cm in length × 3 cm in diameter). Ten days after sowing, 250 second-stage juveniles (J2) of *H. filipjevi* were inoculated into each tube. The developmental stages of *H. filipjevi* were determined 4, 12, 24, 36, 48 and 60 days after nematode inoculation. Nematodes penetrated in all cultivars 4 days after inoculation. The highest number of nematodes per seedling was recorded in the two susceptible cvs Kutluk (42) and Bezostaya (39). The lowest numbers of J2 were found in the roots of the moderately resistant cv. Silverstar (7). Twelve days after inoculation, males were observed in the roots of all cultivars, except in cv. Bezostaya. At harvest, 60 days after inoculation, nematode reproduction in terms of cysts was 3 times higher on susceptible cultivars than on moderately resistant cultivars. The highest reproduction factor (RF=Pf/Pi) was found on the two susceptible cvs Bezostaya (9) and Kutluk (7) and the lowest was recorded on the resistant cv. Silverstar (0.18). Similar results were reported from a field experiment. In conclusion, inhibition of juvenile penetration, delayed nematode development and increased numbers of males are considered the modes of action of the resistant cultivars in this study.

S11–P9

Resistance screening of national and international spring wheat against the cereal cyst nematode (*Heterodera avenae*) and the root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) under controlled conditions in Turkey

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The cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are known to be economically important nematodes of wheat production systems and in particularly under rainfed environments. In Turkey, the three main species commonly occur together in cereal production systems and the main method of control is through some of the traditional cultural practices and production of resistant germplasm. However, long-term control is best achieved through genetic improvement, which provides both economic and environmental benefits. Intensive screening under controlled conditions is facilitated in Turkey, in order to identify sources of resistance in wheat cultivars. Both national and International institutes have exploited many sources of identified resistance which nowhave entered the international wheat nursery for seed distribution to wheat breeding programmes globally. Breeding lines were screened under controlled *in vitro* conditions against RLN (*P. thornei* and *P. neglectus*) and CCN (*H. avenae*), from different institutes including CIMMYT International Mexico. Each line was replicated seven times and seeds were pre-germinated and planted individually in open small tubes (80 g) filled with soil mixture. Each tube was inoculated with either 400 nematodes for *P. thornei, P. neglectus* trials or 200 second-stage juveniles for *H. avenae* trials. Plants were harvested 9 weeks after nematode inoculation and the number of CCN cysts or RLN per plant and soil were counted and resistance was assessed based on known standard control lines. Through integrated efforts of breeders and nematologists several wheat lines with multiple resistance to CCN (*H. avenae*) and RLN species (*P. thornei* and/or *P. neglectus*) have been identified. The promising wheat lines have been shared with several wheat breeding programmes globally (as part of an International Spring Wheat Nursery) and offer a significant contribution to genetic resistance against cereal nematodes.

S12 – Quarantine nematology: new threats, pest risk analysis

Convenors: Nicole Viaene & Geraldine Antoine

S12–T1

The races of *Meloidogyne chitwoodi* in Turkey

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The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden *et al*.) is of economic importance to several crops, especially potato which seriously reduces tuber quality. *Meloidogyne chitwoodi* was first identified in Turkey from infected potato tubers collected from Nigde in 2009 and after that it was determined in Nevşehir, İzmir and Bitlis. *Meloidogyne chitwoodi* is currently reported to consist of two host races and two pathotypes which cannot be distinguished morphologically. The races are recognised based on the reproduction on carrot and alfalfa and the pathotypes are recognised based on the reproduction on *Solanum bulbocastanum* SB22.

Fifty eight geographic isolates of *M. chitwoodi* were collected from Niğde (45), Nevşehir (12) and Aksaray (1). The pure cultures of these isolates were used to evaluate their ability to reproduce on carrot (Red cored chantenay), alfalfa (Prosementi) and *Solanum bulbocastanum* SB22 for race and pathotype determination. Host status ranged from nonhost to good host based on reproduction factor (R) (R: 0—0.09, non-host; R: 0.1—0.9, poor host; R: 1—2, moderate host; R>2, good host). Carrot was a good host for all populations. Alfalfa was a poor host for 29 populations and non-host for 29 populations. *Solanum bulbocastanum* SB22 was a non-host for all populations. According to these results only *M. chitwoodi* race 1 was found in Turkey and also there was no evidence for the existence of host race 2 and pathotypes. These results are found to be parallel to similar studies performed in Europe.

S12–T2

Influence of temperature on the life cycle of the potato cyst nematodes

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The potato cyst nematodes (PCN) *Globodera rostochiensis* (Stone) and *Globodera pallida* (Woll.) are major parasites of potatoes and other members of the *Solanaceae* family. They are listed in the EU Plant Health Directive 2000/29/EC and are regulated by the European PCN Directive (2007/33/EC). In the UK, management of PCN relies on long rotations, nematicides and resistant cultivars, although for *G. pallida* there are few cultivars available with high levels of resistance. Climatic conditions differ around the UK and there is a trend towards increasing temperatures and changes in rainfall associated with climate change. The aim of this study is to investigate the relationship between soil temperatures and the PCN life cycle and population multiplication.

Hatching tests with water and potato root diffusate were conducted over a temperature gradient from 5—29°C. The optimal temperature for *G. rostochiensis* was 21°C and for *G. pallida* 13—23°C. *Globodera rostochiensis* hatched more quickly and had a delayed hatch at ≥25°C. In pot experiments conducted in growth cabinets with soil temperatures of 14, 17 and 20°C, males were present at 5 weeks at 17 and 20°C. A second generation of juveniles was observed by 10 weeks with the susceptible cv. Désirée with both species. Field experiments in Scotland (Luffness, East Lothian) and England (Newport, Shropshire) are in progress to monitor PCN development at monthly intervals during the growing season. Data obtained are being used to develop a dynamic temperature-based model for the life cycle of PCN and to understand the risk from a second generation of PCN.

S12–T3

PCN-free tulip bulbs produced in *Globodera pallida* infested soil

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In July 2010 EU legislation concerning potato cyst nematodes (PCN) was changed. Requirements for EU countries are less tight than for countries outside the EU. Within this scope discussion started whether or not PCN-free flower bulbs can be exported although grown in PCN infested fields. This question is also relevant because availability of suitable fields for bulb production may be limited in future.

In order to guarantee bulb quality in future and to maintain the present export position in the world, research was performed to examine the possibilities of preventing dispersal of PCN in tulip bulbs. Four tulip cultivars were grown in a marine loamy field, which was heavily infested with potato cyst nematodes (PCN) *Globodera pallida*. One year before tulip planting the initial infestation of approximately 900 cysts per kg of soil was created by a susceptible potato crop which is a good host for these nematodes. Four tulip cultivars were selected for special properties and together they cover the complete scale of tulip bulb varieties. The selected varieties were Yokohama, with a very tight skin, Negrita with a loose skin, Leen van der Mark with main bulb and side bulbs and Barcelona, a tulip cultivar with small plant size bulbs which produces ‘pears’. Bulbs were planted in November 2010 and harvested in July 2011. After harvest, tulip bulbs were washed in three different ways: standard prewashing, export washing and the combination of prewashing and export washing. All tulip bulbs were free from soil after washing irrespective of the washing technique used. Assessments for PCN cysts were made under the binocular microscope, per cultivar and washing technique of 1000 bulbs. No PCN cysts were detected, either under the skin or on the skin of all four cultivars export washed and also on Yokohama and Leen van der Mark standard washed. This study shows that there is no risk for PCN cysts in Dutch tulip bulbs for export.

S12–T4

Virulence differences of European and South American populations of *Globodera pallida* assessed on European potato cultivars

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European Plant Health legislation (EU Control Directive 2007/33/EC) makes a distinction between "European" and "non-European" populations of potato cyst nematodes. This distinction is very important as it is now commonly accepted that only a fraction of the virulence towards potato found in the White Potato Cyst Nematode (*Globodera pallida*) was introduced into Europe from South America. European potato breeding programmes and resistance testing schemes only test resistance of potato against potato cyst nematodes for a limited set of populations found in Europe. Should populations with new virulence characteristics be introduced into Europe this could have serious consequences for the control of potato cyst nematodes which largely depends on the use of resistant cultivars. Earliest evidence for new virulent populations was produced in the 1970s when pathotypes not found in Europe were identified in South America. More recently a number of studies have clearly demonstrated that molecular differences between populations present in Europe and South America exist. However, little information is available on the virulence of these populations towards European potato cultivars. Three experiments were carried out under greenhouse conditions to evaluate resistance/susceptibility of potato cultivars to several populations of potato cyst nematodes. Results of these experiments will be presented.

S12–T5

Survival and infectivity of *Meloidogyne chitwoodi*, *M. fallax* and *M. minor* in the absence of a host plant at different temperature regimes

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Survival of second-stage juveniles (J2) of the temperate root-knot nematodes *Meloidogyne chitwoodi*, *M. fallax* and *M. minor* in the absence of a host plant was examined at 4, 10 and 20°C. The number of surviving J2, their lipid reserves and their infectivity (penetration into roots of tomato seedlings) were studied over time. At 4 and 10°C, *M. chitwoodi*, *M. fallax* and *M. minor* showed a similar survival pattern during the first 10 weeks with more than 60% of the J2 still moving. After 14 weeks at 4°C, survival of *M. chitwoodi* was still 54%, whereas for *M. fallax* and *M. minor* this was only 25 and 30%, respectively. At 10°C, a higher survival of *M. fallax* (32%) was observed compared with *M. chitwoodi* (23%) and *M. minor* (22%). A distinct difference between the species was shown at 20°C; survival of *M. chitwoodi* started declining after 2 weeks, whereas for *M. fallax* and *M. minor* decline did not start before 6 weeks. At this temperature, no *M. chitwoodi* or *M. fallax* survived after 14 weeks. For *M. minor* 11% mobile J2 were still present at 20°C after 14 weeks, indicating a better adaptation to higher temperatures. The depletion of lipid reserves in J2 of the three species was similar at 4°C, but at 10 and 20°C lipid reserves in J2 of *M. fallax* decreased faster than in *M. chitwoodi* and *M. minor*. Also, infectivity of surviving *M. fallax* was very low, and this at all three temperatures. Infectivity of the surviving J2 of *M. chitwoodi* was higher than for *M. fallax* and increased with temperatures. *Meloidogyne minor* retained its lipid reserves the longest and surviving J2 could still penetrate root seedlings after 12 weeks at the three temperatures.

S12–P1

Detection and quantification of viable eggs and juveniles of potato cyst nematodes using propidium monoazide (PMA)

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Potato cyst nematodes (PCN), *Globodera pallida* (Stone) Behrens and *G. rostochiensis* (Wollenweber) Behrens are obligate parasites ofsolanaceous plants, causing severe losses in several potato growing areas of Cyprus. Management of PCN is related to nematode population densities estimated as eggs/g of soil. In classical nematology the standard method to determine the viability of PCN is based on microscopic visualisation of nematodes stained with the vital Meldola's Blue (MB) stain. Although MB seems to be reliable in staining embryonated juveniles within eggs and cysts, it is a time and labour consuming assay, which also leads to overestimation of the total numbers of PCN able to infect plants. Furthermore, molecular assays such as real-time PCR using Taqman probes cannot directly assess the viability of PCN inocula, since DNA of both live and dead cells can be amplified and the total amount of DNA present in a sample is quantified. In this work, we report a Real-Time based method for the quantification of viable PCN with the aid of Propidium Monoazide (PMA), a photoreactive, DNA-intercalating dye. The novelty of the method lies in the fact that PMA is nearly completely cell membrane-impermeable; thus, it can be selectively used to intercalate only exposed DNA from dead cells rendering it unable to amplify. Thus, only DNA from viable/intact cells is PCR-amplified and detected. Quantitative analysis of DNA from viable eggs can be performed by using species-specific Taqman probes and primers.

S12–P2

A survey of potato fields for root-knot nematode in Central Anatolia, Turkey

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Nematodes of the genus *Meloidogyne* that cause root-knots are considered to be the world's most damaging nematodes. *Meloidogyne chitwoodi* and *M. fallax* are quarantine organisms in Europe and special requirements exist for planting and movement of seed potatoes. *Meloidogyne chitwoodi* was first identified from infected potato tubers collected from Nigde in 2009 and after that it was determinated in Nevşehir, İzmir and Bitlis.

In this study a survey was conducted to determine the *Meloidogyne* spp. in Central Anatolia, the most important potato production area of Turkey. In total seventy-two populations were collected from infected tubers from Niğde (49 samples), Nevşehir (18 samples), Aksaray (2 samples), Isparta (2 samples) and Kayseri (1 sample). The populations were characterised using morphology and molecular identification by PCR in order to ascertain their identity. Fifteen populations were morphologically identified based on the perineal pattern of mature females and measurements of juveniles and eggs. Besides all populations DNA was extracted from egg masses of nematodes and SCAR primers (JMV1, JMV2 and JMVhapla) were used in multiplex PCR. Specific SCAR fragments were obtained from the extracted DNA. The multiplex PCR reaction produced only the 540bp fragment for *M. chitwoodi*. Both the morphological and molecular methods showed that all populations were *M. chitwoodi* and there was no evidence for the existence of *M. fallax* and other *Meloidogyne* species.

S12–P3

Management of potato cyst nematode PCN (*Globodera* spp.) in Norway

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In Norway PCN was first recorded in 1955. Since then surveys have been made to map the distribution of *Globodera rostochiensis* (yPCN) and *G. pallida* (wPCN). Both species are quarantine pests. The legislative regulation of PCN was introduced in 1956 and prohibits introduction and spread of the nematode with soil or plant materials. In Norway all pathotypes of wPCN and most pathotypes of yPCN except Ro1 are considered virulent. Non-virulent yPCN is managed by crop rotation using non-host crops, alternating susceptible and resistant cultivars, while infestations by wPCN or virulent yPCN results in 40-years ban on growing potato. In the past 50 years great emphasis has been placed on documenting freedom from PCN in the certified seed potatoes production. Infested fields are subjected to a strict quarantine. To prevent introduction of PCN, import of seed potato is forbidden. Nematicides have been banned for more than 40 years, so the use of resistant potato cultivars becomes important, and requires correct information on species and pathotypes. Other important information concerns PCN field decline rates, occurrence of antagonists, the performance of trap crops such as early potato and sticky night-shade *Solanum sisymbriifolium*. Better information on resistant potato cultivars on the market, and thehost–parasite relationship would further improve PCN management.

S12–P4

Quantitative detection of foliar nematodes (*Aphelenchoides* spp.) in complex DNA backgrounds

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Foliar nematodes, a subset of species within the genus *Aphelenchoides*, are plant parasites causing considerable economic damage to a number of important food (rice, strawberry) and ornamental (flower bulbs) plants. However, they constitute only a (numerical) minor group among a majority of fungivorous species. Distinction between (mostly harmless) fungal feeding *Aphelenchoides* species and high impact plant parasites such as the quarantine organism *A. besseyi*, and *A. fragariae, A. ritzemabosi* and *A. subtenuis* is severely hampered by the scarcity of informative morphological characters. Only taxonomic experts can distinguish between the foliar and harmless environmental species. Therefore, based on (nearly) full-length small subunit ribosomal DNA (SSU rDNA) sequences (≈1,700 bp), a phylogenetic tree was generated, and the four target species appeared as distinct, well-supported groups. As the use of the ITS regions for species detection purposes requires a thorough inventory of the intraspecific variation, we preferred to use SSU rDNA, a relatively conserved coding region within the ribosomal DNA cistron with a – for these four *Aphelenchoides* species– low intraspecific variation. PCR primers were developed with high, identical annealing temperatures (63°C). None of the close non-target species tested gave a significant PCR signal after 60 cycles. These primers can be used for the rapid screening of plant material and soil for the presence of one or multiple foliar nematode species and will be used by the Dutch Plant Protection Service (NVWA).
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S12–P5

Susceptibility of *Capsicum annuum* and other solanaceous plants to *Globodera pallida* and *Globodera rostochiensis*

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The potato cyst nematodes *Globodera pallida* and *G. rostochiensis* parasitise several plants in the family Solanaceae. Of these, potato (*Solanum tuberosum* L.) is the most important host crop but Annex I of the EU Control Directive for Potato Cyst Nematodes (2007/33/EC) also lists *Capsicum* spp., *Lycopersicon lycopersicum* (L.) Karsten ex Farw. and *Solanum melongena* L. as host plants for potato cyst nematodes. The most widely cultivated *Capsicum* species belong to *C. annuum* (pepper). However, the status of this plant species as a host for the potato cyst nematodes is dubious. In order to investigate the level of susceptibility of several solanaceous crops to potato cyst nematodes two greenhouse experiments were carried out. In experiment one, four varieties of pepper, four varieties of tomato, two varieties of eggplant and one variety each of potato and tobacco were included. In experiment two, eight varieties of pepper and five varieties of tomato were included. In both experiments the standard nematode populations for *G. rostochiensis* Ro1 and *G. pallida* Pa3 as listed in Annex IV of the EU Control Directive for Potato Cyst Nematodes (2007/33/EC) were used. The results of these experiments will be presented.

S12–P6

Globodera in Poland – from nematode resistance of potato varieties to the distribution of pathotypes

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Globodera rostochiensis and *G. pallida* belong to potato cyst nematodes (PCN) responsible for damage in cultivated solanaceous plants all over the world. In the European Union PCN have a status of quarantine organisms, instituted by EU Control Directive (68/465/EEC; CEC Council 1969). Cysts of *Globodera rostochiensis* were first found in Poland near Gdansk in 1946 (Wilski, 1956). Later, the regular regional surveys conducted since 1968 up to now have shown PCN to be widely distributed in Poland with the highest infestation throughout the Northwest. Currently, there is only one pathotype of *G. rostochiensis* Ro1 reported in Poland (Karnkowski, 2006; Przetakiewicz 2011, unpublished data). Membership of Poland in EU community and the opening of plant exchange market gives an opportunity of field contamination by other pathotypes of PCN.

Laboratory of Quarantine Organisms in Radzikow conducts research focussed on the assessment of resistance to five pathotypes of *G. rostochiensis* (Ro1—Ro5) and three pathotypes of *G. pallida* (Pa1—Pa3) of all Polish potato varieties, breeding lines and clones according to EPPO recommendations (Bulletin EPPO, PM7/40). The other task we undertake, in cooperation with the Main Inspectorate of Plant Health and Seed Inspection, is the identification of PCN pathotypes in soil samples coming from infested fields to prepare an information map of the distribution of pathotypes and resistance of potato varieties to the highest accuracy, mainly for farmers.

S12–P7

Some like it relatively hot: controlling *Ditylenchus dipsaci* in tulips by a conditioned hot water treatment

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A hot water treatment (HWT) of 4 h at 47°C after presoaking in water of the bulbs is used to kill *Ditylenchus dipsaci* in daffodil bulbs. However, a HWT above approximately 45°C is harmful to tulip bulbs and therefore not tried further.

To determine the temperature tolerance of tulip bulbs, preferably in the range of 4 h at 47°C, a number of combinations consisting of different pre-treatment temperatures (PTT), HWT temperature and HWT duration have been tried. To determine the optimal combination of HWT duration and temperature at which *D. dipsaci* was totally eradicated, trials were executed using *D. dipsaci*-infected tulip bulbs. The numbers of surviving nematodes in the bulbs were determined after incubation in a mist chamber. It was shown that tulip bulbs require high PTT in order to survive HWT at higher temperatures. For instance a HWT of 4 h at 47°C preceded by pre-treatment of 1 week at 27°C, 30°C or 33°C resulted in an average yield of good quality tulip flowers amounting 39%, 64% and 92%, respectively. The higher the PTT, the better the bulbs could tolerate the HWT. All nematodes were killed when incubated for 4 h at 47°C after a pre-treatment of 1 week at 30°C. Surprisingly, tulip bulbs appeared to be quite tolerant of HWT at temperatures of 46°C and 47°C, provided that bulbs received a pre-treatment and HWT shortly after harvesting the bulbs. The best results were obtained after a pre-treatment at 33°C. Bulbs that were subjected to this treatment were planted in the field; almost all plants produced good quality flowers. This relatively easy, cheap and fast method will be a practical tool for the tulip growers to treat their *D. dipsaci*-suspected planting stock.

S12–P8

Testing bulb lots for *Ditylenchus dipsaci*: swim and sink!

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Ditylenchus dipsaci causes one of the largest problems in the tulip and daffodil culture in The Netherlands, not in the least because it is a quarantine disease. This nematode is spread by planting infested bulb lots and *via* planting of healthy bulbs in infested soil. The significant economic losses are due to the obligatory destruction of infested bulb lots on one hand, and the expensive measures to be taken to disinfest or leave fallow the infested planting areas on the other hand. Besides the sampling of soil from suspected areas, a diagnostic test on bulb lots to detect *D. dipsaci* would help to prevent further spread of *D. dipsaci*. Therefore, a diagnostic test for large amounts of suspected tulip bulbs will be developed. The test is based on the characteristic that living *D. dipsaci* will swim out of the bulbs when incubated in a mist chamber or when submerged. The submerging of flower bulbs for 2 days proved to be most effective. *Ditylenchus dipsaci* could be detected by microscopical evaluation or by real time PCR on DNA extracted from the sediment. The recovery of *D. dipsaci* was evaluated by spiking bulb lots with a known number of nematodes. Target material for this diagnostic-bulb-lot-test are large amounts of harvested tulip bulbs, discarded because of symptoms which might be caused by *D. dipsaci*. Future actions will be directed at validating the developed test and application of the protocol in practicepractice. Tulip bulb lots from farms with a (recent) *D. dipsaci* incidence will be tested.

S13 – Molecular basis of the compatible interaction: plant response to nematode infection

Convenors: Julia Hofmann & Florian Grundler

S13–T1

Metabolic flux analysis in nematode-induced syncytia

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The *Arabidopsis thaliana—Heterodera schachtii* pathosystem has been shown to be characterised by local and systemic metabolic re-modelling of the hosts (Hofmann *et al*., 2010). Changes in metabolite levels, however, do not necessarily reflect the flux properties of the producing and consuming reactions. Stable isotopes may be used to follow metabolic fluxes and thus to understand solute partitioning. In the current work, ^{13}C-labelled sucrose was applied on shoots of nematode infected plants. First, two time points were identified when ^{13}C-sucrose levels were at steady state in all studied tissues. Second, the distribution of the isotope was followed from the shoots, into roots, syncytia and the sedentary nematodes in the polar and non-polar fraction of the obtained extracts as well as in starch and the non-soluble pellet. Further, the polar fraction was analysed by GC-MS so that the ^{13}C-flux could be followed into 63 metabolites.

These data enabled us to study metabolic fluxes locally and systemically from the source leaves to the feeding nematodes. Shoots and syncytia showed high isotope levels in the polar fraction, especially in sugars and sugar-phosphates, organic acids and some amino acids. In root ^{13}C-levels were low and nematodes were characterised by high isotope enrichment in the non-soluble pellet and therefore in large proteins and membrane fragments. The data further allow construction of operational metabolic networks. Finally, networks of the analysed tissues were compared in order to elucidate their role during nematode parasitism.

S13–T2

The role of AAA+ ATPase family gene *At1g64110* in the establishment and development of syncytia induced by *Heterodera schachtii*

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A transcriptome study of nematode-induced syncytia by *H. schachtii* in *Arabidopsis* roots showed a significant up-regulation of various genes including *At1g64110*, which is a member of a small gene family (including *At5g52882* and *At4g28000*) within the large group of AAA+ ATPases. The results from RT-PCR and qRT-PCR and *in situ* hybridisation of 5 and 15 dpi syncytia confirmed the data of the transcriptome study. The qRT-PCR showed that the gene *At5g52882*, which is not covered by the gene chip, is also expressed in syncytia, while expression of *At4g28000* was not detected. Promoter::GUS fusion lines were developed for *At1g64110*. One representative GUS line was selected. GUS staining was found in syncytia at 5, 10, 15 and 20 dpi. Moreover, histochemical GUS assays displayed GUS staining in different plant parts especially in imbibed seeds and sperm cells. Expression of *At1g64110* was modified by developing artificial microRNA (amiRNA) lines using the constitutive 35S promoter as well as *Pdf2.1* and *Miox5* promoters with strong expression in syncytia. The knockdown lines from all promoters showed silencing of *At1g64110* in syncytia compared with wild type syncytia which resulted in significantly reduced female number, female size and syncytial size. Gene silencing of *At1g64110* in different knockdown lines showed strong positive correlation with the nematode resistance. The T-DNA knockout mutants of gene *At1g64110* also showed resistance against nematodes. The double mutant of *At1g64110* and *At5g52882* demonstratedlower numbers of nematode as compared to the single mutants. The results indicate that the AAA+ ATPase family gene *At1g64110* has an important role in the establishment of nematodes and development of feeding sites in *Arabidopsis* roots.

S13–T3

Transcriptome and metabolome profiling reveal commonalities and specificities of the processes involved in accommodating rhizobial symbionts and parasitic root-knot nematodes

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Medicago truncatula is a plant model to study symbiosis with its bacterial partner *Sinorhizobium meliloti* and a host for plant-parasitic nematodes, such as *Meloidogyne incognita*. During these compatible interactions, root-knot nematodes induce the redifferentiation of root cells into specialised feeding cells called "giant cells". Hyperplasia and hypertrophy of the surrounding cells lead to the formation of root galls. Bacteria induce the development of root nodules and chronically infect plant cells from zone II before differentiating into atmospheric nitrogen-fixing bacteroids. Using laser-assisted microdissection, we specifically monitored, at the cell level, *Medicago* gene expression in nodule zone II cells and in giant cells and their surrounding cells. We revealed an important reprogramming of several pathways in both interactions, which may play key roles in nodule and gall neoformation. In addition, detailed analysis of glutathione (GSH) and homoglutathione (hGSH, a legume GSH analogue) metabolism demonstrated the importance of these compounds for the success of both interactions in *M. truncatula*. Depletion of (h)GSH content impaired nematode egg mass formation and modified the sex ratio. Gene expression and metabolomic analyses of (h)GSH-depleted galls suggest that these major antioxidant molecules have a key role in the regulation of giant cell metabolism.

S13–T4

Mining the active proteome of nematode-induced feeding cells in roots of *Arabidopsis thaliana*

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The cyst nematode *Heterodera schachtii* infects roots of *Arabidopsis* and parasitises them by modifying root cells to a hypertrophic syncytial feeding cell system. Nematodes secrete effectors that manipulate host protein activities in a network of interactions including post-translational modifications *e.g.*, inhibition and activation. Transcriptomic and proteomic approaches cannot display this functional proteomic information. Activity-based protein profiling (ABPP) is a method to investigate the activity of proteome using activity based probes (ABPs). We applied ABPP using three different probes (MV151, FP, MV101) to display differential enzyme activities in syncytium induced by *H. schachtii*. Our analysis shows that the activity of several groups of enzymes is differentially regulated in syncytium. Among those specifically suppressed in syncytium are proteasomal subunits (β1, β2, β5), several Papain-like cysteine proteases (PLCPs i.e. Cathepsin, RD21, AALP, XCP, etc.) and vacuolar processing enzymes (VPEs). An analysis of transcriptional data for proteasomal subunits revealed an accumulation of transcripts in syncytium. These results imply suppression of proteasome activity in syncytium. Similarly, activity of a serine carboxypeptidase-like protein (SCPL), a S-formyl-glutathione hydrolase (SFGH) and methylesterase is specifically up-regulated in syncytium. We characterised the role of some of these differentially regulated enzymes (Cathepsin, VPEs, RD21, AALP, XCP) by using T-DNA insertion knock-out mutants. Our analysis provides a first insight into functional proteomics of nematode-induced syncytia.

S13–T5

Role of phytohormones in rice host-immunity against root knot nematodes

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The importance of phytohormone balance is increasingly recognised as central to the outcome of plant-pathogen interactions. Recently, gibberellins (GA) and brassinosteroids (BR), in addition to their critical role in plant growth and development, have been shown to act in plant innate immunity. In this study, we examined the role of GA and BR in rice (*Oryza sativa*) in plant defence against the root-knot nematode (RKN) *Meloidogyne graminicola*. Exogenous treatment of GA_3 on wild type plants induces disease defence to *M. graminicola* both locally and systemically. On the other hand, applying low concentrations of exogenous epibrassinolide (BL), the source of BR, induced susceptibility in the roots whereas high concentrations of BL enforce systemic defence against this nematode. Mutants in the GA and BR biosynthesis or signalling pathway accumulate higher levels of the immediate JA-precursor 12-oxo-phytodienoic acid (OPDA). Co-application of 0.1μM BL with the JA-biosynthesis inhibitor ETYA resulted in 20% more susceptibility relative to treatment with either compound alone, suggesting that BL acts as a stronger inhibitor of the JA pathway than ETYA alone, or that BL employs additional actions through other defence pathways. Collectively, these results suggest that the balance between the BR and JA pathway is an effective influencer for the outcome of the rice—*M. graminicola* interaction. Our data also suggest that the lower susceptibility of GA biosynthesis and signalling mutants is at least partly due to increased endogenous JA levels, especially OPDA. qRT-PCR confirmed that exogenous GA promotes the production of JA and/or OPDA and JA is known to induce defence against RKN.

S13–P1

Cell wall ingrowths in nematode induced syncytia require *UGD2* and *UGD3*

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Cyst nematodes induce syncytia, specialised feeding cells, in the roots of their host plants, which are the sole source of nutrients during their life. Since the nematodes continuously withdraw nutrients from syncytia, they are strong sinks for water and assimilates within the host plant and have to be continuously "refilled". One mechanism that ensures an increased transport into syncytia associated with female nematodes, which have a higher demand for nutrients than males, are cell wall ingrowths at the interface with xylem vessels. They increase the surface area of the plasma membrane and therefore increase short distance nutrient transport into syncytia. We studied the role of UDP-glucose dehydrogenase genes in Arabidopsis and found that these cell wall ingrowths are missing in syncytia developing in Arabidopsis ΔΔ*ugd23* double mutants, apparently leading to decreased nutrient import into syncytia. Consequently, these syncytia remain smaller and support fewer and smaller female nematodes.

S13–P2

Heterodera schachtii changes early signalling events in Arabidopsis

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Plant-parasitic nematodes infect roots of host plants and form highly sophisticated feeding sites such as syncytia. During early stages of infection process they activate signalling cascades, which alter plant defence responses to the nematodes' advantage. Mitogen-activated protein kinase (MAPK) pathways transduce the extracellular signals into the nuclei and induce defence-related gene expression. MAPK specificity can be modulated *via* protein phosphatases. Here, we investigated early signalling events in syncytia induced by the beet cyst nematode *Heterodera schachtii* in roots of *Arabidopsis*. By use of the GUS reporter gene system we showed a specific activation of the phosphatase AP2C1 promoter at the onset of syncytium formation. In further analysis we investigated the role of two MAPK kinases, MPK3 and MPK6, during nematode infection and development. Our results suggest that MAPK signalling may play an essential role during plant—nematode interaction.

S13–P3

Functional analysis of root-knot nematode genes during *Arabidopsis* infection

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Plant-parasitic nematodes are a huge agricultural problem on many of the world's main food crops, and one of the most damaging of the plant-parasitic nematodes is the root-knot nematode (*Meloidogyne* spp.). These nematodes pose a serious agricultural threat due to their large host range and because many crop plants lack natural nematode resistance. During the susceptible interaction, root-knot nematodes invade host roots where they choose plant cells to convert into metabolically-active feeding sites. The root-knot nematode's manipulation of the plant cell, and in particular how the nematode is able to regulate host plant pathways, is not well-understood. Here we report on the initial findings from a novel effector screen using a heterologous bacterial expression system to functionally analyse the roles of putative root-knot nematode effectors. We describe our early steps in characterising a repertoire of secreted root-knot nematode proteins and their possible roles in the compatible interaction with *Arabidopsis thaliana.* By using the genetic and genomic resources available for *Arabidopsis*, we hope to further our understanding of the nematode's relationship with the plant.

S14 – Interactions of nematodes with other organisms: non-compatible interactions (including biocontrol)

Convenor: Johannes Hallmann

S14–T1

Low temperature scanning electron microscopic studies on the interaction of *Globodera rostochiensis* Woll. and *Trichoderma harzianum* Rifai

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Golden nematode of potatoes (PCN) has been recorded since 1881 as an important and cosmopolitan pest of potatoes, the damage being very serious and world wide. Chemical applications has been one of the most reliable methods in pest management; however, resistance to most of the conventional chemicals has developed. Control of deep infestation of PCN in the soil is far more difficult with nematicides. Furthermore, use of resistant or tolerant cultivars can favour growth of numbers of nematodes that can selectively grow on that cultivar and this is the case with *Globodera rostochiensis* and *G. pallida* pathotypes. Biological control agents have been used as alternative to chemicals. Among various groups of antagonists, fungi have received much attention because many can be easily cultured and grown on large scale, and stored before use. Low temperature scanning electron microscopic (LTSEM) studies revealed that *Trichoderma harzianum* infected mature potato cysts nematode eggs by penetrating directly the cyst wall or *via* natural opening of mouth. The start of infection by *T. harzianum* on the cyst wall to the killing of juveniles inside the egg observed with LTSEM is reported in this paper. Mycelial penetration on cyst wall or egg surface has been seen. The penetration of cyst wall or egg surface was either chemical or mechanical (directly or with appresorium) or both. Freeze fractionation showed the presence of mycelia inside the eggs.

S14–T2

Hatching inhibition of *Globodera rostochiensis* by some fungal isolates

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Globodera rostochiensis is an important potato parasite, a cyst nematode of quarantine concern. In order to establish parasitic relationship between the nematode and a potato plant, the invasive juveniles begin to hatch and invade the host plant. Hatching is sometimes partially or completely inhibited due to the presence of antagonistic fungi. The cyst, as a specific "microcosmos", contains (beside juveniles and eggs) numerous fungal and bacterial species. The fungal isolates were identified by morpho-dimensional analysis using slide-cultures. Only *Mycelia sterilia* was identified by biomolecular analysis. In this study, hatching inhibition of *G. rostochiensis* in *in vitro* conditions is evaluated. The presence of isolated fungal species inhibited hatching of the golden nematode by 98% to 100%. Nearly all fungal isolates performed antagonistic activity indicating their potential use as potato cyst nematode biocontrol agents.

S14–T3

Development of farmer and eco-friendly methods for the application of *Verticillium chlamydosporium* (Goddard) to manage root-knot nematodes *Meloidogyne incognita* (Kofoid and White) in tomato

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Verticillium chlamydosporium has been used against the root-knot nematode, *Meloidogyne incognita*. A total of 35 localities were surveyed in Khyber Pukhtunkhwa province of Pakistan for the isolation of *V. chlamydosporium*. Six isolates of the fungus were maintained on potato dextrose agar. Culture filtrates of all *V. chlamydosporium* isolates were evaluated for their nematicidal activity, showing significant variation in their ability to inhibit hatching and kill second-stage juveniles of *M. incognita* at various concentration levels. Maximum inhibition of hatching was recorded in the culture filtrate of VC-6 followed by VC-1. Mortality of second-stage juveniles was maximum in VC-1 followed by VC-6. Isolates of *V. chlamydosporium* also showed significant variation in parasitism of *M. incognita* eggs and juveniles. On the basis of *in vitro* hatching inhibition and juvenile mortality, VC-1 and VC-6 were selected for further studies.These isolates were tested under screen house conditions at different rates and methods of application. Both the isolates at the rate of 6×10^3 chlamydospores per g of soil per plant were effective in reducing the nematodes population and improving plant growth and yield of tomatoes. Field trials were carried out during two consecutive cropping seasons, *i.e*., summer and winter tomatoes. Both isolates did not exhibit a significant effect in the summer crop. However, a significant effect was observed in the winter crop. Both isolates were found to be more effective at the rate of 6×10^3 chlamydospores per g of soil per plant. Root coating with chlamydospores suspension significantly reduced number of galls on roots. Little effect of both isolates was observed with mycelial root coating. No lesions were noticed on *V. chlamydosporium*-inoculated roots of tomato plants.

S14–T4

Effects of a fungal and a bacterial biocontrol agent on the colonisation and development of *Meloidogyne incognita* on tomato in a synchronised infection assay

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Plant resistance toward the root-knot nematode *Meloidogyne incognita* can be enhanced by both the endophytic fungus *Fusarium oxysporum* strain Fo162 and the endophytic bacterium *Rhizobium etli* strain G12. It is well described that inoculation of tomato seedlings with one of these antagonists leads to a reduction in the number of juveniles that penetrate the root and ultimately the number of galls and egg-masses produced. Little is known about the effects of both endophytes on the development of the nematodes that still are able to colonise the roots. By a synchronised nematode infection assay, in which tomato plants were only exposed to *M. incognita* second-stage juveniles for 2 days, we confirmed that fewer nematodes had penetrated the roots when the plants had been inoculated with either G12 or Fo162. Of the nematodes that had penetrated, we could determine that the development into the third-stage juvenile as well as into the adult stage was delayed in both the Fo162 and G12 inoculated plants when compared to the control. In addition, inoculation with one of the endophytes also reduced the number of eggs per female. Although completely different organisms, the various plant responses elicited by the bacterial and the fungal biocontrol agent may be very similar, not only causing a reduction in root penetration by *M. incognita* but also negatively affecting their development and reproduction.

S14–T5

Developing a biological control agent for molluscs in South Africa

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European molluscs (slugs and snails) have become significant pests in South Africa, affecting both the agricultural and horticultural industries. These pests were introduced to the country in the eighteenth and early nineteenth century, during the migration of European settlers to Africa. The success of these pests is not completely understood, but the lack of associated parasites may play a significant role in the invasion of these European molluscs. Current methods for controlling these pests rely on chemical molluscicides, such as metaldehyde and carbamate compounds. Both metaldehyde and methiocarb are poisonous to a wide range of vertebrates and studies have shown that methiocarb is toxic to a number of beneficial invertebrates, including earthworms and carabid beetles. Therefore, it is important that a method of biological control is identified. The most effective commercial method for the biological control of molluscs in Europe is the mollusc-parasitic nematode *Phasmarhabditis hermaphrodita*. *P. hermaphrodita* is currently mass produced by Becker Underwood UK Ltd (Littlehampton, UK) and sold under the trade name of Nemaslug®. To date, this product cannot be sold in South Africa due to current legislation (amendment of Act 18 of 1989 under the Agricultural Pest Act 36 of 1947). Therefore, this work presents a systematic survey of mollusc-parasitic nematodes present in South Africa with the aim of developing a new biological control agent for molluscs in South Africa.

S14–P1

Nematodes of bark beetles of genus *Ips* De Geer, 1775 (Coleoptera: Scolytinae) in the Czech Republic and central Europe

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The relationships between nematodes and bark beetles are described in various ways including commensalism, various types of parasitism, saprobiotism and predation. Phoresy is also very widespread in nematodes related to the bark beetles (Scolytinae), with the nematodes using their hosts as means for colonising new environments. Phoretic nematodes are found on the bodies of beetles. They are most frequently located in clusters under the elytra, on the wings or between the segments of the body and they are often found on the bodies of beetles in anabiosis. Sometimes, they take specific formations called nematangia such as do representatives of the genus *Ektaphelenchus*, which locate under the elytra and on the wings. By contrast, parasitic nematodes are found in the bodily cavities of adult beetles, larvae and pupa. In the area of the Czech Republic, seven species of genus *Ips* occur (*Ips acuminatus, I. amitinus, I. cembrae, I. duplicatus, I. mannsfeldi, I. sexdentatus, I. typographus*). Examples of phoretic nematodes of bark beetles of *Ips* genera in the Czech Republic and central Europe include representatives of the genera *Bursaphelenchus*, *Fuchsnema, Ektaphelenchus, Neoditylenchus* and *Micoletzkya*. Examples of parasitic nematodes include representatives of the genera *Contortylenchus, Parasitylenchus* found free in the haemocoel. Representatives of the genera *Parasitaphelenchus* and *Parasitorhabditis* are found in the lumen of the intestine but also moving freely in the body cavity. Members of the genus *Cryptaphelenchus* are found on the surface of the body and also in the Malpighian tubules.

S14–P2

Use of *Trichoderma harzianum* as a biological control agent against *Meloidogyne* sp. in summer tomatoes

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To study the efficacy of the selected *Trichoderma* isolates, field trials were conducted in the root-knot nematode-infested areas of Dargai and Swat, Pakistan. Four isolates of *T. harzianum* viz, Th1, Th2, Th9 and Th15 were tested against root knot nematodes on summer tomatoes under field conditions. The *T. harzianum* isolates, grown on wheat grains substrate, were applied at 8 g per plant, either alone or in different combinations. The root weight of tomato plants was reduced by Th9 as compared to 26.37 g in untreated control. Isolate Th1 was found to enhance shoot and root lengths to the maximum levels of 78.76 cm and 19.59 cm, respectively. Tomato shoot weight was significantly increased (65.36 g) in Th1-treated plots as compared to 49.66 g in control. Maximum (156) number of flowers per plant and highest (48.18%) fruit set per plant was observed in Th1 treated plots, while there were 87 flowers and 35.50% fruit set in the untreated control. Maximum fruit weight (70.97 g) per plant and highest (17.99 t ha^{-1}) marketable yield were recorded in the treatments where *T. harzianum* isolate Th1 was used, in comparison to 51.33 g tomato fruit weight and 9.90 t ha^{-1} yield noted in the control plots. It was observed that *T. harzianum* isolates significantly reduced the nematode populations. The fungus enhanced plant growth and yield in all the treated plots. Jabban isolate (Th1) was found to be the most effective in nematode suppression followed by Shamozai (Th9) isolate.

S14–P3

The interaction between root-knot nematodes (RKN) and soft rot enterobacteriaceae (SRE) in potatoes

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Potato (*Solanum tuberosum*) is one of the most important crops worldwide. Root-knot nematodes (RKN), *Meloidogyne* spp., are an important pest of potatoes in tropical and subtropical regions. Compared with other plant pathogens in the soil, RKN are relatively large and present in large numbers. The combined effect of secreted plant cell wall degrading enzymes and repeated thrusting of the stylet on plant roots leads to wounds and openings that can be used by other pathogens to enter the host roots. After penetrating the roots of the host, RKN physiologically modify the roots by forming galls that are used as nutrient sinks by RKN and other plant pathogens. Thus, complexes and synergies have been demonstrated between RKN and other soilborne pathogens. To date, no such interaction has been demonstrated between *Meloidogyne* spp. and soft rot-causing agents, *Pectobacterium* spp. Both the RKN and soft rot erwinia are important pathogens of potatoes resulting in massive yield losses annually. There is no known chemical treatment for soft rot erwinia and chemical nematicides, which are generally used for effective control of RKN, are slowly being phased out due to mounting pressure from environmental and health lobbyists. Hence, the aim of this study was to determine whether there is an interaction between the two pathogens. Results indicated that *Pectobacterium* spp. can attach on to the surface coat of *Meloidogyne* spp. and the potential of RKN to disseminate *Pectobacterium* spp. was also demonstrated. In glasshouse trials, the presence of *Meloidogyne* spp. in the rhizosphere of potato plants cv. Mondial was shown significantly to increase disease severity and incidence caused by *Pectobacterium* spp.

S14–P4

Pseudomonas putida UW4 producing ACC deaminase is a potential biocontrol agent for pine wilt disease

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Pine wilt disease, caused by the nematode *Bursaphelenchus xylophilus*, is responsible for devastation of pine forests worldwide. Until now, there are no effective ways of dealing with this serious threat. The use of ACC deaminase-producing plant growth promoting bacteria has been shown to be a useful strategy to reduce the damage due to biotic and abiotic stresses. *Pinus pinaster* seedlings inoculated with the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 strain showed an increased root and shoot development and reduction of *B. xylophilus*-induced symptoms. By contrast, *P. putida* UW4 ACC deaminase mutant was unable to promote pine seedling growth or to decrease *B. xylophilus*-induced symptoms. This is the first report of the use of ACC deaminase-producing bacteria as a potential biological control agent for tree diseases, thus suggesting that the inoculation of pine seedlings grown in a tree nursery might constitute a novel strategy to obtain *B. xylophilus*-resistant pine trees.

S15 –Behaviour and physiology

Convenors: Roland Perry and David Chitwood

S15–T1

Plant compounds for nematode control: From optimism to reality

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Plants possess a vast array of compounds toxic or otherwise antagonistic to phytoparasitic nematodes. Although these or related compounds or plant products are potentially useful for nematode management, their actual utilisation in agriculture has often been impractical. Nonetheless, because plant-based products or compounds are often safer than synthetic ones and receive less regulation, researchers are still in active pursuit of perfect phytochemical nematicides. Like any nematode control agent, such a compound or materials would need to be inexpensive, be effective only against agricultural pests and pathogens, and be able to move in soils and persist in a manner that is agriculturally effective yet environmentally safe. In pursuit of the ideal candidate, researchers face critical choices in experimental approach and design. For example, the plant material should be easily produced if not widely available already. If crude plant extracts show promise against nematodes, researchers would be better served by a bioassay-guided fractionation scheme than by inferential guessing about the nature of the suspected nematode-antagonistic phytochemical. The fractionation and purification scheme should not focus on lipophilic compounds because these are not likely to move in soil after application. The best bioassay organism would be a phytoparasitic target of control, not a microbivorous species, which ideally would be unaffected. Finally, high minimally effective concentrations are not usually appropriate for future greenhouse or field experimentation, except in unusual circumstances.

S15–T2

Targeted lipidomics in biotrophic plant–nematode interactions

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Lipids are essential components of cellular life and play an important role in structuring the plasma membrane and endosomal compartments. In addition they are responsible for energy storage and serve as signalling molecules mediating biotic and abiotic stress responses. Some plant-parasitic nematodes induce feeding cell complexes within the roots of host plants that serve them as the sole source of nutrients. The induction and expansion of these feeding sites leads to profound local and systemic changes in the plant. We were interested if these changes are accompanied by differences in the lipid composition. Therefore, we utilised the axenic *Arabidopsis thaliana–Heterodera schachtii* model system and quantified several lipid classes in leaves and syncytia using targeted qTOF mass spectrometry. The leaves of infected and control plants showed no significant differences in total amounts of lipid among the classes analysed. However, significant differences between several molecular species of phospholipids could be detected. Interestingly,stress-induced lipid accumulation like an increase in phosphatidic acid could not be observed. The syncytium contained more lipid per fresh weight and the composition of several lipid classes differed compared with the control root. Generally, the composition shifted frompoly-unsaturated fatty acids towards mono- and unsaturated fatty acids. In conclusion, *H. schachtii* infection affects the lipid composition of *A. thaliana* systemically and the local changes in the syncytium suggest that it provides the lipids required by this nematode.

S15–T3

Variation in eclosion and hatch of eggs among geographic isolates of the reniform nematode, *Rotylenchulus reniformis*

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Studies published previously have documented the role of weed exudates on the eclosion and hatch of eggs of *Rotylenchulus reniformis* and differences in eggs per gram of root tissue produced by pathologically variable geographic isolates of *R. reniformis* from Louisiana (LA), Mississippi (MS), Texas (TX), Hawaii (HI) and Arkansas (AR) in the USA. Laboratory studies conducted subsequent to microplot-based pathogenicity experiments evaluated more precisely the eclosion and emergence of juveniles of *R. reniformis* from eggs representative of the five geographic isolates. For each isolate, six replicates of 50 hand-picked, undifferentiated, freshly-extracted eggs were placed into 20 ml of sterile distilled water (pH 7.1—7.3) contained in a 60 × 15mm Petri dish and incubated at 28°C. Contents of each dish were examined daily for 13 days and categorised into six developmental stages: undifferentiated egg; 8—16 cell stage egg; juvenile inside egg; hatched juvenile; infective female; and male. The study was repeated twice and data analysed over the three runs of the trial. At 96 h, some eggs of all populations had reached the 8—16 cell stage, although development was significantly less so for the populations from HI and AR. By day 7, hatched juveniles and/or juveniles in eggs were present in all populations, but only the MS population contained infective females. At 10 days, the majority of eggs from all populations except those from HI were hatched. At 13 days, populations from LA and MS contained only juveniles, infective females and males. Those from TX, HI and AR remained 10—25% mixed egg stages, most of which did not hatch by 20 days when monitoring was terminated. These differences in egg and juvenile development among populations correlate well with final population densities and subsequent pathogenicity data from full-season microplot trials with cotton and soybean.

S15–P1

Survival of the root-knot nematodes (*Meloidogyne* spp.) without feeding

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Root-knot nematodes (RKN) cause severe crop loses, feeding on thousands of different plant species. Second-stage juveniles (J2) infect plant roots. J2 do not feed in the soil until they locate the host plant roots. The aim of this study is to find out how many days they can survive without feeding under laboratory conditions. This study was carried out with two root-knot species, *Meloidogyne incognita* and *M. hapla*. *Meloidogyne incognita* and *M. hapla* were extracted from tomato and Taiwan lettuce, respectively, by using the funnel spray method (mistifier). After extraction they were counted and stored in 100 ml tap water, then placed in dark room at +4°C and tap water was added each week to supply oxygen. About 10 million *M. incognita* and 15 million *M. hapla* were extracted on 22 different occasions between March and May. Assessment of survival was conducted every three days until 111th day after collection and placement in the cold room. The survival of *M. hapla* was 100% until 69 days in the cold room, and 80% were still alive after 104 days. All *M. hapla* J2 were dead by the 108th day. Although the first group of *M. incognita* were dead by the end of the 23rd day, the longest surviving *M. incognita* group had a 5% survival by the 99th. *Meloidogyne incognita* did not survive as well as *M. hapla*. According to these results, *Meloidogyne* species can live more than three months without feeding. It is also believed that nematodes live longer if enough oxygen is supplied to them. After extraction, nematodes should be put into the cold room as soon as possible to enhance survival.

S15–P2

The neurobiology of *Globodera pallida*

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This project aims to investigate the neurobiology involved in the host-seeking behaviour of plant-parasitic nematodes. Bioassays are being developed to examine the ability of the potato cyst nematode *Globodera pallida* to reach an attractant. The drugs Ivermectin, Levamisole and Fluoxetine, which affect glutamate-gated chloride channels, nicotinic acetylcholine receptors, and serotonin re-uptake, respectively, have a detrimental effect on nematode movement. The assay will determine the extent to which these drugs affect the ability of *G. pallida* to move towards an attractant.

The genome of *C. elegans* is sequenced, and its food-seeking behaviour and the components involved in this chemosensory response are well understood. We are currently examining the recently determined sequence of the *Globodera* genome to identify orthologues of those *C. elegans* genes coding for the receptors of neurotransmitters and genes involved in the synthesis of neurotransmitters. Analysis of transcriptome data confirms that these genes are expressed preferentially in second-stage juveniles and adult males, the two motile stages of *G. pallida.* The genes have been cloned and their role is being investigated using RNAi together with bioassays that assess the ability of the nematode to migrate towards an attractant. *C. elegans* mutant lines will be transformed with the respective full-length copies of the *G. pallida* genes, e.g. transforming an *unc-38 C. elegans* mutant with the putative orthologue of *unc-38* from *G. pallida,* to determine whether or not the mutant phenotype can be recovered.

S15–P3

Effect of daily temperature fluctuations on the hatching response of potato cyst-forming nematode *Globodera rostochiensis*

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The study of temperature influences on the establishment of host-parasite relationships in the system 'potato–potato cyst-forming nematodes' and host resistance showed that pre-treatment of seed material (seedling and tubers) by short-term low temperatures led to a 3-fold decrease in the number of 'newly-formed' cysts as compared with the control. Besides the direct effect on the physiology of the developing juvenile in the plant roots, short-term low temperatures influenced eggs and juveniles inside newly-formed females. There was no hatch of juveniles in the presence of host plant root leachates after short-term low temperatures, yet *Globodera rostochiensis* juveniles from laboratory cultures hatched actively. In addition, the stimulatory effect of root leachates of temperature-treated plants on the hatching process of laboratory culture juveniles was investigated. Newly-formed cysts contained eggs containing juveniles that failed to hatch under favourable conditions due to diapause initiated by signals from the host plant to the female and hence to the developing juveniles. An assay of cyst contents showed that the number of eggs and juveniles per new cyst and their viability were high. Temperature fluctuations altered plants before nematodes invaded, and perturbed the synchronization between host plants and *G. rostochiensis* life-cycle, particularly the hatching process.

S15–P4

Negative gravitactic behavior of *Caenorhabditis japonica* dauer larvae

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Gravity is a constant stimulus on Earth and many organisms are able to perceive and respond to gravity. However, there is no clear evidence of nematodes exhibiting this ability. In this study, we demonstrated and characterised negative gravitaxis in dauer larvae (DL) of *Caenorhabditis japonica* that engage in phoretic association with the burrower bug *Parastrachia japonensis*. The negative gravitactic behaviour of nematodes was evaluated using the negative gravitactic index (NGI), which was calculated by the following procedure. After inoculation of nematodes at the centre of a 9-cm diameter nematode growth medium (NGM) plate, the plate was set vertically and the ratio of nematodes that migrated upward to the total number of inoculated nematodes was calculated. NGI values of *C. japonica* DL showing nictation, a typical host-finding behaviour, were high, whereas those of DL showing no nictation were low. The NGI values of nictating *C. japonica* DL collected from younger nematode cultures were higher than those collected from older cultures. A 24-h incubation of these nictating DL in buffer did not alter the NGI values but a longer incubation resulted in lower NGI values. These results are indicative of negative gravitaxis in nictating *C. japonica* DL, which is maintained for at least 24 h once initiated and seems to be influenced by the physiological and/or neural conditions of nematodes.

S15–P5

Temperature dependant reproduction of the root-knot nematode *Meloidogyne ethiopica*

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Root-knot nematode (RKN) *Meloidogyne ethiopica* Whitehead is a member of the tropical *Meloidogyne* group. The species was recently included in a group of 12 important root-knot nematodes considered to be important agricultural pests. The pest could be very important because it is very damaging species; it has a wide host range including perennial crops such as grapevine, kiwi and stone fruits. It was also shown that the pest is able to survive open-field winter conditions in Europe. It was listed on the EPPO Alert list of harmful organisms in 2011. The length of the reproduction cycle of RKN is highly dependent on the soil temperature. *Meloidogyne ethiopica* required 67, 48 and 36 days to complete its reproduction cycle at mean daily temperatures of 18.3, 22.7 and 26.3°C, respectively, in a growth chamber controlled conditions. At 13.9°C, the species was not able to reproduce. Additionally, the base temperature of nematode entry in the host roots and further development was established at 14°C. The data were used for calculating the correlation between temperature and the reproduction cycle duration. The mathematical equation obtained covers temperatures ranging between 14°C and 26.3°C. An additional experiment was set up to test applicability of the reproduction curve in the open field conditions where soil temperatures vary. Tomato plants were used as host plants, which were inoculated with *M. ethiopica* eggs. The obtained data proved the usefulness of the reproduction curve, which can be used for modelling for tests such as host status testing and searching for resistance in wild plants as well as prediction of the extent of *M. ethiopica* reproduction during the plant growing period.

S15–P6

Migration of bacterivorous nematodes towards food spot as affected by presence of other nematodes (conspecifics *vs* other species)

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Nematodes can extract different types of information from their immediate environment, chemoreception being the primary neurosensory tool to detect food sources, potential hosts, noxious compounds, etc. Whether and how interspecific differences in migration towards food affect their species interactions and assemblage structure remains unclear. A laboratory experiment was set up to determine the influence of other nematodes on the migration of two bacterivorous nematodes, *Diplolaimelloides meyli* and *D. oschei*, towards food spots. We hypothesised that: (a) bacterivorous nematodes can sense food spots from a distance; (b) the presence of conspecific nematodes in food spots enhances nematode migration; and (c) the presence of other species inhibits nematode migration as a result of priority effects. Both species exhibited stronger migration towards food spots with existing nematode populations. *Diplolaimelloides oschei* migrated more towards spots with conspecifics than spots with *D. meyli*, whilst *D. meyli* migrated at similar rates towards feeding spots with either species. The results indicate that the presence of active nematodes enhances migration of other nematodes towards food spots, but that this effect is species-specific. It also supports previous studies showing antagonistic interactions between congeneric monhysterid nematodes and the idea that *D. meyli* is competitively stronger than *D. oschei*. Our data do not indicate the importance of priority effects in food-finding and early population establishment in these nematodes, but there is need for a longer-term follow-up of nematode population development to confirm this conclusion.

S15–P7

A method to study nematode behaviour in three dimensions

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Nematode behaviour has been studied mostly in two dimensions, either horizontally or vertically. Here a method was developed to study the searching behaviour of entomopathogenic nematodes in three dimensions. A cube was constructed and filled with moist sand and a single *Galleria* larva was placed as the host cue on the side, top or bottom of the cube. Infective juveniles were then similarly applied to the top, bottom, side or middle of the cube and their subsequent distribution determined after sub-dividing the cube into blocks. Results and shortcomings of the method are discussed.

S16 – Molecular basis of non-compatible plant nematode relationship; resistance and virulence

Convenors: Erin Bakker & Anna Tomczak

S16–T1

Structure-informed analysis of Gpa2 and Rx1 recognition specificity

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The potato cyst nematode resistance gene *Gpa2* confers resistance to *Globodera pallida*. *Gpa2* belongs to the class of CC-NB-LRR resistance genes and is located on a complex locus that also harbours the closely related Potato Virus X (PVX) resistance gene *Rx1*. *Rx1* and *Gpa2* are 88% identical at the amino acid level, yet they confer resistance to completely unrelated pathogens. For both R proteins, the pathogen elicitor that triggers the resistance response is known. *Gpa2* recognizes the secreted *Globodera pallida* protein RBP1, whereas *Rx1* detects the PVX coat protein. This makes *Gpa2* and *Rx1* an excellent model system to investigate how the recognition specificity is determined in NB-LRR proteins.

To determine which amino acids may play a role in *Gpa2* and *Rx1* specificity, we combined sequence information of 35 closely related homologues derived from wild *Solanum* species, functional data and a consensus *in silico* 3D model obtained in previous studies. For both *Gpa2* and *Rx1*, two residues were detected which are located in the region that is crucial for elicitor recognition. In the 3D model, they map together on the LRR surface, suggesting a potential role in pathogen recognition. The functional validation of these residues is in progress.

S16–T2

The *Ma* gene for complete-spectrum resistance to root-knot-nematodes in *Prunus*: biological features, positional cloning and validation

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Stone fruit crops, *Prunus* spp., are severely damaged by root-knot nematodes (RKN) *Meloidogyne* spp. and breeding for RKN resistant-rootstocks is a promising control alternative to nematicides. Resistance (R) genes have been identified and mapped in plums, peach and almond. Because the perennial status of *Prunus* increases the risk for resistance breaking, R genes are being pyramided for durable resistance in interspecific rootstocks. Among the genes identified, *Ma* from Myrobalan plum has been shown to confer a complete-spectrum, high-level and heat-stable resistance to both mitotic (*M. arenaria, M. incognita*, *M. javanica* and *M. enterolobii*) and meiotic (*M. floridensis*) RKN. The *Ma* gene triggers a hypersensitive-like reaction characterised by the total absence of galls and fixed nematode stages. The positional cloning of the *Ma* locus in accession P.2175 has been performed using a high resolution mapping developed in two successive steps totalling over 3000 segregants. The *Ma* locus interval has been reduced to a 32-kb cluster of three TIR-NB-LRR genes (TNL1 to TNL3) including a pseudogene (TNL2) and a truncated gene (TNL3). Using *A. rhizogenes* transformed hairy roots and composite plants, the best candidate gene, TNL1, comprising the genomic sequence preceded by its native promoter region (15.3 kb), has been validated as *Ma* as it conferred the same complete-spectrum and high-level resistance as in the donor clone P.2175. The full-length cDNA (2048 aa) of *Ma* is the longest of all R genes cloned to-date. Its TNL structure is extended by a huge C-terminal post-LRR (PL) region (1088 aa) comprising five repeated C-terminal PL exons.

S16-T3

ROS, a two-faced Janus in plant responses to pathogens?

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Reactive oxygen species (ROS) are produced at and around infection sites during plant defence responses. NADPH oxidases, also referred as respiratory burst oxidases homologues (RBOH) have been shown to play an important role in ROS production in plants. Of the 10 RBOH genes (A—J) in *Arabidopsis thaliana*, AtRBOHD and AtRBOHF are known to be involved in defence responses. The cyst nematode *Heterodera schachtii* infects roots of *Arabidopsis* plants and parasitises by modifying root cells to a syncytial feeding cell system. The aim of this work is to understand the role of AtRBOH-mediated ROS during plant—nematode interaction. Visualisation of ROS production using DAB (Diaminobenzidine), CM-H2DCFDA and transgenic plants encoding H_2O_2 sensor HyPer revealed a distinct pattern during migration, syncytium induction, and feeding. Our results suggest that AtRBOHD and AtRBOHF are required for this pathogen-induced ROS production. Unexpectedly, knock-out mutation of atrbohd/f reduced development of female nematodes by 90%, a situation resembling incompatibility. Treatment of plants with DPI (diphenylene iodonium), an inhibitor of NADPH oxidase, gave similar results. Similarly, overexpression of AtRBOHD increases the suitability of plants to nematodes. Further analyses of atrbohd/f revealed up-regulation of plant defence response genes (PR1, PR2, PR3 and PR5) in syncytia. Taken together, our findings suggest a novel role of AtRBOH mediated ROS in the function of compatible plant-pathogen interactions.

S16–T4

The tomato somatic embryogenesis receptor Kinase 3A and 3B are required for root-knot nematode defence

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The plant Somatic Embryogenesis Receptor Kinase 3 (SERK3)/Brassinosteroid Insensitive 1-Associated Kinase 1 (BAK1) is required for pattern-triggered immunity (PTI). Tomato (*Solanum lycopersicum*) has three *Sl*SERK members and two of these members have sequence identity to *At*SERK3 and therefore were named *Sl*SERK3A and *Sl*SERK3B. In a previous study, we had identified a role for the third member *SlSERK1* in *Mi-1*-mediated resistance to potato aphids but not root-knot nematode (RKN; *Meloidogyne incognita*). We used virus-induced gene silencing (VIGS) to identify roles for *SlSERK3A* and *SlSERK3B* in tomato defence. Silencing both *SlSERK3A* and *SlSERK3B* triggered necrotic lesions in tomato. Plants silenced for the individual *SlSERK3A* or *SlSERK3B* were evaluated for infection by RKN and the bacterial pathogen *Pseudonomas syringae* pv. *tomato hrcC*$^-$ mutant. Silencing either *SlSERK3A* or *SlSERK3B* resulted in increased RKN infection and *Pst hrcC*$^-$ bacterial titre indicating that both *SlSERK3*s are positive regulators of defence and suggesting that nematode recognition functions through PTI. The flagellin-derived peptide flg22-induced ROS was suppressed in plants silenced for either *SlSERK3A* or *SlSERK3B*. *Sl*SERK3A and *Sl*SERK3B are active kinases and localised to the plasma membrane. In addition, both *Sl*SERK3A and *Sl*SERK3B interact with the Flagellin Sensing2 (*Sl*FLS2) receptor in a flg22-dependent manner.

S16–T5

The genetic background plays an important role on durability of plant major resistance genes to nematodes

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Root-knot nematodes, *Meloidogyne* spp., are extremely polyphagous plant parasites and are present throughout the world. Since the use of most chemical nematicides is being prohibited, genetic resistance is an efficient alternative way to protect crops against these pests. However, resistance genes (R-genes) are limited and nematode populations are able to overcome them in time. Good management of these valuable resources is thus a key point of R-gene durability. In pepper, *Me3* is a dominant major resistance gene, currently used in breeding programmes, which controls *M. arenaria*, *M. incognita* and *M. javanica*, the three main root-knot nematode species. In this study, it was introgressed in either a susceptible or a partially resistant genetic background in either homozygous or heterozygous allelic status. Confronting these genotypes with a high inoculation pressure of an avirulent *M. incognita* isolate or a *Me3* virulent laboratory-selected population (obtained by successive re-inoculation on a *Me3* R-pepper line) demonstrated i) that the genetic background plays an important role, *Me3* is overcome more easily in a susceptible genetic background than in a partially resistant one, and ii) that the allelic status has no effect. Experiments are now underway to detect and localise QTLs of resistance to root-knot nematodes explaining the differences observed between susceptible and partially resistant genetic backgrounds, and to determine the effectiveness of their 'protective' role on the major R-genes. All these results are of importance for the creation of new varieties by breeders who have to take into account the plant material used and the resistance gene they want to introgress.

This research was supported at the national level by 1/ the Agriculture Ministry with a CTPS (permanent technical committee of the selection of the crop plants) project on the durability of resistance to RKN in Solanaceae (2007—2010) and 2/ the Ministry of Higher Education and Research with a CIFRE contract (PhD funded by six private seed companies). At the European level, this research was supported by the European network for the durable exploitation of crop protection strategies, acronym ENDURE (2008—2010).

S16–P1

Suitability of selected potato cultivars to *Meloidogyne enterolobii* and three other *Meloidogyne* spp. occurring in Florida

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Meloidogyne enterolobii was reported in the USA in 2002 infecting ornamental plants. The objective of this study was to determine the suitability of potato cvs Atlantic, La Chipper and Red LaSoda as host for *M. enterolobii, M. floridensis, M. incognita* race 3, and *M. javanica* race 3 in a duplicated experiment in a greenhouse. Each plant was inoculated with 5000 eggs of each nematode species. Tomato cv. Rutgers was included as a susceptible host to all nematode species. At harvest, numbers of galls and egg masses per root system, the number of eggs per gram of fresh roots, and the reproduction factor were determined. All potato cultivars were susceptible, and there were no differences in the susceptibility among the potato cultivars to the nematode species ($P > 0.05$). No differences were observed in the root galling induced by *M. enterolobii*, *M. floridensis*, and *M. javanica* race 3 among the potato cultivars ($P > 0.05$). *Meloidogyne incognita* had a lower gall rating on La Chipper than the other two potato cultivars. *Meloidogyne enterolobii* and *M. javanica* race 3 produced the highest egg mass numbers on Atlantic and Red LaSoda, whereas *M. incognita* race 3 and *M. floridensis* produced the highest numbers on Atlantic. Red LaSoda and Atlantic sustained more eggs of *M. incogntia* and *M. javanica* than La Chipper. *Meloidogyne enterolobii* produced the highest number of eggs per gram of roots on Atlantic (7833), whereas *M. floridensis* produced the highest on Red LaSoda (2334) ($P < 0.05$). In summary, all the potato cultivars were good host of the *Meloidogyne* spp. included in this study.

S16–P2

Determination of the effectiveness of biological agents against root-knot nematodes in vegetables

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The aim of this study was to investigate the effects of some biological agents against root-knot nematodes. Susceptible (SC2121) tomato plants, three fungal agents (Bionematon, Trichoflow, Mycotal) and one bacterial agent (Serenade) were tested and Fenamiphos was used as control in the experiment conducted in greenhouse. Plant height, leaf number, wet and dry weight of the shoot, root weight, gall index, egg number per plant, females per plant, nematode number of total root and reproductive index were performed, 8 weeks after inoculation by applying 2000 or 0 egg per pot. Plant growth parameters showed significant differences between pots with biological agents and pots without. The highest values for plant height, leaf number, wet and dry weight of the body, root weight were determined in Fenamiphos- and Bionematon-applied plots and followed by Trichoflow and Serenade. The lowest values were obtained in pots with Mycotal and the positive control, which were statistically different from the high values. Serenade was the only biological agent enhancing plant growth and development and has the highest values, even greater than the negative control. When infested plants were compared with each other for gall index, egg number, females on roots and total nematode numbers in roots and reproductive index, highest values were obtained in the control plots, followed by Trichoflow and Serenade, and the lowest values on the Bionematon and Fenamiphos plots. Therefore, biological agents may reduce the damage of root knot nematodes in vegetable growing areassurelyexperiment.

S16–P3

Screening of winter/facultative wheat germplasm against the cereal cyst nematode *Heterodera filipjevi* in Turkey

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Cereal cyst nematode is considered one of the major plant-parasitic nematodes affecting cereal production worldwide and causes huge damage and losses. The most reported pathogenic species are *Heterodera avenae*, *H. filipjevi* and *H. latipons,* each having different pathotypes. At least 12 pathotypes have been described for *H. avenae*. Several control options are used to protect against CCN damage; however, one of the most cost effective, environmentally friendly and easily adopted control measures is the use of genetic host resistance which will maintain nematode populations below economic threshold for damage. Many sources of resistance against cereal cyst nematodes (CCN) rare known but their effectiveness and usability is dependent on the reaction of the specific species and pathotype present in different regions. CIMMYT, in collaboration with partners in Turkey, have screened about 3000 wheat entries against *H. filipjevi* and *H. avenae* under controlled conditions. Up to the present, more than 100 genotypes with resistance to CCN (groups 1 and 2 out of five based on number of cysts per plant root) have been identified. The resistant germplasm represents a broad geographical spectrum of breeding lines and varieties from Turkey, Europe, Central Asia and IWWIP program. The best resistant germplasm is being tested in the field under high and low nematode pressure to evaluate their yield performance and tolerance to CCN. The current research aims to identify possible molecular markers for resistance to CCNs through association mapping. A set of 278 lines with variable levels of resistance to CCN has been selected for this study.

S16–P4

Comparison of molecular markers linked to *Mi-1* gene in tomato

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Tomato is one of the most important vegetables worldwide. Root-knot nematodes cause serious yield losses of tomato in the world. Resistant tomato varieties are used for control of nematodes. The *Mi-1* gene, which was mapped on the short arm of chromosome 6 and cloned, controls resistance to root-knot nematodes in tomato. Molecular markers tightly linked to the *Mi-1* gene have been developed in previous studies. These markers have been used for screening for the *Mi* gene in tomato lines, potential and commercial hybrids carrying Ty-1. Also, they were compared with each other. All markers Rex, PMi12 and Mi23 gave consistent results in plants bearing Ty-1. However, Rex-1 produced false positive results in other samples including Ty-1, except for four plants bearing Ty-1. Our results showed both PMi12 and Mi23 markers can be used for screening of *Mi*-1 gene in tomato breeding.

S16–P5

Resistance and tolerance in lily cultivars for the root lesion nematode, *Pratylenchus penetrans*

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Dutch lily growers suffer severe damage from root lesion nematodes (*Pratylenchus penetrans*). Therefore, they often choose to apply metam-sodium to the soil before lilies are planted. Using resistant and tolerant lily cultivars could be a good tool to control *P. penetrans*. However, not much is known about this. It was assumed that all lily cultivars are good hosts for *P. penetrans* until results from a pot experiment in 2010 showed that cv. Siberia was actually a bad host for this nematode with final nematode population densities lower at all initial population densities (Pi) tested. However, cv. Siberia could suffer 100% yield loss at the highest nematode densities. It is possible that more cultivars are bad hosts and/or have a higher tolerance level. It would be interesting to screen a wide range of cultivars. However, the costs of doing this with pot experiments using a wide range of Pi or with field experiments are too high to test large numbers of cultivars. Therefore, a cheap, but scientifically valid, test is needed. In order to develop such a test a first pot experiment, including five lily cultivars at ten Pi, has been started. The population dynamics will be modelled, in order to find out whether downscaling to one or a few Pi will be possible and to pinpoint which Pi(s) are optimal for this test.

S16–P6

Host suitability level of brassica plants for *M. incognita* and *M. arenaria*

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Biofumigation with Brassica plants could be an alternative method to suppress root-knot nematodes. It is important to select poor- or non-host Brassica genotypes for *Meloidogyne* spp. to investigate the efficiency of biofumigation. Forty local cultivars were screened for host suitability level to *M. incognita* and *M. arenaria* in pot experiments. Seedlings of each genotype were inoculated with 2000 or 0 eggs per plant. Pots were arranged in a completely randomised block design with 5 replicates in a controlled greenhouse at 20±1°C for 60 days. The experiment was repeated twice. Host suitability was based on the gall index (GI), egg masses index and nematode developmental stage. No galls were observed on roots of arugula (*Eruca sativa* cv. Istanbul). The presence of plants with GI ≤ 2 was observed on roots of 12 genotypes for both species of root-knot nematodes. Among the evaluated genotypes, 14 genotypes did not allow any egg production, and the gall index of six genotypes was less than two for both root-knot nematodes. When all parameters were evaluated, 14 genotypes may have research potential for biofumigation.

S16–P7

Systemic Acquired Resistance (SAR) can be induced by salicylic acid in tomato–root knot nematode interaction

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Salicylic acid (SA), and its chemical homologue BTH, have been proved to restrict root-knot nematode (RKN) infestation on tomato, taking into account a number of variables, such as chemical dosages, application times and methods, and soil composition. SA is known to elicit SAR by inducing the expression of a set of inducible defence-related genes (*PR*-genes). In this study, susceptible tomato plants untreated or treated with 150 ppm (µg SA/g soil) were inoculated in pots with a high density inoculum of *M. incognita* (3 J2/cm^3 soil). Infestation level was estimated by determining infectivity, female fecundity, and damage potential in untreated and SA-treated plants. Moreover, induction of SAR was detected, in roots and shoots, by the expression of two SA-inducible *PR*-genes, *PR*-2 and *PR*-5, which encode for a glucanase and a thaumatin-like protein, respectively. Glucanase and catalase enzyme activities, as well as phenol content, were also assayed. Comparable tests were carried out on roots and shoots of uninfested and infested *Mi-1.2*-carrying resistant tomato. *PR*-gene expression was induced in roots and repressed in shoots of infested SA-treated susceptible plants, whilst in infested resistant plants *PR*-genes were down-regulated in roots and up-regulated in shoots. Glucanase activity showed the same trend in both cases. Data suggest that SAR may be induced in a compatible plant—nematode interaction by SA treatment, although it occurs only in roots, once tomato plants have been infested by nematodes. By contrast, SAR seems to be induced in shoots during incompatible tomato—RKN interactions.

S16–P8

Use of Mi23 and Pmi SCAR markers for *Mi*-1.2 gene in pure tomato lines and F2 populations

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Root-knot nematodes have a wide range of host plants and cause important yield losses in many crop plants. The resistance gene *Mi* was introduced to the cultivated tomatoes from the wild tomato species *Solanum peruvianum* in 1940. The gene confers resistance to *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. Molecular methods are used extensively in tomato breeding programmes. Developed SCAR markers linked to the *Mi-1.2* gene, the co-dominant gene Mi-23 and Pmi markers have been used in tomato breeding programmes. In this study, tomato genotypes including pure lines, F1 and F2 were screened for their resistance as susceptible, homozygote or heterozygote resistant by using Mi23F-Mi23R and Pmi specific primers for *Mi-1.2* gene. Using these markers in Alata Horticultural Research Institute, 40 pure tomato lines yielded homozygote resistance bands, F1 and some F2 heterozygote resistance bands yielded markers. These findings suggest that these markers can be used to develop nematode resistant standard and hybrid tomato cultivars.

This work waswas supported by TÜBİTAK (Project number: 109G096).

S16-P09

Daily temperature fluctuations and nematode defense-gene expression

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Plant defences and resistance against pathogens can be affected by changing environmental conditions, particularly temperature. Although temperature has long been known to influence plant resistance to disease, it is only recently that the molecular mechanisms by which temperature-generated signals intersect with immune pathways are being uncovered (Wang et al., 2009). Climatic variation influences the trade-off between plant growth and disease resistance. Mechanisms of potato plant responses to infestation by *Globodera rostochiensis* Ro1 (10 cysts/plant) under daily fluctuating temperatures DFT (low hardening temperature of 5°C for 2 h) was investigated. It was shown that DFT enhanced both plant chilling tolerance (Sysoeva et al., 2005) and resistance to *G. rostochiensis*. Elevated level of expression of COR-gene *ci7* responsible for cold resistance formation and R-gene *H1* responsible for resistance to nematode in plants was established. Evidence suggests that chromatin remodelling affected by DFT might play a role in determining the kinetics of defense-related gene expression in plants. It is possible to suggest that DFT primes the potato plant for the battle with the nematode. This work was supported financially by the Russian Foundation for Basic Research (№ 10-04-00097a) and Biology Department of RAS (№ 01201262103).

S17 – Plant-parasitic nematodes in temperate crops: management of plant nematodes

Convenor: Galip Kaskavalci

S17–T1

Effect of crop rotation on control of root lesion nematodes in rain-fed wheat system

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Wheat is cultivated on a large scale as a rain-fed systems in the west parts of Iran. Root lesion nematodes (RLN), *Pratylenchus thornei* and *P. neglectus,* are widespread in wheat fields with high population density, particularly where cultivation is intensive and wheat is grown after wheat without rotation. In order to investigate the effect of crop rotation as one of the control measures for RLN, a four year experiment was conducted during 2008—2012 with 4 replications in a completely randomised block design arrangement in a naturally infested field in Ekbatan agricultural station (Hamadan Province) with 300 mm annual precipitation. Six selected treatments were included: wheat—wheat, wheat—fallow, wheat—chickpea, wheat—canola, wheat—barley, and wheat—wheat along with adding 20 tonnes of manure per hectare. Population density of RLN was counted before planting and after harvesting by sampling of soil from each plot. Moreover crop yield, number of tiller, stem length, and thousand grain weights were calculated every year. On the basis of analysed results, planting wheat after wheat and wheat after canola were the most unsuitable rotations and increased RLN population density and significantly reduced wheat yield. Wheat after fallow and wheat after barley were the best crop rotations and significantly reduced RLN population density, although the treatment of wheat after chickpea showed the maximum wheat yield among the six treatments but increased nematode population to a low level. With regard to the findings of these experiments RLN have a major effect in reducing rain-fed wheat yield in west of Iran and crop rotation with selected crops such as barley or legumes or fallow, could be considered one of the most appropriate and practical methods to control the RLN and reduce crop damage.

S17–T2

Management of *Meloidogyne hapla* in organic farming using an overwintering green legume as trap crop

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Plant-parasitic nematodes such as *Meloidogyne hapla* are a major threat to organic vegetable production. In particular, legumes that are important for N fixation often foster the build-up of damaging population densities. The objective of the present research project was to develop a long-term rotation scheme based on overwintering legumes that ensures N fixation but prevents multiplication of *M. hapla* at the same time. The results of three year field studies on commercial farms indicated that this is possible if legumes are grown as a green manure crop and are seeded late August/early September and incorporated no later than June 10th. Results further indicated that development of *M. hapla* was interrupted by low winter temperatures and newly hatched juveniles first needed to infest the legumes in spring to cause multiplication. The time period required by *M. hapla* to complete its cycle can be predicted by a temperature sum (sum of mean daily soil temperature above 8°C) of 450°C after winter. However, to avoid *M. hapla* multiplication in practice, it is recommended to incorporate the overwintering legume at the latest at 350°C after winter, *i.e.* around June 10th. By then, sufficient nitrogen has been fixed. To avoid nitrogen losses, Italian ryegrass was grown afterwards and onions were seeded in March the next year. Regarding onion yield, plots with lowest nematode numbers yielded the highest amount of marketable onions. Associated greenhouse experiments confirmed that the first offspring of *M. hapla* appeared at a temperature sum of 330°C; however, main reproduction occurred at 450°C. Temperatures below 5°C disturbed nematode development. In conclusion, legumes can be successfully grown as an overwintering crop to fix nitrogen and reduce *M. hapla* densities at the same time.

S17–T3

Will *Meloidogyne minor* become a threat to our agriculture?

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For several years, a new species of root-knot nematode, *Meloidogyne minor*, has been reported from parts of The Netherlands, Belgium, UK and Ireland. So far, this species causes most problems on golf courses but has also been reported from a potato field in Zeijerveld (The Netherlands) where it caused strong growth reduction in potato plants, but no damage to potato tubers. As The Netherlands is a potato-producing country, field experiments were set up to evaluate the potential risks this species poses. In a 4-year field experiment the host status of potato, rye, annual ryegrass, perennial ryegrass, creeping bent grass, white clover, sugar beet, maize, fodder radish and rapeseed was tested. In general, potato seemed to be a good host for this nematode species with a Pf/Pi-ratio about 1.5. However, there was no reduction in potato production, neither in yield nor in tuber quality. In addition, only perennial ryegrass and white clover were good hosts for this nematode. From these results one might conclude that this nematode will not become a major threat to European agriculture.

S17–T4

Lost competence in nematology may cause recurrent crop damage

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The decrease in lecturing and field training of students in nematology threatens recruitment, scientific development and awareness of plant nematodes as parasites. This results in plant-parasitic nematodes being overlooked as factors of crops failure. This allows damage to go on for years to the disadvantage of the farmers. In Scandinavia the most common species of cereal cyst nematodes are *Heterodera avenae* and *H. filipjevi*. Both have pathotypes with differing host preferences. Serious damage seems to appear with about 40-years intervals. Since this is equal to the career of a scientist, loss of competence may result in recurrent peaks of damage, and a need to repeatedly re-build lost expertise and know-how. Another example relates to the stem nematode *Ditylenchus dipsaci*, the causal agent of "stock" in clover. This condition was described in 1819 and remained a problem over the years. In Scandinavia the nematode was common in 1940—1950. In 1950s resistance breeding was significantly improved in efficacy by new techniques of testing and selection. The use of resistant varieties of clover and alfalfa essentially solved the stem nematode problem in Nordic countries, and the resistance testing was abandoned. Today, the increasing use of clover in organic farming may over time cause the stem nematode to reappear as an important pathogen requiring new action to be taken.

S17–T5

The effect of cationic surfactants on plant-parasitic nematodes *in vitro* and *in vivo*

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North Western Europe is undergoing climatic change and, as a result, the average minimum temperatures in Northern Ireland have increased steadily over the last twenty years. It is known that changing patterns of temperature and humidity will allow species to shift their habitat or geographical range or it may allow for increased reproduction, leading to greater numbers of pests and ultimately to greater feeding damage in crops. This has the potential to increase the number of nematode pests appearing which were previously undiscovered such as has been apparent with the emergence of *Meloidogyne minor* in Europe.

A number of nematicides having been deregulated in recent years due to the implementation of the European Union authorisation Directive 91/414/EEC (Europa 1991) there is a demand for new and novel treatments to manage nematode populations in amenity, horticulture and agriculture. This study reports on the effects of liquid cationic surfactants on both cyst and migratory plant-parasitic nematodes. Concentration levels needed to induce nematode mortality are examined *in vitro* and the results of this extended to plot trials. Potato cyst nematode, *Globodera pallida,* cysts when treated with a 5% solution of these compounds reduced the number of juveniles hatching by 89% and in a subsequent field trial, the multiplication rate of *G. pallida* was reduced by 74%. On an amenity site, the overall nematode burden under grass was reduced by 87% over a six week period. Evidence has shown that this formulation can assist in the management of cyst and vermiform nematode populations. Further studies are required to establish its full potential and its mode of action.

S17–P1

Soil solarisation for control of quarantine nematode *Globodera rostochiensis* in potato fields of Hamadan province

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The potato golden cyst nematode *Globodera rostochiensis* was observed and reported in some potato field in Hamadan Province for the first time in 2008. In spite of all quarantine activities, the disease spread over a large area and damaged infested fields with up to 100 juveniles and eggs per gram of soil. Cysts are the resistant stage of nematode and can survive 20—30 years in soil and disease control is very difficult, expensive and time consuming. Farmers grew susceptible potato variety *Marfouna* constantly, which encouraged nematode population. As the fields are fallow during the warm months of summer, it is possible to implement soil solarisation for killing second stage juveniles in cysts; for this purpose an experiment was done in a completely randomised blocks design with 4 replications in an infested potato field of Bahar city. There were six treatments: 4, 6 and 8 weeks of solarisation by covering plots with 20 micrometer thick plastic covers which was compared with fumigated and free plots as controls. Before and after solarisation the number of juveniles and eggs in soil of each patch was counted. During the soil solarisation, the temperature under the plastic cover was measured in 0—10, 10—20 and 20—30 cm depths of soil by special thermometers. The highest temperature recorded was 82.7°C under plastic on 4 Aug. 2011 while the average daily temperature was 31°CC. Analysed results of the test by SAS statistical software revealed that number of juveniles and eggs in different treatments were significantly different and soil solarisation for 8 weeks with application of 10 tonnes poultry manure per hectare before soil solarisation decreased nematode population up to 48%. With findings of this test and similar experiments soil solarisation is a practical and effective way to control the disease with respect to environmental aspects.

S17–P2

Effects of organic soil amendments on population reduction of *Globodera rostochiensis* in greenhouse condition

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Potato cyst nematode (PCN) *Globodera rostochiensis,* a major pest of potato worldwide, has caused considerable damage in potato production region of Hamadan in Iran. Addition of organic materials to infested soil was shown to control plant-parasitic nematodes by releasing toxic compounds, increasing microfauna and microflora, and by improving soil structure, which has a positive effect on plant growth. Oil seed cakes are amongst the most frequently used and their effects sometimes were comparable to that obtained with chemicals. In this work, canola and soya meal amendments were examined on PCN behaviour on potato. Different rates of canola and soya cakes were incorporated into infested soil with PCN and after 2 weeks pots were planted with potato. Metam sodium as pretreatment and resistant/tolerant potato cultivars to PCN were included for comparison with non-treated infested soil with nematode. At harvest, plant growth parameters and PCN population density were measured. The results show promising effects of amendment on nematode reproduction and density in treated soil.

S17–P3

Effects of some agronomic crops on decline rate of *Globodera rostochiensis*

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Since its first report in 2008, rotation has been the main method recommended for controlling potato cyst nematode (PCN) *Globodera rostochiensis* infestation in Hamadan Province in Iran. In this respect different agricultural crops suitable for the region are being tested in the field. To evaluate the superiority between the plant species, experiments were conducted to monitor the influence of some of these plants on decline rate of PCN *in vitro* and in a pot test. During the growth period, root diffusates of all plants were obtained by drenching soil with distilled water and collected liquid was filtered before use. Cysts of PCN were placed in root exudates of chosen plants such as garlic cv. Hamadani, oil seed canola commercial cv. Hyola, corn cv. KSC 301, coriander, white and red onions and cucumber; hatched juveniles were collected weekly and final percentage hatch was determined after 8 weeks. In glasshouse experiment, the same plants were grown in sterile soil infested with cysts of PCN. At harvest plants were up-rooted, soil samples were taken and decline rates of eggs within cysts were analysed and compared between different crops. All pots were planted with a susceptible potato cultivar; at harvest, potato yield, nematode density and reproduction rate were determined. The results so far show that root diffusates of all plants stimulated hatch to some extentextent; the highest number of juveniles emerged in corn root diffusates, white onion and coriander. The resultswill be discussed further.

S17–P4

The management of *Meloidogyne incognita via* arthobacter (ROA) and *Tagetes patula* (French marigold) extracts

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Meloidogyne incognita is a highly virulent pathogen of many crops including cucumbers and tomatoes in Turkey. A greenhouse study was conducted to determine the affects of an Arthobacter (ROA) and plant extracts of *Tagetes patula* (French marigold) on controlling this pathogen on tomatoes (*Lycopersicon lycopersicum*) Safir F1 and cucumber (*Cucumis sativus*) Yakamoz F1 compared to Iprodione (Devguard 500 SC) nematicide. The experiment consisted of two nematode inoculum levels, two plant varieties, three treatments replicated five times. Additionally, untreated controls with no nematodes (-) and treated controls with nematodes (+) were included. One week after the transplanting four-week old seedlings, the pots were inoculated with 1,000 eggs and (or) second-stage juveniles/pot and 2000 eggs and (or) second-stage juveniles/pot for each plant variety. Throughout the experiment plant height and at the harvest, the percentages of roots galled and plant fresh weight per root system were recorded for all treatments. Percentages of root galled per root system differed among the treatments in both nematode inoculum levels for both plant varieties. *Tagetes patula* extracts treated plant roots revealed the lowest galled roots percentages (28%) among other treatments except for Iprodione treated plant roots (9%). Fresh weight and height of plants did not differ significantly among treatments.

S17–P5

Soil nematodes occurring in nurseries of ornamental trees and shrubs in Poland

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Ornamental plant production in Polish nurseries is a new branch of horticulture, whose development began in the 1990s. At present, nursery production involves about 45% of the total value of horticultural production and ornamental shrubs and trees make up a considerable part of this production. In 2007—2008 samples of soil were taken from 26 species of trees and shrubs at 15 nurseries located over Poland. The root-knot nematodes (*Meloidogyne* spp.) occurred occasionally, whilst root-lesion nematodes were often recorded in ornamental nurseries. Based on our research, *Pratylenchus penetrans* is the most important nematode, especially in nurseries where *Robinia pseudoacacia* is produced. The occurrence of semiendoparasites in ornamental plants nurseries was frequently noted. The most common nematode species was *Helicotylenchus digonicus*, which occured mainly in soil taken from rhizosphere of *Betula pendula* and *Robinia pseudoacacia*. *Rotylenchus robustus*, the most dangerous nematode damaging trees and shrubs, was not found frequently in the soil taken from nurseries. However, the highest level of pest population was noted in cultivation of *Tilia cordata* (52 individuals/200 g soil).

In general, ectoparasites were numerous in the soil taken from ornamental trees and shrubs nurseries. Among them the most common species was *Bitylenchus dubius* but it did not occur in large numbers. In the cultivation of ornamental trees and shrubs the most dangerous nematode is *Paratylenchus projectus* – especially for *Berberis thunbergii, P. bukowinensis* and *Acer pseudoplatanus*. *Gracilacus straeleni* seems to be the most important plant-parasitic nematode in cultivation of *Tilia cordata*. Nematodes from Criconematidae family are also present in the soil, but not in large numbers. The soil in nurseries may also be infested by nematodes transferring plant virus diseases, but they occur in low numbers and only rarely. However. *Trichodorus viruliferus* appeared in large numbers in the soil taken from rhizosphere of *Tilia cordata* (56 individuals/200 g soil).

S17–P6

Effect of treatment of tomato seeds and root-knot nematode egg sacs with various doses of γ-irradiation on the development of plants and nematode

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Radioactivity is a process which has influenced life on our planet since organic matter has been organised. One of its components is gamma rays, which have great biological effects on all living organisms. Gamma-rays can induce various mutations, and activate biochemical systems and resistance of organisms. Investigations of the effect of ionizing radiation on host—parasite systems make clear various aspects of interactions between parasite and its host and it is of great importance to increase the plant resistance to parasitic attacks.

The influence of various doses of γ-irradiation (90, 700 and 1800 mGy) on cv. Tiny Tim tomato plants and developing eggs sacs of root-knot nematode *Meloidogyne arenaria* were investigated. Ionizing radiations of tomato seeds by low dose (90 mGy) stimulate development of plants. High doses of γ-irradiation (700 and 1800 mGy) suppress development (height, root and shoot weight) of tomato plants. High irradiation doses (700 and 1800 mGy) retarded the growth of nematodes. Metric characteristics of *M. arenaria* females, mainly body size, were smaller. The highest experimental dose (1800 mGy) prevented the development of females of *M. arenaria* (J4) to mature forms. A change of female to male ratio under the influence of γ -ionizing radiation has been observed, resulting in a decrease in males. These results show aspects for future research into the application of γ-irradiation in management of root-knot nematodes.

S17–P7

Distribution of nematodes related to onion cropping in Karaman province in Turkey

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Onion is produced in a 25,000 ha area in Karaman province, providing 3.4% of Turkey's production. The bulb and stem nematode, *Ditylenchus dipsaci,* damages onions in Karaman province. A survey was conducted to determine the distribution of plant-parasitic and free-living nematodes on the onion cropping area in Karaman province. Plant and soil samples were collected from onion planted fields. Nematodes were extracted using the "Modified Baermann Funnel" technique from three plants and 100 g of soil from each sample. *Ditylenchus* spp. were extracted from 15% of the plant samples. Nematode numbers ranged between 0—140, on average 5 nematodes per 3 plants. Sixty-one percent of the soil samples were found infected with *Ditylenchus* spp. Nematode numbers were between 0—165 per 100 g of dry soil, on average 33 nematodes per 100 g of dry soil. Other abundant nematode genera were *Paratylenchus* (56%) and *Tylenchus* (49%) in sampled fields. The most abundant free-living nematodes were bacterivorous nematodes which were found in 98% of samples. *Cephalobus* and *Eucephalobus* were the most frequently found Genera of bacterivorous nematodes. Eighty-six percent of samples contained fungivorous nematodes belonging to the genera *Aphelenchus* and *Aphelenchoides*.Omnivorous nematodes in Dorylaimida were found in 23% of samples.

S17–P8

Population dynamics of *Pratylenchus neglectus* on cereal and rape crops

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Pratylenchus neglectus is a migratory endoparasitic nematode species often isolated from the root zone of mono- and dicotyledonous plants including agricultural crops, vegetables, ornamentals and weeds. This nematode species causes serious damage in cereals, especially barley and wheat.

The influence of winter and spring forms of cereal and rape crops (*Triticum durum, T. spelta, T. aestivum, Avena sativa, Hordeum vulgare, Secale cereale, Brassica napus*) on changes in population density of *P. neglectus* was investigated in an outdoor pot experiment. Both spring and winter forms of cereals caused an increase of nematode population density (Pf/Pi >1). In rape crops population density decreased (Pf/Pi <1). Observations on the dynamics of juvenile stages and adult forms in plant roots of wheat and *Brassica napus* were conducted in a pot experiment in a glasshouse. In wheat crops all developmental stages of *P. neglectus* were more numerous in root tissues of *T. durum* and *T. spelta* and differences were observed among populations of the nematode species. No differences were observed among populations of *P. neglectus* in roots of spring as well as winter forms of *Brassica napus*.

S17–P9

Root-knot nematodes in Norway (*Meloidogyne* spp.)

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In Scandinavian countries the northern root-knot nematode *Meloidogyne hapla* has been the species of principal focus. In recent years observations in Norway indicate that the damage caused by *M. hapla* has become more frequent in field-grown vegetables. At the present time, *M. hapla* occurs mainly in the southern-coastal areas. In addition to *M. hapla* the species *M. ardenensis* has been recorded on wild vegetation in Kristiansund (mid-Norway). In 2008 the species *M. naasi* was recorded in summer wheat (cv. Zebra) and on other graminaceous plants and weeds in Vestfold County (southern-Norway). This was the first record of a root-knot nematode on cereals in Scandinavia, and could reflect a change to a warmer climate in southern Norway. We suggest that monitoring the current spread of *Meloidogyne* spp. in Norway should be a high priority. The recent expansion of *M. hapla* suggests that the detection of this species in potato is imminent. To the best of our knowledge, Scandinavia is currently free from the quarantine pests *M. chitwoodi* and *M. fallax*. However, the monitoring of these species is urgent since both of them can be expected to survive and cause damage in the whole region.

S17–P10

Roles of soil amendment in control of plant-parasitic nematodes

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Plant-parasitic nematodes and declining soil fertility are among the main causes of low productivity of crops in Iran. Soil amendments can both improve tolerance of the plant to nematodes and also reduce nematode populations. A controlled experiment was conducted to evaluate the suppressive effect of soil amendment compost, applied at five doses (0%, 1%, 2.5%, 5% and 10% w/w), on tomato to control different genera of plant-parasitic nematodes in potting mixtures. Six genera of plant-parasitic nematodes were identified in the control and treated pots. Significant reductions were detected in the soil nematode populations of the amended soils. Population reductions due to soil amendments ranged from 45—83% compared with the control. The best reduction of number of plant-parasitic nematodes was found for the nematode genera *Helicotylenchus, Ditylenchus, Meloidogyne* and *Paratylenchus* by compost. All tested rates of compost significantly reduced the number of nematodes compared with no amended soil (control) ($P = 0.01$). However, a reduction of populations of *Geocenamus* and *Pratylenchus* genera was only observed at 5% and 10% rates, respectively. Relationship between applied doses and number of nematodes showed a significantly negative correlation. The results indicated that organic soil amendments can be useful in mitigating the negative effects of nematode infestation and poor soil fertility.

S17–P11

PESTOLIVE: an historical and ecological approach for understanding and managing soil-borne parasite communities on olive in the Mediterranean basin

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PESTOLIVE (*Contribution of olive history for the management of soil-borne parasites in the Mediterranean basin*) is a project funded by ARIMNet, an ERANET action supported by the 7th European Framework Programme and by Mediterranean non-European countries.
PESTOLIVE aims at producing knowledge and tools for new and efficient management of plant-parasitic nematodes (PPN) and plant-pathogenic fungi (PPF) in olive (*Olea europaea* L.) cropping systems and nurseries, while reducing the use of pesticides. Because of the anthropic continuum from *Olea* post-glacial refuges to oleasters (domestication) and then to olive-trees (breeding and cropping), the fragmentation of the PPN and PPF communities and of their natural enemies could explain the scattered diversity of the control techniques (especially resistant rootstocks, biocontrol, cropping strategies) developed and applied all around the Mediterranean basin. The novelty of PESTOLIVE is based on i) the analysis and the management of the parasite diversity (ecology of communities) instead of controlling emblematic species (population approach) and ii) the involvement of knowledge about the historical co-adaptation of soil-borne parasite and natural enemies communities to olive-tree domestication (origins and past assemblages) and breeding that follows the history of *O. europaea* around the Mediterranean basin.

S17–P12

Determination of efficiency of alternative methods to control root-knot nematodes (*Meloidogyne* spp.) in cucumber grown greenhouses

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This study was conducted on autumn-planted cucumbers in greenhouses infected with root knot nematodes, *Meloidogyne javanica* and *Meloidogyne incognita,* in Western Anatolia (Izmir-Menderes) Turkey in the years 2010 and 2011. The aim of the study was to determine efficiency of some alternative methods to registered chemicals used to control root-knot nematodes (*Meloidogyne* spp.). In the study, it was evaluated whether preparations containing different doses of active substances such as sesame oil, broccoli, mycorrhizal fungi and azadirachtin had an effect on *Meloidogyne* spp. and total crop yields. Trials were set up as a randomised block design with 11 characters and 4 repetitions. At the end of the growing season, the efficiency of the applications was determined according to the gall index of the roots based on the 0—10 Zeck scale.

According to results of the trials, it was found that soil structure and the seedling planting period can significantly affect nematode population in soil and root galling. The first year trial was set up in a greenhouse with a 5 scaled root galling index value. The average root gall indices from 0.55 to 2.78 were obtained in plots with applied alternative methods and this was found to be less than the control plots value (5.72). The highest effects were obtained from sesame oil (84.26%–%–90.38%), broccoli (80.77%–%–82.00%) and azadirachtin (68.00%–%–86.88%) applications. The second year trial was set up in a different greenhouse with an 8 scaled root gall index. As a result of this experiment, while in the control plots the average root galling index value was determined as 8.9, alternative methods applied to plots resulted in index values ranged from 6.8 to 8.2. The highest effects of the alternative methods were obtained from sesame oil (20.22%–%–23.60%) and broccoli (20.22–%–21.35%) applications.

According to these results, sesame oil and broccoli applications are considered to be promising as alternative control methods to the registered nematicides in greenhouses infested with *Meloidogyne* spp.

S17–P13

Occurence of the northern root-knot nematode in Republic of Srpska, Bosnia and Herzegovina

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During 2010 and 2011, surveys on potato cyst nematodes were conducted in the Republic of Srpska, Bosnia and Herzegovina. In the 120 samples that were processed each year, no cysts of *Globodera* spp. were found. However, potato producers from two areas in the highland complained about damage on tubers. Symptoms suggested the presence of root knot nematodes. Additional sampling for root-knot nematode was performed in October 2011. Soil samples were extracted using the Oostenbrink elutriator and nematode genera were identified using a compound microscope. In 13 out of 13 samples from Nevesinje, second–stage juveniles (J2) of root-knot nematodes were detected. In Rogatica, J2 of *Meloidogyne* sp. were detected in 12 out of 20 fields. In Nevesinje the highest number of nematodes per 100 ml soil was 225, while in Rogatica it was 1090. Molecular identification was performed using species-specific primers. This technique revealed that the species was *Meloidogyne hapla*, the northern root-knot nematode. Tests on resistance and tolerance of potato varieties is in progress.

S17–P14

Determination of the effects of plant-parasitic nematodes on potato plant development in İzmir, Turkey

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This study was conducted with the aim of determining the effect of plant-parasitic nematodes on potato plant development. The field experiments were conducted in a field naturally infested with plant-parasitic nematode species in İzmir-Ödemiş (Bozdağ) during 2007 and 2008. The experiments were arranged as a randomised block design with eight characters and five replications and repeated twice. Population dynamics and their effects on plant growth in potato varieties such as cvs Agria, Granola, Marabel and Marfona were investigated. In plots infected mainly with *Globodera rostochiensis* and *Pratylenchus penetrans*, it was found that the plant growth parameters, such as plant yield, tuber size, maturation period and plant heights, were affected differently by these nematodes. Yield data obtained from both first and second year work showed that Marfona (with 49.17% and 111.20%, respectively) and Marabel (with 52.17% and 96.77%, respectively) were the most affected potato varieties. Granola species with 21.30% (first year) and 33.48% (second year) was the least affected variety.

As a result of this study, it would be recommended that cv. Granola could be planted in the fields infested by the nematode species under the Izmir conditions. Also it was suggested that the use of nematicides in infested fields will increase potato yield.

S17–P15

Survey of plant-parasitic nematodes in the main seed potato production areas in Heilongjiang Province of China

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In China, potato is the fourth most important crop after rice, wheat and maize. Potato acreage and industry are increasing very rapidly. The production area in 2011 was 5.2 million ha with a total yield of 81.5 million tonnes. The economic value of this crop was RMB 21000 (2600 Euro) per ha. In Heilongjiang the climate and the fertile soils are highly suitable for seed potato cultivation. In 2011 seed potato was cultivated in 285,500 ha with a yield of 7 million tonnes. The average yield in China per ha is 1.1 tonnes but in Heilongjiang is 1.7 tonnes. A previous survey in potato fields in Liaoning Province recorded 29 genera of plant-parasitic nematodes. The most important were *Ditylenchus destructor*, *Pratylenchus coffeae, Meloidogyne* sp., *Trichodorus* sp., *Paratrichodorus* sp., *Longidorus* sp. and *Xiphinema* sp. Plant-parasitic nematodes could be a factor affecting potato production in Heilongjiang, but so far no systematic studies on plant-parasitic nematodes have been made. As a priority for HAAS, the main objective of the project is to study the occurrence, distribution and importance of species of parasitic nematodes of economic concern in seed potato in Heilongjiang.

The study is financed by: The Ministry of Science and Technology of the People's Republic of China, project nr. S2012ZR0328, and by HAAS-Bioforsk.

S18 – Plant-parasitic nematodes in tropical crops

Convenor: Danny Coyne

Sponsored by Syngenta

S18-T1

Occurrence of plant parasitic nematodes on banana in Lebanon and the effect of plant extracts and essential oil of *Origanum* sp. on burrowing nematodes

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During a survey of banana plantations in the coastal area of Lebanon, 93 samples were collected for plant-parasitic nematode assessment. Nematodes were recovered from 96.7% of soil samples and 88.2% of the root samples. The soil infestation ranged between 0 to 39 nematodes/g soil, while in roots densities ranged between 0 to 72.5 nematodes/g roots. *Radopholus* sp. were recovered from both soil and root samples, in addition to *Rotylenchulus* sp., *Xiphenema* sp., *Longidorus* sp., *Meloidogyne* spp., *Helicotylenchus* sp., and *Pratylenchus* spp. Crude oil and eight pure components of *Origanum* were tested against *Radopholus* sp., of which thymoquinone (LC_{50}=0.045 µl/ml), thymol (LC_{50}=0.18 µl/ml), and carvacrol (LC_{50}=0.9 µl/ml) proved most toxic against *Radopholus* sp. following 4 h of exposure, followed by p-Cymol (LC_{50}=6.3 µl/ml), and (1S)-(-)-α-Pinene(LC_{50}=36 µl/ml). However, caryophyllene (LC_{50}=49 µl/ml), terpinene (LC_{50}=58 µl/ml) and (1R)-(+)-α-Pinene (LC_{50}=58 µl/ml) were less effective, even after 24 h of exposure. Exposure to crude oil (LC_{50}=4.8 µl/ml) also resulted in a high degree of nematicidal activity on *Radopholus* sp. after 4 h of treatment. In pot experiments, plant extracts provided significant reduction in the number of nematodes, with *Cucurbita* sp. (89%) followed by *Chrysanthemum coronarium* (88%), *Melia azadirachta* (85%), *Origanum syriacum* (77%), *Foeniculum vulgare* (68%), *Inula viscosa* (66%) and *Allium sativum* (44%) proving most effective. The highest concentration of essential oils (5—6%) was detected in leaf extracts of *O. syriacum.*

S18–T2

Biology and histopathogenesis of the root-knot nematode, *Meloidogyne incognita,* on Egyptian cotton, *Gossypium barbadense*

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Root-knot nematodes, *Meloidogyne* spp. are considered among the most important global pests to crops. In Egypt these nematodes have a wide host range, affecting fruits, vegetables and field crops, such as cotton. The Governorate of Fayoum is considered an important region of Egypt in terms of cotton production, which has been shown to be heavily affected by root knot nematodes. The species involved, however, has been unclear and so the current study was undertaken to determine which species are attacking cotton. From the examination of perineal patterns of populations from Fayoum, *M. incognita* race 3 was the only species detected. Pot studies involving inoculation of the nematode, in increments of the initial population up to 2000 second stage juveniles per pot, showed serious damage to cotton growth with destruction of cortical layers, endodermis, pericycle and vascular tissues evident. Using the susceptible cotton cultivar Giza 83, inoculated second stage juveniles reached maturity after 35 days incubation at 30±5°C, while the resistant cultivar Giza 86 required over 60 days to reach maturity.Such information is important when designing a pest management program to address root knot nematode damage to cotton in Egypt.

S18–T3

Root-knot nematodes as a key constraint for intensive vegetable production systems in West Africa: identification and management strategies

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Investigations into vegetable pest problems in different agro-ecological regions of Benin have established that plant-parasitic nematodes, especially root-knot nematodes (RKN), are the most prevalent soil-borne pests attacking roots of a wide range of vegetables. The most common species, based on morphological identification, were *Meloidogyne javanica*, *M. incognita* and *M. arenaria*. Also recorded for the first time in West Africa were *M. exigua, M. chitwoodi,* and *M. artiella*. Seventeen isolates of *Trichoderma* spp., four of *Pochonia chlamydosporia* and three of *Aspergillus allahabadii* were isolated from RKN egg masses and/or rhizosphere soils. To identify a strategy for biological control, the best performing isolates of *Trichoderma* spp., *P. chlamydosporia*, *A. allahabadii*, and indigenous arbuscular mycorrhizal fungi, were assessed for their RKN control potential on (peri-)urban vegetable fields in the coastal area of Benin. The fungi were applied individually or in combination using a coconut husk carrier substrate and compared against the synthetic nematicide Furadan® (5 g/m²) in a double-cropping system of tomato—carrot and carrot—lettuce, under farming conditions. Although results were variable across sites, application of some native isolates resulted in significant suppression of RKN multiplication and root galling. Crop yields and carrot quality were also improved following the application of some isolates. This study provides evidence that beneficial microorganisms native to West Africa can provide better protection of vegetables against RKN damage than the current practice of using synthetic nematicides.

S18–T4

Distribution of plant-parasitic nematodes associated with cut-flowers in Ethiopia

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The floriculture sector in Ethiopia is flourishing due to the favourable and diverse agroclimate. However, cut-flower production faces pest problems that reduce both quality and yield. Among these are nematodes that are economically important pests attacking floriculture crops around the world. Nematodes infect most plant parts including flowers, buds, leaves, stems and roots. To initiate management strategies, information on the occurrence, biodiversity and damage potential of plant-parasitic nematodes on cut-flowers is required. However, in spite of the existence of damage that is repeatedly being mentioned by the growers, there is no published information in Ethiopia. Therefore this survey was carried out to monitor the occurrence, distribution, and abundance of plant-parasitic nematodes associated with cut-flowers. The wet season survey was done from July to September 2011 covering 14 commercial flower farms representing the different regions, agroclimates and cut-flower species. 10 to 14 soil samples composed of 40 soil cores from the top 20 cm were collected randomly per farm for rose, friesia, carnation, gypsophila, and statice making a total of 152 samples. Nematodes were extracted from 200ml aliquots of soil using the modified Baermann technique and they were then heat killed and fixed in TAF before they were brought to JKI for morphological analysis. From the surveyed rhizosphere soil of five different cut-flower species vis. rose, gypsophila, carnation, friesia and statice, a total of 21 nematode genera were recovered. All the genera, namely *Helicotylenchus, Hemicyclophora, Meloidogyne, Paratylenchus, Pratylenchus, Criconemella, Rotylenchulus, Rotylenchus, Trichodorus, Tylenchorhynchus, Xiphinema, Longidorus, Ditylenchus, Radopholus, sAphelenchoidess, Merlinius, Psilenchus, Aphelenchus, Malenchus, Filenchus* and *Tylenchus,* were encountered associated with rose. *Helicotylenchus* was detected associated with all plant species surveyed with a 77% frequency of occurrence and PV value of 83.1. Conversely, *Meloidogyne* spp. was found restricted to roses with a frequency of occurrence of 46.2% and PV of 73.8. The population density per 100 ml of soil ranges from 6 for *Aphelenchus* spp. to 389 for *Helicotylenchus* spp. Indeed, the detection level seems variable between sampling sites which might be as a result of the pesticide applied. Nevertheless, this survey shows the presence of potential plant-parasitic nematodes in the cut-flower farms of Ethiopia and that is a start for future nematode management strategies.

S18–P1

A study into the causes of litchi tree dieback

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Litchi dieback is becoming a serious problem in orchards where adult trees get sick and eventually die. Several fungi have been identified from affected trees but none were directly linked with the disease. Additionally factors like nematodes, drought stress and other biotic factors were never really considered to play an important role. An intensive survey was conducted in all litchi producing areas where both healthy and sick trees were sampled. In addition to nematodes and fungi present on roots and in the soil, other data collected included irrigation type, soil nutrients, age, cultivar and size of trees. Fungi identified were *Pythium, Phytophthora* and *Cylindrocladium* with *Pythium* being the most abundant species found in all areas and in 88% of the samples including samples from healthy trees. Although several nematode species like *Xiphinema, Hemicycliophora, Meloidogyne, Helicotylenchus* and *Pratylenchus* were present in the samples, the ring nematode *Hemicriconemoides mangiferae* was associated with all the orchards confirming it as the most important nematode species on litchi in production areas in South Africa. However, with the principal component analysis only three parameters, namely *Hemicycliophora, Pythium* and Ca+Mg/K, were significantly different between diseased and healthy trees. *Hemicycliophora* was only found in diseased trees indicating its pathogenicity when present. The survey confirmed that litchi dieback is directly linked to trees under stress which results in infection by *Pythium* and *Phytophthora*. Factors that could induce litchi tree decline are nematodes, water and nutrients. Although nematodes are not the primary cause of litchi dieback they can play an important role in the development of the disease.When nematode numbers are high they should be controlled to reduce the risk of litchi dieback.

S19 – Interactions of nematodes with other organisms: micro-organisms associated with nematodes

Convenor: Patrick Tailliez

S19–T1

Co-existence and niche separation of two subspecies of *Photorhabdus temperata* associated with the entomopathogenic nematode *Heterorhabditis downesi,* at a dune grassland site

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Entomopathogenic nematodes of the genus *Heterorhabditis* are mutualistically associated with the insect pathogenic bacteria *Photorhabdus* spp. The free-living stage of the nematode, the infective juvenile, carries cells of its symbiont in its gut. When it enters an insect it releases the bacteria which proliferate, assist in killing the insect and convert it to a nutritive soup that supports development and reproduction of the nematodes. *Heterorhabditis downesi* is associated with at least two subspecies of *Photorhabdus temperata, P.t. cinerea* and *P.t. temperata.* The two subspecies co-occur at a dune grassland site, where they are spatially segregated. The nematodes that carry the different subspecies are not distinguishable as separate strains, and can readily feed on and carry either subspecies of *P. temperata*, irrespective of which they were associated with when isolated from the wild. We expect that each of the bacterial subspecies excels in the conditions prevailing where it predominates. *Photorhabdus t. cinerea* predominates closer to the beach where soil organic matter is low and risk of desiccation is higher; nematodes survived prolonged desiccation better when they were in cadavers colonised by *P.t. cinerea* than when in cadavers colonised by *P.t. temperata*. *Photorhabdus t. temperata* occurs further inland in more established grassland with higher organic matter levels and biotic activity. Contrary to expectation, it was *P.t. cinerea* that showed superior antibiosis against a range of bacteria and fungi. The ecological significance of bacterial symbiont diversity will be discussed.

S19–T2

Entomopathogenic nematodes as disseminating agents for *Yersinia pseudotuberculosis*: a laboratory model

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The existence of biological micro-reservoirs for pathogenic microorganisms explaining the long-term survival of these pathogens in the environment has long been speculated. The capacity of soil invertebrates to act as intermediary hosts was the starting question of our study and entomopathogenic nematodes (EPNs) were investigated in this respect. EPNs are able to invade, kill and feed on insect cadavers thanks to a species-specific symbiotic bacterium belonging to the family Enterobacteriaceae (*Xenorhabdus* or *Photorhabdus* spp.). The symbiont provides a number of biological functions that are essential for its EPN host including the production of entomotoxins, of enzymes able to degrade the insect constitutive macromolecules and of bacterial toxins able to prevent the growth of competitors in the insect cadaver.

We wondered whether notorious mammalian pathogens taxonomically related to *Xenorhabdus* were able to substitute for or to "hack" the symbiotic relationship associating *Xenorhabdus* and *Steinernema* EPNs. To deal with this question, we studied a dynamic laboratory model consisting of *Galleria mellonella* insect larvae, an African *Steinernema* EPN species with its natural *Xenorhabdus* symbiont and *Yersinia pseudotuberculosis*, the etiologic agent of a gastro-intestinal disease affecting animals and humans, which was injected in the haemocoel of the insect larvae prior to infection with EPNs.

Our results show that the number of *Y. pseudotuberculosis* CFUs retrieved from EPNs after 7 consecutive infection cycles – lasting for about 2 months – is comparable to the initial inoculum. In other words, the laboratory model demonstrates the capacity of EPNs to act as a micro-reservoir ensuring maintenance and dissemination of the pathogen. We also show that not all *Enterobacteriaceae* behave like *Y. pseudotuberculosis* inside EPNs. The capacity of *Y. enterocolitica* to survive infection cycles inside EPNs is somewhat reduced compared to *Y. pseudotuberculosis*. By contrast, *Escherichia coli* and *Salmonella enteritidis* seem to have no capacity at all to survive EPN infection cycles. Genetic determinants that allowed *Y. pseudotuberculosis* to maintain inside EPNs are currently under study. The potential implication of the recently discovered type 6 secretion system components as well as that of other genes shared by both *Yersinia* and *Xenorhabdus* spp. is systematically investigated.

If they turn out to have an environmental significance, these findings may reveal an unexpected biotic reservoir explaining the long-term persistence and dissemination of pathogenic bacteria in the environment.

S19–T3

Nematode bacteria interactions in potato fields of Damaneh in Isfahan province of Iran

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Potato tuber rot is a common and important disease that has recently occurred in potato fields of Damaneh in Isfahan province of Iran. Skins of the infected tubers are usually cracked and darkened with cavities containing nematodes observed in the tuber flesh. Beside these cavities, affected tubers had soft rot symptoms with brown margins as described for pectolytic bacteria. To identify the pathogens involved in the development and spread of potato tuber rot and to find out the interactions between them in the region, a large number of rotten potatoes with nematode and bacterial symptoms together were collected from the potato fields of Damaneh between 2009 and 2010. Microscopic examination of the cracked skin tubers showed the presence of many nematodes in white pockets of flesh of these samples. The majority of nematodes were identified as *Ditylenchus destructor* based on morphological and morphometric characters and molecular methods. Fifteen pectolytic bacteria with green metallic colonies on EMB medium were isolated from the tubers showing surface cracking and soft rot symptoms, simultaneously. These characteristics of the isolates together with results of molecular methods were the basis for diagnosis of *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*). To determine the role of each pathogen occurring in the potato tuber rot complex, an appropriate amount of *D. destructor* and *Pcc* were inoculated alone and together in potato plants grown in greenhouse conditions. Tuber rot symptoms identical to those observed in the field appeared on all tubers inoculated with *D. destructor* but not on the *Pcc* inoculated tubers. Co-inoculation of *Pcc* with *D. destructor* resulted in tubers with surface skin cracking symptoms and soft rot symptoms simultaneously. Based on these results, it was concluded that the nematode and bacteria interact together; *D. destructor* nematodes alone created the wounds which possibly allowed *Pcc* bacteria to penetrate, and the interaction together caused soft rot in the tissues.

S19–T4

Histopathology of tomato roots ınoculated with *Verticillium chlamydosporium* and *Meloidogyne incognita*

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Histopathological studies on the interaction of *Verticillium chlamydosporium* and *Meloidogyne incognita* on tomato roots revealed cellular alteration among treatments. Plants treated with *M. incognita* and *V. chlamydosporium* showed fewer galls and the galls were of smaller size, with empty giant cells and less abnormality in the vascular bundle as compared to treatments where the plants were inoculated with nematodes only. The fungus developed in the intercellular spaces of epidermal and cortical cells in plants treated only with *V. chlamydosporium*. Their vascular bundles remained intact. The fungus was observed in the nematode-affected vascular regions in plants inoculated with *M. incognita* and *V. chlamydosporium*. Numerous mature females feeding on giant cells were seen in infected roots. No histological alterations were observed in untreated healthy plants.

S19–T5

Defences in tomato and the *Arabidopsis* model system induced by endophytic *Fusarium oxysporum* against root-knot nematode

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Plant-associated microorganisms can support plants coping with biotic and abiotic stress factors. The endophytic fungus *Fusarium oxysporum* isolate Fo162 has been shown systemically to repress the colonisation of sucking insects and both sedentary and burrowing nematodes in various plant species. Although the exact mode of action is currently poorly understood, split-root experiments show that the fungus initiates certain systemic plant defence responses. By combining biological, molecular and biochemical approaches, we aim further to unravel the mechanisms leading to such plant responses, e.g. by gene expression studies and finding parallels with the described induced systemic resistance (ISR) or systemic acquired resistance (SAR) mechanisms against other plant pathogens in the important crop plant tomato. Because of the extensive molecular knowledge and the availability of a significant number of well-characterised mutants, the *Arabidopsis* model system is a highly suitable addition for this type of research. Results show that Fo162 grows endophytically in *Arabidopsis*, promotes plant growth and reduces colonisation by the sedentary root-knot nematode, *Meloidogyne incognita*, in a systemic way. The model plant *Arabidopsis* is therefore also suitable for further studying the molecular and biochemical mechanisms responsible for the endophyte-induced defences against nematode infection.

S19–P1

Interaction between *Steinernema* sp., their symbiotic bacteria and *Spodoptera littoralis*

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Entomopathogenic nematodes of the genus *Steinernema* and their symbiotically associated bacteria, *Xenorhabdus* sp., are used for the control of several soil-dwelling insect pests. Parasitism starts when nematodes enter natural openings of the insects and release their bacterial symbionts in the host body cavity. The insects die mainly due to toxaemia and/or septicaemia. Nematodes reproduce in the insect cadaver and feed on the symbiont biomass and insect tissues metabolised by the bacteria. During the final stage of development, the nematode and bacteria reassociate and the nematode subsequently develops into its nonfeeding infective juvenile stage (IJs). The IJs emerge from the insect carcass in search of a new insect host.

The genus *Steinernema* is phylogenetically diverse and the species described until now are distributed within five major clades. Each *Steinernema* species is associated with a unique *Xenorhabdus* species, whereas a *Xenorhabdus* species can be associated with different *Steinernema* species. Here, we compared with bioassays the capability of different *Steinernema* species to kill and develop in *Spodoptera littoralis* larvae taking into account the phylogenetic diversity of the nematodes and their symbiotically associated *Xenorhabdus* species. Results show diverse nematode behaviours suggesting potential applications to specifically control pests or groups of pests.

S19–P2

Light microscopy and Cryo SEM observations on *Pochonia chlamydosporia* parasitism in root-knot nematodes

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The nematophagous fungus *Pochonia chlamydosporia* (Hypocreales, Clavicipitaceae) inhabits the soil as a saprophyte where it occurs as hyphae, mycelium, conidia and chlamydospores. The fungus has received much attention because of successful results in controlling root-knot nematodes (*Meloidogyne* spp.) under specific conditions such as glasshouses and peri-urban agriculture systems, thus supporting its potential as a biocontrol agent in horticultural crops such as tomato, potato and beans. In the rhizosphere, the fungus colonises the roots of host plants and some *Pochonia* species have been reported to have an 'endophytic behaviour'. As a parasite of eggs of sedentary plant endo-parasitic nematodes colonisation of the root surface by the fungus is linked to nematode egg production of cyst (*Heterodera* spp., *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.). *Meloidogyne* spp. eggs embedded in a gelatinous matrix (*i.e.*, egg masses) eventually emerge on to the root galls surface (rhizosphere) where the vegetative hyphae of the fungus can colonise the gelatinous matrix, thus reaching and eventually infecting the eggs via appressoria. Parasitism of cyst nematodes by the fungus can occur as females mature and burst through the root cortex, thus facilitating the fungus reaching eggs inside the cysts. Although there are numerous studies dedicated to the tritrophic relationship (fungus—plant—nematode), fungal diversity, ecology, fungus production and application to soils, there are few studies dedicated to the biology of the fungus, especially microscopy studies of hyphal colonisation of eggs within the gelatinous matrix, conidial attachment to eggs and second-stage juveniles (J2). In the present study, different nematode stages (*i.e*., eggs, egg mass, females, J2) of *Meloidogyne incognita* and their colonisation by the nematophagous fungus *P. chlamydosporia* were studied using cryo-scanning electron microscopy with the cryo-planing technique and light microscopy. Nematode and fungal samples produced in different liquid and solid culture methods, but processed with the same cryo techniques, showed similar morphological features of both organisms and changes associated with parasitism during the nematode/fungus interaction, thus supporting the potential of these techniques to assess, without alteration, the nematode infection process by the fungus. Most studies on nematode fungal colonisation and associated changes in both fungus and nematode have been conducted mainly on eggs. In the present study we have found that similar morphological features related to infection also occurred in infected J2, a subject that deserves further study.

S19–P3

Induction of cereal cyst nematode suppressiveness by the fungi *Pochonia chlamydosporia* and *Fusarium graminearum* in fields

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Distinct differences in CCN population densities were observed among four cereal production regions suggesting the suppressiveness had a biological nature. Results show that the microflora associated with nematodes is very diverse and some exhibited associations with one or more of the nematode population density measurements (eggs or J2). Also some of them are known to be nematode parasites: *Fusarium* sp. and *Pochonia chlamydosporia*. Low densities of *H. avenae* in Siliana and Kef can be explained by the presence of microorganisms associated with cysts that are associated with low rates of multiplication of nematode in these two regions: specifically about 4-6 live eggs/g soil. Indeed significant rates of mortality of eggs and larvae were recorded, on the order of 75% and 30% in Siliana and 51% and 28% in Kef. The most important microorganisms responsible for this parasitism are *P. chlamydosporia*, the most common fungus isolated from four prospected regions in our study, and the association of the latter with *F. graminearum* in the Siliana region. The presence of *F. graminearum* in wheat fields could play an important role in regulating populations of *H. avenae* where they exist. Although our work just highlights the parasitic effect, this remains unconfirmed since no studies have shown the action of *F. graminearum* as a biocontrol agent and its presence in the grain fields has always been attributed to its pathogenicity.

S19–P4

Phytostimulating and nematicidal properties of some secondary metabolites of actinomycetes (*Streptomyces* sp.) isolated from soils of R. Moldova

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Many soil microorganisms including the actinomycetes (*Streptomyces* sp.), which synthesise biologically active substances, possess the ability to stimulate sprouting, plant growth and to suppress the development of plant pathogens based on their chitinolytic activities and antibiotic proprieties. Antagonistic streptomycetes are considered as ideal biological control agents due to their rapid growth, easy handling and production of secondary metabolites. Seventeen strains of streptomycetes isolated from soils of R. Moldova were screened for their potential to increase germination of tomato seeds and rootlet lengths of two popular grades Leana and Novelty of Dniester and to depress the invasive second-stage juveniles of root-knot nematode *Meloidogyne incognita in vitro*. The effect of secondary metabolites of strains *Streptomyces* sp. 11, *Streptomyces* sp. 22, *Streptomyces* sp. 182 on the germination of tomato seeds increased the rootlets and their weight by 15–20% compared with the control. The secondary metabolites of selected strains of streptomycetes showed nematocidal properties in varying degrees against the invasive second-stage juveniles of the root-knot nematode *Meloidogyne incognita.* The nematicidal activities were shown by non-soluble solutions of secondary metabolities of four strains: *Streptomyces* sp. 47, *Streptomyces* sp. 11, *Streptomyces* sp. 22 and *Streptomyces* sp. 76 possessing also the phytostimulating abilities, with a positive influence on germination of tomato seeds and root formation.

S19–P5

Heterogeneous microbial community associated with *Bursaphelenchus xylophilus*

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Pine wilt disease (PWD) is a complex disease integrating three major factors: the causal agent, the pinewood nematode *Bursaphelenchus xylophilus,* the insect-vector *Monochamus* spp. and the host pine tree, *Pinus* sp. Since the early 1980s, the notion that another pathogenic agent, namely bacteria, may play a role in PWD has been gaining attention; however, the role of bacteria in PWD is still unknown. The present work suggests the intriguing possibility that some *B. xylophilus*-associated bacteria may play a significant role in the development of this disease. This is inferred as a consequence of: (i) the phenotypic characterisation of a collection of 35 isolates of *B. xylophilus*-associated bacteria in different tests broadly used to test plant pathogenic and plant growth promoting bacteria, and (ii) greenhouse experiments that infer pathogenicity of these bacteria in maritime pine, *Pinus pinaster*. The results illustrate the presence of a heterogeneous microbial community associated with *B. xylophilus* and the traits exhibited by at least some of these bacteria appear to be related to PWD symptoms. The inoculation of four specific *B. xylophilus*-associated bacteria in *P. pinaster* seedlings resulted in the development of some PWD symptoms suggesting that these bacteria likely play an active role with *B. xylophilus* in PWD.

S19–P6

Studies on nematode vectors of some NEPO viruses in a raspberry and blackberry nursery in Hatay, Turkey

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Establishment of new plantations of raspberry and blackberry have been increasing in recent years in Turkey. The presence of virus diseases in one of the first plantations and a nursery in Hatay, Turkey was reported. Due to lack of knowledge on these new crops and their diseases, many seedlings were shipped all over Turkey from the nursery. In this study some NEPO viruses namely Arabis mosaic virus (ArMV), Raspberry ringspot virus (RRSV-RpRSV), Strawberry latent ringspot virus (SLRSV), Tomato black ring virus (TBRV), Tobacco ringspot virus (TRSV), Tomato ringspot virus (ToRSV) and their dorylaimid nematode vectors were investigated. Some of the viruses and their potential vectors were identified. Further studies are in progress.

S20 – Plant-parasitic nematodes in temperate crops: new issues

Convenor: Leendert Molendijk

S20–T1

The use of Brassica species for the management of potato cyst nematode infestations of potatoes

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The potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis* are the most economically important nematode problems to the UK potato industry. They inflict an annual cost of approximately £50 million on UK potato farmers and have become a threat to the future of the potato crop for many growers. Breeding for resistance since the 1950s has produced a few commercially acceptable varieties with resistance to *G. pallida*. Effective control of *G. pallida* is an essential requirement to maintain the competitiveness of UK potato production. During the past three decades, farmers in the UK have relied heavily on granular nematicides and soil fumigants to control PCN. These fumigants, although effective, pose a variety of negative side effects, which has led to certain products being de-registered (e.g. 1,,3- dichloropropene) or having restricted use. Biofumigation is increasingly being viewed as an effective method for increasing the efficiency of soil-borne pest and disease control. Biofumigation refers to the suppression of soil-borne plant pests and pathogens by biocidal compounds released when plant residues are hydrolysed. In the present study, the effects of brassica green manures on *G. pallida* have been assessed in two field experiments conducted at separate sites in Shropshire, UK. Three brassica species, *Brassica juncea* cv Caliente 99, *Raphanus sativus* cv Bento and *Eruca sativa* cv Nemat, were planted at these locations in areas infested with *G. pallida*. The first field experiment was sown in July 2011, chopped and incorporated in September 2011 and followed by a potato crop in March 2012. The second field was sown in September 2011, overwintered, chopped and incorporated in March 2012 prior to the planting of a potato crop in May 2012. Soil samples were collected pre-sowing of brassica crops (for initial PCN population density estimates), 6-weeks post-sowing of brassica crops, pre- and post-incorporation of brassicaceous residues as well as pre-sowing of the potato crop. At each time of sampling, the viability of the encysted eggs of *G. pallida* was assessed by hatching bioassays and Meldola's blue staining. Leaves, stem and root samples of all three *Brassica* species were collected pre-incorporation of brassica tissues and processed using standard procedures for the analysis of their glucosinolates content *via* High Performance Liquid Chromatography (HPLC). A measurement of PCN root invasion will be undertaken on 6-week-old potato plants. Potato growth and development are being monitored through the growing crop and tuber yield will be assessed post-defoliation and soil samples will be taken to estimate *G. pallida* final population density. Glasshouse and *in vitro* laboratory experiments will be conducted for an in-depth understanding of effects observed in the field. Experiments will be repeated over time and space to check for consistencies.

S20–T2

Developing methodology for screening the relative susceptibility of potatoes with resistance against *M. chitwoodi*

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In The Netherlands resistance genes against *Meloidogyne chitwoodi* became available as a result of the DREAM (EU QLRT-1999-1462) project. Several Dutch potato breeders have used these genes to develop potato genotypes containing a single resistance gene against *M. chitwoodi*. Some of these genes also provide resistance against other *Meloidogyne* species. Currently, a research project has been initiated by Dutch breeders to investigate the impact of these genotypes on the population dynamics, yield loss and tuber infestation with *M. chitwoodi*. Also, the development of a simple and cheap resistance test, as has been realised for PCN resistant potatoes, is pursued. At this moment three large experiments have been conducted to obtain the required basic information. Ten potato genotypes were screened for their resistance against *M. chitwoodi*. The susceptible potato cv. Désirée was used as a susceptible control. Potato genotypes were grown in 5 and 10 l pots in an artificial soil at ranges of initial nematode densities (*Pi*), *e.g.* from 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 to 128 (g of dry soil)$^{-1}$, to enable fitting population dynamical and yield models and estimating relative susceptibility (*rs*), tolerance, yield loss and tuber quality, expressed as root-knot index (RKI). The partial resistance of the genotypes with a single resistance gene was very high > 99%, except for two genotypes with non-*M. chitwoodi* resistance genes (< 50%). Three of the resistant genotypes also showed a remarkable tolerance for high initial population densities of *M. chitwoodi*. A marked improvement of tuber quality was found in eight genotypes with *M. chitwoodi* resistance, with RKI value below 10, the minimum threshold for acceptance of ware potatoes for industrial processing. So far, on nine genotypes screened for resistance, the population dynamical models used to describe relative susceptibility, fitted well. From the comparison of the population dynamics of both the susceptible and resistant varieties it can be concluded that downscaling of the screening method is feasible.

S20–T3

A long term trial on the population dynamics of *Heterodera schachtii* and Pi dependent yield performance of susceptible, tolerant and resistant sugar beet variety types

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The white beet cyst nematode *Heterodera schachtii* is known as one of the most harmful pests in sugar beet production in Europe, where it occurs in growing regions from Spain to Finland. Due to market constraints and increasing energy costs, production areas tend to be concentrated around sugar factories, causing an intensification of production. Nematode-tolerant varieties are available for sugar beet growers in Germany providing a new option in management strategies to control *H. schachtii* damage. A permanent field trial including a three year rotation was set up some 40 years ago in Elsdorf within the centre of Germany's western sugar beet growing area. Since 2005 trials focused on yield effect of different population densities (Pi) and multiplication rates of a susceptible, a resistant and a tolerant variety type. Using susceptible and resistant mustard or radish cultivars prior to the sugar beet season it was possible to establish a wide range of Pi levels between 20 eggs and juveniles/100 ml (EuJ/100 ml) and 6100 EuJ/100 ml. In a comparison between low infestation classes (172—237 EuJ/100 ml) and high infestation classes (2037—2852 EuJ/100 ml) of *H. schachtii*, a tolerant variety lost 8% relative white sugar yield, whereas a resistant and a susceptible variety both lost 17% to 18%. Despite horizontal resistance in tolerant varieties obtained from *B. maritima* a reproduction (Pf/Pi) of 4.9 ± 2.3 at low infestation levels below 300 EuJ/100 ml is evident. Comparing data over the various growing seasons shows a damage effect that was different between the years.

S20–T4

Experimental evidence of the efficiency of two resistance genes deployment strategies – pyramiding or alternating – for sustainable management of root-knot nematodes

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The current restrictions in the use of chemical nematicides have contributed to an increase in root-knot nematode (RKN) problems in horticultural crops. In this context, plant resistance is the most effective and sustainable method of control. In horticultural crops, a few major resistance (R) genes are available, but the possible occurrence of virulent nematode populations able to reproduce on *R*-plants may constitute a severe threat to this control strategy. Here, we tested several *R*-gene deployment strategies in the pepper—nematode interaction to implement a rational management of the *R*-cultivars increasing the sustainable management of RKN. Experiments were conducted in climate-controlled rooms, in greenhouses, and under 3-years-field agronomic conditions comparing i) the succession of the same *R*-gene every year, when introgressed in a resistant vs. a susceptible genetic background, ii) the alternation of single *R*-genes in rotation, iii) the mixture of genotypes bearing single *R*-genes sown in the same plot, and iv) the pyramiding of two *R*-genes in one genotype. Results allow the identification of conditions lowering the emergence of virulent nematodes and assessing the time required for the sustainable improvement of soil health (reduction of parasite populations under their damage threshold) using the *R*-plants as RKN "traps". Alternating different *R*-genes in rotation was confirmed to be efficient to decrease virulent populations in fields due to the specificity of the virulence previously demonstrated in laboratory experiments. Suppression of the emergence of virulent isolates by the pyramiding of two different *R*-genes in one pepper genotype was confirmed in controlled and natural conditions and proved durable during a 3-year-field experiment.

This research was supported at the national level by 1/ the Agriculture Ministry with a CTPS (permanent technical committee of the selection of the crop plants) project on the durability of resistance to RKN in Solanaceae (2007—2010), 2/ INRA with a project on integrated production of vegetable crops (PIClég™), acronym Neoleg2 (2008—2012), and 3/ the French National Research Agency with a project on Ecosystems, living resources, landscapes and agriculture (Systerra), acronym Sysbiotel (2009—2013). At the European level, this research was supported by 1/ the European network for the durable exploitation of crop protection strategies, acronym ENDURE (2008—2010), and 2/ the INTERREG Alcotra cross-border cooperation France—Italy project, acronym Valort – Valorization of cross-border vegetable crops (2010—2013).

S20–T5

Damage thresholds and population dynamics of *Meloidogyne chitwoodi* on carrot (*Daucus carota* L., cv. Nerac) at different seed densities

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Yield loss of carrot (*Daucus carota* L., cv. Nerac) caused by *Meloidogyne chitwoodi* and the population development of this nematode were studied using a range of population densities (0, 0.125, 0.25, 0.5 up to 256 J2 (g soil)$^{-1}$) and three different seed densities, 2, 4 and 18 seeds/pot, similar to 1, 2 and 10 million seeds/ha of normal practice. Carrots were harvested when the controls reached market size. Seinhorst's yield reduction model was fitted to the data of yield and quality loss. The tolerance limits for yield loss (T_y) were 0.34, 0.62 and 0.5 J2 (g soil)$^{-1}$ and those of quality yields (T_q) were 0.01, 0.14 and 0.81 J2 (g soil)$^{-1}$ at 2, 4 and 18 seeds/pot, respectively. However, yield loss model without the parameter T fitted best for quality loss. Minimum yield (m_y) increased with seed density and was 0.25, 0.30 and 0.50 at 2, 4 and 18 seeds/pot, respectively. Minimum quality yield (m_q) was 0.10, 0.08 and 0.15 at 2, 4 and 18 seeds/pot, respectively. Quality loss occurred at each seed density and was expressed by misshapen, forked, stubby, galled taproots and taproots with rot. Of the two population dynamical models fitted, the one for sedentary nematodes (cyst nematodes) fitted best for *M. chitwoodi*, even though this nematode has more than one generation per cropping season on carrot. Both parameters, the maximum multiplication rate (a) and the maximum population density (M) increased with increasing seed density; *M* was 0.98, 1.65 and 4.32 and *a* was 1.17, 3.69 and 6.15 at 2, 4 and 18 seeds/pot, respectively. Carrot cv. Nerac can be considered a bad host. At 2 seeds/pot, initial population densities above 0.5 J2 (g soil)$^{-1}$ declined. The larger values of the parameters *a* and *M* with increasing seed density can be attributed to the increased combined size of the taproots and the root system per volume of soil.

S20–P1

Suppressing effects of solarisation and biofumigation on potato cyst nematode *Globodera rostochiensis*

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Potato cyst nematode *Globodera rostochiensis* has become a major yield restricting factor since its discovery in 2008 in Hamadan Province in Iran, and exploring different control methods is a priority to help farmers avoid losing profit. Mulching with plastic film during the warm season normally elevates soil temperature, resulting in reduction of soil-borne pathogens and weeds. With cyst nematodes, the cyst fails to protect eggs and juveniles from temperature stress more in wet than in dry soil conditions. Combining solarisation with biofumigation may enhance the lethal effect of each method alone; this concept was tested in a pot experiment. Fresh leaves of Brassica plants such as cabbage and broccoli were amended into infested soil with PCN; some were covered with clear plastic sheets. Infested soil with or without plastic covering and metam sodium-treated soil were amongst the treatments. Appropriate treatments were solarised in open air for 3 and 5 weeks, after which all pots were planted with a potato tuber and kept on a glasshouse bench for 3 months. PCN infestation levels in all pots under plastic were reduced; adding biofumigant plants to soil synergised their effect under plastic and reduced nematode density in cysts when compared to either treatment singly compared to non-treated plants.

S20–P2

Determination of biofumigant effects of Brassicaceae plants on root-knot nematodes in vegetables

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The aim of this study was to determine the biofumigant effects of some Brassicacae plants on root-knot nematodes of cabbage plants in greenhouse conditions. Two sets of experiments were conducted; in the first sets, host levels of 15 different varieties of cabbage to *M. incognita* and *M. arenaria* species were researched in the pot experiments by applying 2000 or 0 nematode eggs per pot. Evaluation was performed 8 weeks after inoculation, and weak host and non-host varieties were selected for a second set of experiments. In this experiment,susceptible tomato and seven Brassicaceae genotypes were used in growth chambers and greenhouse conditions to determine the biofumigant effects on root-knot nematodes. These varieties were grown for 2 months in pots infected with root-knot nematodes, then the plants were harvested, mixed with soil in the pots and covered with polyethylene plastic cloths. After waiting 4 weeks, polyethylene cloths were removed and tomato seedlings were transplanted to the pots. After 8 weeks, experiments were harvested, plant growth parameters were obtained and roots were evaluated by using the 0—5 gall index. The highest values for plant growth parameters were determined for tomato plants after transplanting from broccoli Fiesta and chinese cabbage. When infested plants were compared with each other for gall index, egg number, females of root, total nematode number of roots and reproductive index, the highest values were obtained in the control plots, followed by cauliflower cv. Altamira. Thus, some Brassicaceae plants may be suitable for use in the control of root-knot nematodes in infested vegetable growing areas.

S20–P3

Investigation of the host status of barnyard grass and small flower umbrella sedge to rice white-tip nematode (*Aphelenchoides besseyi* Christie, 1942)

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In this study, we investigated whether barnyard grass [*Echinocloa crus galli* (L.) P. Beauv., Poaceae] and small flower umbrella sedge (*Cyperus difformis* L., Cyperaceae), which are major weeds in rice growing fields are host plants for rice white-tip nematode (*Aphelenchoides besseyi*). For this purpose, barnyard grass and small flower umbrella sedge were collected in fields planted to the Halilbey rice variety which is susceptible to white tip nematode and had 77% white tip symptoms on flag leaf at flowering stage and averaged 324 *A. besseyi* per panicle. In this analysis, while no nematode was found in small flower umbrella sedge, an average of 30 *A. besseyi*/10 g seeds, 400 *A. besseyi*/plant with 15 tiller (flag leaf + panicle), 435 *A. besseyi*/plant with 6 tiller (flag leaf + panicle) were found in barnyard grass. These results are the first recorded research data on this topic in Turkey. In addition, *Echinochloa crus galli* is an important disease factor in rice planting as an alternative host for rice blast (*Pyricularia oryzae*). Accordingly, a battle against barnyard grass should be made toward preventing rice white-tip nematode and other pests or diseases from spreading.

This study is part of a research project "Distribution of Rice White tip Nematodes (*Aphelenchoides besseyi* ChristieChristie, Aphelenchida: Aphelenchoididae) In Rice Growing Areas in Thrace Region and Research on Some Control Methods" TAGEM-BS-08-07-04/01-04, supported by the General Directorate of Agricultural Research and Policies (GDAR), Food, Agriculture and Livestock Ministry.

S20–P4

Glucosinolates and resistance in Oilseed Radish against both cyst and root-knot nematodes

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Resistance to cyst and root-knot nematodes is one of the main criteria for farmers to choose their cover crop. Oilseed radish (*Raphanus sativus oleiformis*) varieties with resistance to white (*Heterodera schachtii*) and yellow (*H. betae*) beet cyst nematodes are widely available, with declines in nematode population of more than 90% (Pf/Pi>0.1). Some varieties are registered in The Netherlands and Germany as resistant to the quarantine status Columbian root-knot nematode (*Meloidogyne chitwoodi*); in practice these varieties are also resistant to *M. fallax*. Varieties that are registered as resistant are able to decrease the nematode population by more than 99%. These varieties are a powerful means for farmers to increase soil health in an era when cover crops are an essential component of sustainable agriculture.

Our research focused on the question of whether the glucosinolate (GLS) profile of the roots of the different varieties was related to nematode resistance. GLS are present in all species of the *Brassicaceae* family. Upon tissue damage, GLS are hydrolysed and form isothiocyanates (ITC), which are toxic compounds. A number of varieties differing in resistance were grown under normal field conditions. The varieties were grouped in non-resistant, beet cyst-resistant and both root-knot and beet cyst-resistant. Samples of the roots were taken during the vegetative stage. A HPLC analysis was performed to obtain a GLS profile. The varieties differed mainly in GLS concentration; the GLS profile was relatively stable among genotypes. However, no significant correlation with nematode resistance was found. Further research must reveal whether the varieties differ in shoot GLS profiles or myrosinase activity, which directly influences the ability to form ITC.

S21 – Nematode as Bio-indicators

Convenor: Isabelle Abrantes

S21–T1

Meiofauna as indicator of mercury pollution in Murcielagos Bay, Zamboanga del Norte, Philippines

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Barangay Libay is a coastal barangay of Sibutad, Zamboanga del Norte, the centre of the small scale gold mining operation in Murcielagos Bay. Sediment cores and water were collected from twelve random quadrats of the transects and analysed for total mercury (THg) and meiofaunal population structures. Temperature, salinity of the interstitial water and pH were also determined *in situ.* The site was mapped using the Coastal Transect Analysis Model and Golden Software Surfer 7 and the physico-chemical parameters correlated with the concentration of THg in water and sediment and density and abundance of the meiofauna were assessed, Twenty one major genera were identified from the cores representing three major groups: foraminifera, ostracoda and nematodes. Meiofauna exhibited various tolerances with THg. The foraminifera *Elphidium* was present in all stations and had the highest density of 721 per 10 cm^2 and 25.67% abundance. The nematode *Subanguina* had the lowest density of 7 per 10 cm^2 and 0.25% abundance. *Elphidium* dominated in areas with high concentrations of THg while *Subanguina* in low concentrations. The genus *Peneroplis* exhibited irregular protrusions and expansion of the test to as much as half of the test. The THg from the sediment cores ranged from 108—704 parts per billion (ppb) while in the water it was (68—176 ppb). These levels are beyond the concentrations for the normal background of uncontaminated sediment sand and beyond the concentration level set by DENR- DAO 34—35 which is 12.29—40 ppb.

S21–T2

Heavy metal effects on nematodes: stress responses and uptake characteristics of *Xiphinema vuittenezi*

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Although soil nematodes are increasingly used as biological indicators, most of their known stress responses at the species level have been derived from experiments with an ephemeral bacterial feeder, *Caenorhabditis elegans*. For this reason, it is advisable to involve further species belonging to different feeding types and life history strategies as test organisms in ecotoxicological experiments. Our previous studies revealed that *Xiphinema vuittenezi* (Dorylaimida: Longidoridae), a widely spread plant pest in the Central European region, is quite sensitive to heavy metals such as Cr and Cu. Therefore, we aimed to study heavy metal uptake by this nematode. Adult females treated with solutions of chromium and copper salts were exposed to microanalytical techniques to explore the amount and distribution of heavy metals in them. Total X-Ray Fluorescence (TXRF) was used to explore the quantity of heavy metals in single specimens. Other nematodes were studied with focussed ion beam electron microscopy (FIB SEM) in order to explore copper and chromium distribution along a cross section of the body. Finally, synchrotron induced X-ray fluorescence and X-ray Absorption Near Edge Structure (XANES) measurements were performed. The acquired data and images revealed an increasing heavy metal content in the studied specimens both along a concentration gradient and with the increased exposure time. From our results it appears that the cuticle plays a major role in uptake, but the alimentary canal cannot be excluded either and some local hotspots can be identified. The meaning of our findings will be discussed.

Our research was supported by OTKA K 81401, TÁMOP-4.2.2/B-10/1-2010-011 and TÁMOP-4.2.1.B-11/2/KMR projects.

S21–T3

Ecotoxicity of zeolitic nanoparticles on the nematodes *Caenorhabditis elegans* and *Meloidogyne incognita*

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Many studies have investigated the ability of natural zeolites, zeolitic tuff nanoparticles and other amendments to reduce heavy metals availability in contaminated soils. However, the eco-toxicity of such amendments in the form of nanoparticles remains poorly investigated. Therefore, we aimed to investigate the eco-toxicity of a Jordanian novel nanoparticulate zeolitic tuff on two nematodes, *Caenorhabditis elegans* and *Meloidogyne incognita*. In this assay, two concentrations of 85 and 170 mg/l were used. Results showed that the exposure of the first-stage juveniles of the nematode *C. elegans* to the two nanotuff concentrations did not reduce its survival. Similarly, neither the survival nor the hatching of the second-stage juveniles of the root-knot nematode *M. incognita* were suppressed when exposed to either concentration of the nanotuff. Such non-ecotoxicity effect will favour the use of the zeolite nanotuff as a soil amendment to reduce the heavy metals in contaminated soils.

S21–T4

Toxicity of nanosized TiO_2 to plant-feeding nematodes

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The production of engineered nanoparticles is constantly increasing and they are being used in a wide range of applications. Therefore, these particles are being released into the environment in growing amounts. Despite this, there is still a considerable lack of knowledge about their effects on living organisms. Photocatalytic active TiO_2 nanoparticles are included in toothpastes and sunscreens. TiO_2 particles are suitable for cleaning contaminated soil as well. Data for ecotoxicological effects of nanoparticles on nematodes have been derived only from *Caenorhabditis elegans* tests. From these tests, nanosised TiO_2 particles appeared to be more toxic than larger TiO_2 particles. The toxic effects of TiO_2 nanoparticles were investigated and compared to their bulk form in this study. Adult *Xiphinema* females were extracted from collected soil samples using a modified version of Cobb's decanting and sieving method. All acute mortality tests were carried out in microtitre plates, throughout a period of 7 days and the survival of nematodes was counted. Based on the survival patterns, nanosised TiO_2 particles did not show a toxic effect after 3 h of exposure. Subsequently, results show an increasing trend of toxicity along the gradient of increasing concentration. Our results give warning about the potential toxicity of TiO_2 nanoparticles and support the suggestions for more careful exploration of possible adverse effects caused by releasing nanomaterials in the environment.

Our research was supported by OTKA K 81401 and TÁMOP-4.2.1.B-11/2/KMR.

S21–P1

Toxicity of pesticides and metals in the soil nematode community

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Nematodes play an essential role in soil ecosystems and the structural composition of the nematode community is useful to evaluate toxicological and ecological effects. The aim of this work was to assess the effect of pesticides and metals on a native soil nematode community, free from chemicals, based on taxonomic and structural (trophic groups) approaches. The effects of three pesticides (summer oil, abamectin and fosetyl) and metals (Cd, Zn and Cu) on the total abundance, number of families and structure of the nematodes, were analysed over 15 days, at 21°C. Contaminants in three doses were added to previously defaunated soil followed by an inoculation of 1000 nematodes/replicate. The bacteriophagous nematodes were the dominant trophic group (52.9%), followed by plant-parasitic (41.1%), while fungivorous (Aphelenchidae) were the less abundant (1.3%). Concerning the response of the nematodes to the contaminants, a clear dose-response was detected in all compounds. Significant effects were found in the total abundance of nematodes and the trophic structure decreased significantly with increasing doses, with the abamectin the being the most toxic. This approach, based on the effects of stressor agents on the structure of the nematode community, was revealed to be effective to evaluate the toxicicity of chemicals toward nematodes.

S21–P2

Fluctuation of different nematode trophic groups in organic and conventional oil rose (*Rosa adamascena* Mill.) plantations

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Oil rose is a significant agricultural crop in Isparta, Turkey and rose oil production in this area accounts for 60% of total world production. Nematodes have different trophic groups and maintenance of the annual population density of terrestrial nematode groups is important in terms of soil health. In the present study, different nematode trophic groups were followed in organic and conventional oil rose (*Rosa damascena*) cultivation and a natural ecosystem of cedar plantation in Isparta between March 2010 and February 2011. Soil samples were taken from five rows between 0—30 cm depth and from each row a sample was taken monthly. A total of five replications were sampled. Nematodes were extracted *via* a modified Baermann funnel technique and nematodes counted under a light microscope according to the nematode trophic groups. Different trophic nematode groups were compared on organic and conventional oil rose cultivation by using t-test at the 0.05 significance level. Additionally, terrestial nematode biodiversity was analysed by using Shannon index (MVSP 3.13P, Kovach Computing Services, 1985). Nematode trophic groups were found to be higher in the conventional than the organic plantation during the sampling period. Saprophagous nematodes had the highest population density in both organic and conventional cultivation. The density of plant-parasitic nematodes was similar in conventional and organic oil rose plantations except during January. However, phytophagous nematodes had higher density in conventional garden than organic sites in all sampling periods except April. Predator nematode density was the lowest in both types of cultivation. Similarly, omnivore nematodes had low population density compared to other nematode groups but their density was higher in the organic rose plantation than the conventional one. The highest nematode biodiversity was observed in the conventional oil rose plantation. The cedar plantation ecosystem had the lowest nematode biodiversity in the study.

S21–P3

Fluctuation of different nematode trophic groups in organic and conventional apple orchards

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Terrestrial nematodes are important organisms for soil quality and biological indicators reflecting soil health. Community structure of terrestial nematodes directly affects richness and structure of soil health. In this study, the fluctuations of different trophic nematode population densities were followed in organic and conventional apple orchards. Cedar plantation was used as a natural ecosystem and nematode populations were monitored monthly between March 2010 and February 2011. Soil samples were taken as five replications from each orchard. Nematodes were extracted using a modified Baermann funnel technique and nematodes were counted under a light microscope. Different trophic nematode groups were compared on organic and conventional apple orchards by using t-test at 0.05 significance level. Terrestrial nematode biodiversity was analysed using Shannon index (MVSP 3.13P, Kovach Computing Services, 1985). All nematode trophic groups had higher population density in conventional orchard than organic apple orchard in all sampling periods. Predator nematode density was found to be the lowest; however, saprophagous nematodes had highest population density in both cultivation types. Predatory nematodes were generally detected in organic apple orchards. Also, omnivore nematodes had low population density at the same type of orchard. Plant-parasitic nematode populations in conventional orchards were higher than in the organic orchard throughout the sampling period. Nematode biodiversity in conventional orchards was higher than in organic apple orchards. Nematode biodiversity was the lowest in natural cedar ecosystem.

S21–P4

Testing sensitivity of plant-feeding nematodes to acute chromium and copper stress

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Free-living nematodes are among the most important components of soil fauna with respect to both compositional and functional aspects. They play a key role in nutrient turnover processes and in regulation of other soil organisms. The most important group in agriculture are the plant-parasitic nematodes. They are well-known and quite well characterised. Furthermore, free-living nematodes are also known as useful and important bioindicators of environmental pollution. There are established and standardised laboratory toxicity test methods for free-living terrestrial nematodes. These usually involve cultures of two bacterial feeding species, *Caenorhabditis elegans* and *Panagrellus redivivus*, maintained under laboratory conditions. Stress responses of plant feeding nematodes are also little known, despite long traditions of their practical applications. In our experiments, dose-dependent Cr and Cu-effects on plant-feeding nematodes, *Xiphinema* spp. and *Rotylenchus buxophilus* extracted from soil samples taken from natural habitats, were studied in aqueous media. The results showed noticeable differences between sensitivity of species in the same trophic group but with different evolutionary background, with *Xiphinema* being more sensitive than *Rotylenchus*. Our results underline the importance of using free-living nematodes in ecotoxicology, considering the dissimilar sensitivity patterns of nematode species belonging to different taxonomical and life strategy groups.

S21-P5

Metagenomic approach for the analysis of nematode diversity in soils with different health status

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Nematodes are widely recognised as bioindicators of the soil environment health. Analysis of soil nematode community is increasingly used to calculate various ecological indices related to enrichment and trophic status of nematofauna. The soil nematode community from three selected relatively undisturbed and disturbed sites in the Apulia region (Italy) was comparatively studied through both morpho-taxonomic and molecular analysis. Nematodes for both analyses were extracted from 100 g sub-samples from composite soil samples collected at each site. Nematodes were fixed in a 2.5% formaldehyde solution and then identified at family and genus level under an optical microscope. The maturity and trophic diversity indices were determined. For the molecular study, total DNA was extracted from the nematode community of each soil subsample and PCR amplification was performed by using the small subunit (18S) rDNA, as diagnostic marker, for nematode species discrimination. The 300 sequences available at this moment are still under characterisation. Sequencing of further 18S amplicons is also in progress.

Funds provided by Fondazione Cassa di Risparmio di Puglia

S21–P6

Ferroalloys industry and its impact on soil nematode communities

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The metallurgical industry and production of ferroalloys has a relatively long and strong history in the area around Dolný Kubín city (northern Slovakia). The production started in 1952 when OFZ a.s. Istebné was opened and its development continued by opening of similar plant OFZ a.s. Istebné branch Široká, just 10 km away. As a result of processing polymetallic ores, there was a hypothesis that soils near the plants and especially between plants have become heavily polluted by heavy metals originating from released emissions. In order to test this hypothesis chemical analysis of soils for heavy metals (As, Cd, Cr) was carried out. In addition, nematode communities were analysied for their bioindication potential of such disturbances. Soil samples were collected from the permanent grasslands at eight different sites from the area between plants. Nematodes were isolated from the soil samples, identified to genus and classified into trophic and functional groups. Several environmental indices (e.g. SI, EI, MI2—5) were applied to assess the impact of heavy metals on nematode communities as well as the state of maturity of the soil environment. The content of trace elements (As, Cd, Cr) in soil samples were analysed by mass spectrometry (ICP-MS). The results showed significant differences ($P<0.05$) between sites, especially between site A, located close to the pollution source and the rest of sampling sites. Heavy metals exceeded the permitted thresholds in two cases – Cr at site A (closest to the OFZ a.s. Istebné) and Cd at site H (closest to the OFZ a.s. Istebné branch Široká). From the composition of c-p groups within the nematode communities the most abundant groups were c-p1 and c-p2 with peaks at opposite ends of the observed transect. From ecological indices no significant differences ($P>0.05$) were observed in development of Shannon-Weaver index or in the number of genera in samples. According to MI2—5, the highest maturity of the ecosystem was found at site A near plant OFZ a.s. Istebné with the highest chrome level. Structural and Enrichment Indices showed that ecosystems with their food web conditions in the majority of study sites are maturing with a relatively low magnitude of disturbance. The rest of the sites (mainly in the middle between plants) were characterised as relatively well structured without any significant disturbances which could affect nematode community structure. However, we did not confirm our hypothesis which we postulated; our results showed that simultaneous use of ecological indices based on nematode community structure together with c-p group profiles affords enough information about status of the observed ecosystems and their range of disturbance in comparison with the level of heavy metal contamination.

S22 – Nematode genomics and transcriptomics: High Throughput Sequencing and *de novo* Assemblies

Convenors: Etienne Danchin & Ann Burnell

S22–T1

Genomics and functional genomics of the pine wilt nematode *Bursaphelenchus xylophilus*

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Bursaphelenchus xylophilus is an important plant pathogen, responsible for an epidemic of pine wilt disease in Asia and, increasingly, in Europe. *Bursaphelenchus xylophilus* has acquired the ability to parasitise plants independently from other economically important nematodes and has a complex life cycle that includes fungal feeding, a stage associated with an insect and plant parasitism. We have sequenced the genome of *B. xylophilus* and used it as a resource to understand disease mechanisms and the biological basis of its complex ecology. The assembled genome size is 74.5 Mb and predicted to contain 18,074 genes, a slightly smaller number than those present in *C. elegans.* An expansion in digestive and detoxification proteins was observed in the genome, which may reflect the unusual diversity in foods it exploits and environments it encounters during its life cycle. Additionally *B. xylophilus* possesses a unique complement of plant cell wall modifying proteins acquired by horizontal gene transfer, underscoring the impact of this process on the evolution of plant parasitism by nematodes. Together with the lack of proteins similar to effectors from other plant-parasitic nematodes, this confirms the distinctive molecular basis of plant parasitism in the *Bursaphelenchus* lineage. The genome sequence of *Bursaphelenchus* represents an important step forward in understanding its biology, and will contribute to efforts to control the devastating disease it causes.

S22–T2

The genome sequence and transcriptome of the potato cyst nematode *Globodera pallida*

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The potato cyst nematode, *Globodera pallida*, is the most economically important nematode to UK arable agriculture and one of the most damaging nematode pests of potato in temperate climates worldwide. To provide a valuable resource for molecular research, a UK consortium comprising the University of Leeds, the James Hutton Institute, Rothamsted Research and the Wellcome Trust Sanger Institute initiated a project to sequence and annotate the genome of *G. pallida*. The basis for the genome sequence is a standard Pa2/3 pathotype population "Lindley" that is typical in terms of its virulence and genetic characteristics of a broad range of *G. pallida* populations found throughout the UK. The initial draft genome assembly is now complete. The sequencing strategy utilised a combination of 454 and Illumina sequencing technologies to produce in excess of 100-fold coverage of the genome. Shotgun libraries of 3, 8 and 20kb inserts were used for unpaired and paired-end reads to enable contigs and scaffolds to be assembled. The assembly consists of 132 Mb contained within approximately 9000 scaffolds and encodes just over 21,000 predicted genes. Illumina transcriptome sequencing of eight different developmental stages of *G. pallida* has allowed analysis of expression profiles for all predicted genes. Clusters of genes with similar expression profiles can be identified so providing insights into gene function.

S22–T3

The 959 nematode genomes initiative and a phylum-wide phylogenomic analysis of Nematoda

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With steadily increasing throughput and decreasing costs of sequencing technologies, researchers can now generate a useful annotated draft genome in a few months for their favourite nematode. The 959 Nematode Genomes (959NG) initiative was created to keep the research community informed of ongoing genome and transcriptome sequencing projects for species of the phylum Nematoda. To encourage collaborations between groups interested in the same nematode or clade, a wiki was set up at 959.NematodeGenomes.org. On the wiki, sequencing projectscan be tracked at every stage, and the contact information of the researcher in charge of the project is available. Furthermore, a BLAST server is provided for preliminary assemblies allowing the quick use of data that have not been submitted formally to sequence repositories.

Utilising the data available at 959NG as well as other public repositories (NCBI, EBI, WormBase, FlyBase, and the Broad Institute), we produced a phylum-wide phylogenetic tree for nematodes. To account for the high rate of homoplasy between species and the effects of adaptive radiations, we applied multi-locus phylogenomic methods to improve topology resolution. We combined data from over 100 genes and 27 species in a supermatrix approach analysed with maximum likelihood and Bayesian inference methods. The phylogenetic tree derived manages to resolve topologies both at order and genus levels, providing a new molecular evolutionary framework for the nematodes.

S22–T4

Analysis of the transcriptome of *Hirschmanniella oryzae* to explore survival strategies and host-nematode interactions

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The rice root nematode *Hirschmanniella oryzae* is the most abundant nematode in flooded rice fields all over the world. Although it is economically less important compared to sedentary nematodes, it can cause severe yield reductions and economic losses in specific environmental conditions. No transcriptome data for this genus was available up till now. We have performed 454 sequencing on a mixed population to generate expressed sequence tags (EST) to gain more insight into the nematode—plant interaction and nematode survival. The results of two assembly strategies were combined to reduce redundancy of the dataset. The data was screened for putative (new) plant cell wall modifying proteins which help the nematode migrate through the host roots. Furthermore a set of LEA (Late Embryogenesis Abundant) proteins was discovered which could help *H. oryzae* to survive desiccation stress in dry soils in between growing seasons. Thaumatin, a protein that could be involved in abiotic stresses or antifungal activity, was found to be one of the most abundantly expressed genes. A potential secreted chorismate mutase could alter the salicylic acid synthesis pathway to lower the host defences upon infection. Further analysis of these genes will enable us to gain more insight into the biology and survival strategies of the nematode.

S22–T5

The identification of genes involved in anhydrobiosis and cryobiosis in the nematode *Panagrolaimus superbus* using RNA-Seq technology

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Panagrolaimus superbus is an anhydrobiotic nematode which can also survive freezing when its tissues are fully hydrated. These adaptations make *P. superbus* an excellent system for studying the molecular basis of environmental stress tolerance. We prepared 18 independent RNA-Seq libraries (Illumina Tru Seq) from cDNAs isolated from *P. superbus* which had been exposed to one of the following treatments: Desiccation – 98% Relative Humidity (RH) for 12h (T2) or 36h (T3); Recovery from Anhydrobiosis – rehydration for 2h (T4); Cold – exposure to 4°C for 24h (T5) or exposure to 10°C for 11d (T6); Control – unstressed nematodes (T1). Three biological replicates were prepared for each treatment.These treatment time points were selected based on the results of quantitative PCR using putative candidate genes involved stress and recovery pathways. The RNA-Seq reads were aligned to the *P. superbus* transcriptome (see O'Mahony Zamora poster) using TopHat. The DEseq package was used to identify differentially expressed genes. The number of differentially expressed genes (> than 2 fold up-regulated, $p<0.01$) identified in a comparison between T1 (control) and each of the other treatments were as follows: T2 85; T3 503; T4 606; T5 269 and T6 322. These gene sets contain homologs of stress-responsive genes from other organisms as well as novel sequences. Cluster analysis shows that the gene sets recovered from the cold and desiccation treatments are distinct from each other, implying that the nematodes appear to utilise distinct gene repertoires when responding to desiccation and cold stress.

S23 – Nematode genomics and transcriptomics: Functional analyses

Convenors:Ann Burnell & Etienne Danchin

S23–T1

Identifying specific parasitism genes in root-knot nematodes by genome mining

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Root-knot nematodes (RKN) are able to infect the roots of almost all cultivated plants, and constitute one of the most damaging crop pests in the world. The genomes of two root-knot nematodes, *Meloidogyne incognita* and *M. hapla,* have been sequenced and annotated. Comparative genomics and transcriptomics analysis against other species allows identification of genes specifically from parasites. These genes might be involved in functions essential for establishment and maintenance of a successful parasitic interaction. Furthermore, because they are restricted to the genomes of parasites, these genes constitute interesting targets for the development of specific control means.

We compared predicted proteins of root-knot nematodes against those of 25 other eukaryotic genomes (including plants and vertebrates) and against the NR database. Because our goal is to identify druggable parasitism genes, we discarded all genes that had a predicted ortholog in species that could be unintentionally damaged (e.g. plants, chordates, pollinator insects).

Using a series of bioinformatic screens, we selected nematode genes that (i) passed the taxonomic filter, (ii) are present in at least another parasite, (iii) had a transcriptional support from EST or RNA-seq data, (iv) had a signal peptide but no transmembrane region. A total of 41 proteins satisfied these criteria among which 15 were further analysed with design of siRNAs and infestation assays after silencing. In total 10 out of the 15 inactivated genes showed a significant reduction (as much as 60%) in the number of egg masses or gall numbers compared to the control.

S23–T2

Application of RNAi to develop plant resistance to nematode pathogens

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The aim of this work is to develop and apply RNA interference (RNAi) technology to establish host resistance in cereal, grass and dicotyledonous crop plants of economic importance. The focus is on resistance to root lesion nematodes (*Pratylenchus* spp.) that reduce yields of wheat, barley and sugarcane crops by 7—15% or more, and the beet cyst nematode (*Heterodera schachtii*), which is a major pest of brassica and beet crops. Using new sequencing technologies we have undertaken transcriptome analyses of *P. thornei*, *P. zeae*, and *H. schachtii,* and following annotation and comparative genomic analyses (Nicol *et al*. 2012), a series of potential target genes were identified which if silenced would confer host resistance. Two approaches to test the effects of silencing these target genes were undertaken – 'soaking' J2 nematodes in dsRNA, and delivery of dsRNA to nematodes via transgenic plants. Methods were established to generate transgenic plants of wheat, sugarcane and Arabidopsis, and for analysis of RLNs after soaking experiments (Jones *et al.*, 2009). Replicated lines of different transgenic events were challenged in soil or in sand with J2s of *H. schachtii* (Arabidopsis) and mixed stages of RLNs (wheat and sugarcane) of the different nematode species. With reductions in nematode replication of 90% or more, the results provide clear proof-of-concept that RNAi can be used to confer host resistance to nematode pathogens both in dicotyledonous and monocotyledonous crop plants.

Jones, M.G.K. *et al*. (2009). Towards developing transgenic resistance to nematodes in wheat. In 'CCN: status, research and outlook' Eds Riley, I.T. *et al*., Proc. First Workshop of the ICCNI, Antalya, pp 191-194.

Nicol, P. *et al*. (2012). *De novo* analysis and functional classification of the transcriptome of the root lesion nematode, *Pratylenchus thornei,* after 454 GS FLX sequencing. *Int. J. Parasitol*. 42, 225-237.

S23–T3

Gene silencing in root lesion nematodes

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We have used Roche 454 technology to sequence the transcriptome of mixed stages of the root lesion nematode (RLN) *Pratylenchus thornei*, and have subsequently assembled and annotated it (Nicol *et al.*, Int. J. Parasit. 42, 225-237, 2012). In the current work RNA interference in RLNs was assessed to study gene function in *P. thornei* and *P. zeae*. Conditions were optimised for 'soaking experiments', in which the RLNs were treated with double stranded RNA (dsRNA). Mixed stages of both *P. thornei* and *P. zeae* took up dsRNA in a basic soaking solution (M9 buffer, 0.05 % gelatin) containing 10—50 mM octopamine, 1—6 mM spermidine and 0.1—1 mg/mL FITC for 16 h without detrimental effects. Soaking in spermidine phosphate salt hexahydrate rather than spermidine or spermidine trihydrochloride improved uptake by nematodes, and also resulted in more effective gene silencing. Silencing of the genes *pat-10* and *unc-87* of both nematode species resulted in paralysis and slow and uncoordinated movements, although to a greater extent in *P. thornei*. QPCR analysis showed that there was also a greater reduction in transcripts of both genes in *P. thornei*. Following dsRNA treatments, replicated axenic carrot 'mini' discs (10 × 10 mm) were inoculated and cultured for 8 weeks: nematodes were extracted and counted from replicates each week. Untreated *P. thornei* and *P. zeae* increased in number, 32-fold and 73-fold respectively over 8 weeks. However, for *P. thornei* treated with dsRNA of *pat-10* and *unc-87*, there was a substantial reduction in replication, with 81% and 77% reduction in numbers of nematodes respectively at 5 weeks. These results show that RLNs are amenable to gene silencing.

S23–T4

Silencing the effectors of RNA silencing

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Root-knot nematodes (RKN, *Meloidogyne* spp.) are crop pests. These sedentary endoparasites induce and feed from host cells (giant cells). Using RNA interference (RNAi) specific genes can be silenced by introduction of double stranded RNA (dsRNA) similar to such genes into an organism. This technology has been used widely to study gene function in the free living nematode *Caenorhabditis elegans,* including identification of genes involved in the small interfering RNA (siRNA) and micro RNA (miRNA) pathways. In plant-parasitic nematodes, RNAi has been used to study gene function either by *in vitro* feeding of juveniles with dsRNA or *via* a plant producing corresponding dsRNA/siRNA. However, there appear to be differences in the mechanisms of RNAi between free living and plant-parasitic nematodes.

The aim of this project is to study the differences that may exist in the RNAi pathways between plant-parasitic nematodes and that of *C. elegans*. Genomic data mining and comparative bioinformatics have been used to identify similarities and differences in genes involved in the siRNA and miRNA pathways of these nematode groups. Protein domains and structures of identified RNAi effectors provide insights into their functions. *In vitro* feeding experiments to silence RNAs encoding protein domains of RNAi effectors followed by characterisation of expression of related genes and the fitness of nematodes can provide insights into the RNAi mechanism of RKNs. This study will identify candidate genes with potential for control of RKN *via in planta* delivery of dsRNA/siRNA, and this can be extended to broader resistance to more than one nematode species.

S23–T5

Identification and functional characterisation of effectors from the potato cyst nematode *Globodera pallida*

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The potato cyst nematode *Globodera pallida* induces complex changes in its host and effectors secreted from the pharyngeal gland cells are thought to be important in these processes. The genome sequence of *G. pallida* is now complete. We are currently identifying the full effector complement of this species. Key findings that have emerged to date include the fact that, apart from the cell wall degrading enzymes, there are almost no shared effectors between root-knot nematodes and cyst nematodes. *Globodera pallida* homologues of effectors from other cyst nematodes have been identified.In addition, over 100 potential new effectors (novel secreted proteins that are upregulated in parasitic stages) have been identified for further analysis. Many effectors in *G. pallida* are present as substantial gene families.Most notably, the SPRYSEC gene family in *G. pallida* consists of over 300 different genes. Further diversity within this family is generated through alternative splicing. We are currently using a whole life stage transcriptome dataset to compare expression profiles of the various members of this gene family. Effectors that suppress host defence responses are a particular focus for our current work and several candidates have been identified that may be important in this process.

S23–P1

Molecular characterisation of putative effectors in *Meloidogyne hispanica*

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The root-knot nematode *Meloidogyne hispanica* (Mhi) is a destructive endoparasite of several crops in different countries. Nematode secretions and proteins from the surface coat are likely to be the first signals perceived by the plant. These molecules, known as effectors, are thought to affect the host defence response, facilitating the penetration of the second-stage juveniles (J2) in the host plant, and to contain pathogenicity factors that induce the feeding site formation. Eight putative effectors from *M. incognita* and *M. hapla* were selected and specific primers, designed on the basis of the conserved regions between *M. incognita* and *M. hapla* genes, were used to amplify the genomic DNA obtained from *M. hispanica* J2. The eight partial gene sequences were successfully amplified by PCR and confirmed by sequencing, demonstrating the presence of these effectors in *M. hispanica*. They were characterised here for the first time and designated as Mhi-eng-1; Mhi-vap-2; Mhi-MnSOD; Mhi-vap-1; Mhi-map-1; Mhi-cpl-1; Mhi-sec-2 and Mhi-CRT-1. The results revealed a higher identity of these genes with those of *M. incognita* than with those of *M. hapla* and the phylogenetic analyses showed that each *M. hispanica* gene clustered with that of *M. incognita* and *M. hapla*. The characterisation of these putative effectors from *M. hispanica* will lead to further understanding of the infection process.

S23–P2

PCR-based cloning of four β-1,4-endoglucanases from the root-lesion nematode *Pratylenchus vulnus*

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Endo-1,4-β-glucanases have been found in numerous plant-parasitic nematodes (PPN) and play key roles in nematode—plant interactions.

Four β-1,4-endoglucanase encoding transcripts were cloned and characterised in the root-lesion nematode *Pratylenchus vulnus*. The *P. vulnus* endoglucanases show high similarity to other endoglucanases found in other nematodes belonging to glycosyl hydrolase family 5 (GHF5). All deduced proteins from the cloned sequences contained the predicted signal peptide for secretion and three of the four endoglucanases did not contain a carbohydrate-binding module (CBM). Real-time PCR experiments suggested that two of endoglucanase transcripts are expressed through the second-stage juveniles (J2), J3—J4 juveniles, males, and the adult females at different amounts confirming that all life stages are able to penetrate the host plant. *In-situ* hybridisation showed that both transcripts of endo-1,4-β-glucanases accumulated specifically in the pharyngeal subventral gland cells of all *P. vulnus* stages, thus suggesting the parasitic behaviour of each life stage. Recent data on these characterised genes will be presented and discussed.

S23–P3

Silencing of tomato *NGB* and *NAB* genes disturbs the development of syncytia

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Plant cyst nematodes are common pests of many crops causing substantial losses in agriculture. A potential alternative to nematicides is silencing of nematode or host plant genes crucial for pathogenesis. From the previously identified 150 tomato genes being up-regulated during *Globodera rostochiensis* migration and syncytium development we focused on *NIK*, *NGB* and *NAB*. The highest mRNA expression level of these genes was observed in roots containing syncytia at 7 dpi. Transcripts of *NGB* were *in situ* localised only in young syncytia while transcripts of *NAB* were found in 3—14 dpi syncytia and in surrounding cells. The regulatory regions of studied genes were cloned upstream of the *uidA* reporter gene, analysed in tomato and potato roots and were found to show changes in expression profiles upon infection. Functional analysis was supplemented by the RNAi of selected genes. Silencing of the *NIK* gene in tomato caused a dramatic decrease in regeneration ability and fertility of transgenic plants, whereas silencing of *NAB* or *NGB* genes slightly decreased plant fertility and changed fruit or leaf morphology. The number of *G. rostochiensis* females was reduced by 57—86% in *in vitro* tests and by 30—46% in pot trials. The observations of the development and ultrastructure of syncytia induced in transgenic lines revealed retarded growth, electron translucent cytoplasm, smaller vacuoles, as well as a reduced number of plastids, mitochondria and ER structures. These results demonstrate that *NGB* and *NAB* genes play an important role in the development of syncytia.

This work was supported by Polish Ministry of Science (grants no. 2P06A03230, PBZ089/P06/2003 and NN302-593938).

S23–P4

Silencing of *msp18*, a dorsal oesophageal gland gene of *Meloidogyne incognita*, through host delivered dsRNA in eggplant (*Solanum melongena*)

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Oesophageal gland secretions delivered through the stylet of *Meloidogyne incognita* play a major role in plant parasitism. Even though many genes specifically expressed in the oesophageal glands have been identified, the function and role of several novel genes in parasitism need to be established. Gene silencing by RNA interference is a powerful tool for functional genomics of genes both under *in vitro* and *in vivo* conditions. In the present paper, we report the effect of silencing by RNAi of the *msp18* gene coding for a novel secretory protein specifically expressed in the dorsal esophageal glands of parasitic second-stage juveniles (J2) and the late parasitic third stage juveniles. *In vitro* RNAi by soaking the J2 in dsRNA solution significantly interfered with their attraction towards host roots. *In vivo* RNAi through host delivery of dsRNA of *msp18* in eggplant was found to be very effective in reducing infection including development and reproduction. An *in vivo* assay revealed that the number of females produced per plant was reduced by about 73% in transgenics over the wild type control. Likewise, the total number of egg masses and eggs per egg mass was reduced by 80 and 40% respectively. The gene integration in these transgenics was confirmed by PCR and Southern hybridisation. Expression of the transgene has been verified by qRT-PCR. The present findings demonstrate that all stages of parasite cycle are affected due to silencing of *msp18* resulting in overall reduction of population build up at the end of the disease cycle. Disease management under infected field conditions is effective only when nematode multiplication (PF/PI) is drastically affected resulting in effective disease management. Thus, the transgenic eggplants generated are promising tools for management of root knot nematode under field conditions.

S23–P5

Horizontal gene transfer events in *Bursaphelenchus xylophilus*

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Bursaphelenchus xylophilus is a migratory endoparasite with the ability to feed on fungi and living plant cells. It is the causal agent of the pine wilt disease (PWD), a devastating disease that threatens wood lumber related industries in Portugal. Although the molecular mechanism of this disease is not completely clarified, horizontal gene transfer (HGT) has been pointed out as a probable mechanism to equip *B. xylophilus* with new genes facilitating its propagation in pine trees. A transcriptome project using pyrosequencing has been conducted at Biocant to study the pathogenicity of the nematode. The assay generated a total of 16,297 transcripts, among which we found genes with bacterial and fungal origins, leading to the possibility of finding new HGT events in *B. xylophilus.* To accomplish our search, we established a data analysis pipeline composed of several filters aiming to keep only genes of bacterial or fungal origin. Transcripts were filtered based on the organism of origin, statistical significance (E-value) and assigned protein function. A final screen was performed by removing all hits matching the microbial community of the nematode. Our methodology outlined three HGT candidates, a β-1,3-endoglucanase, already described for *B. xylophilus*, an alcohol dehydrogenase, previously described for *C. elegans* and a new gene belonging to the short-chain dehydrogenase/reductase family, with an undetermined role in the nematode biology and disease mechanism.

S23–P6

Assessment of assembly strategies for Roche/454 transcriptome data using the stress transcriptome of the anhydrobiotic nematode *Panagrolaimus superbus*

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Using the Roche/454 Titanium platform we have sequenced the stress transcriptome of *P. superbus* in an effort to better understand the pathways involved in anhydrobiosis and cryotolerance in this nematode. To increase the range of transcripts recovered, particularly stress-related transcripts, cDNA was prepared from pooled populations of mixed stage nematodes that had been exposed to one of the stress conditions: cold (24 h @ 4°C), desiccation (60h @ 98% rh, 20°C), heat (24 h @ 32°C), oxidative stress (38μM paraquat, 24h 20°C), as well as unstressed control nematodes. Two cDNA libraries were constructed and sequenced separately: a standard library prepared using a Mint cDNA synthesis kit (Evrogen, Moscow) and normalised library prepared using a cDNA normalisation kit (Trimmer NK001, Evrogen). Previously sequenced Sanger ESTs were also included in the final assembly. The reads from both libraries were combined, along with previously sequenced Sanger ESTs for the *de novo* assemblies. We carried out *de novo* assemblies using the following software: Newbler versions 2.3, 2.5 and 2.6 using the both the +URT and –URT parameters (urt = "use read tips"); CLCBio; Celera and Mira versions 301r2 and 3.4.0.1. We have also used the hybrid assemblers (Kumar and Blaxter, *BMC Genomics* 11, 571) CAP3 and Phrap on the various Mira and Newbler assemblies. Our data suggest that hybrid assemblers may not be optimal for *de novo* assembly of the *P. superbus* transcriptome and that cDNA normalisation increases the number of unigenes recovered from a transcriptome.

S23–P7

Effect of host delivered dsRNA of two FMRFamide like peptides, *flp-14* and *flp-18* on *Meloidogyne incognita* infecting tobacco

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FMRFamide-like peptides (FLPs) are neuropeptides that have been demonstrated to be fundamentally important to nematode biology. Disruption of FLP neurosignalling in plant-parasitic nematodes represents a novel form of pest control. We report silencing of two FLP genes through host delivered RNA interference that can affect infection of *Meloidogyne incognita* in transgenic tobacco. Southern hybridisation, PCR and qPCR confirmed stable integration and expression of double stranded RNA of *flp-14* and *flp-18* of *M. incognita* in tobacco. Quantification of the expression of both the genes using qRT-PCR indicated a fold expression difference of 484 to 9549 times in different transgenic events expressing *flp-18* over the wild type control plants. Similarly a fold difference ranging from 84.5 to 3821 was observed in various transgenic events expressing *flp-14*. The phenotypic consequence of RNAi was reduced nematode reproduction in terms of total number of egg masses per plant and eggs per egg mass although no significant difference was observed in the number of females between the transgenic and the wild tobacco plants. Nematode multiplication factor (PF/PI) is a key epidemiological determinant for disease intensity from crop to crop. The reduction in multiplication factor in different transgenic events of *flp-14* and *flp-18* ranged from 44 to 68 and 28 to 87 per cent respectively. Reduction in multiplication factor in the transgenic plants over the controls is promising for potential use under field conditions.

S23–P8

Molecular markers for the assessment of genetic diversity of pinewood nematode isolates from distinct geographic locations

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Bursaphelenchus xylophilus, the pinewood nematode (PWN), is the causal agent of the pine wilt disease, a devastating pine disease recently introduced into Portugal. The transcriptome of seven isolates, from Portugal (4), China (1), Japan (1) and USA (1), was established through next generation sequencing. While investigating the expression of genes related to the pathogenicity of the nematode, such as cellulase, expansin, pectate lyase or chitinase, intra- and inter-isolate diversity was detected. Upon detailed analysis of 26 transcripts, we identified a total of 155 single nucleotide polymorphisms (SNPs). The SNPs were confirmed by Sanger sequencing in the isolates referenced above and in seven additional isolates, enlarging our study to six geographic locations: Portugal-Continental (4), Portugal-Madeira Island (2), China (2), Japan (3), Korea (1), and USA (2). A total of 136 SNPs, in 10 contigs, were used to study the population structure and phylogeny. Discriminative SNPs were found for the Chinese, Portuguese, Japanese and American PWN isolates and even within the Portuguese isolates. These molecular markers clearly correlate the genetic diversity with the geographic location, and, based on it, a genotyping assay was developed, combining mismatch amplification mutation assay (MAMA) and Real Time-PCR. Our results revealed that Portuguese and Korean isolates share the same SNPs, cluster together and are very close to the Chinese isolates.

S23–P9

The road to identification of a core Tylenchid genome: Genome sequences from diverse species enables comparative analysis

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Plant-parasitic nematodes are a remarkably diverse and adapted group of pathogens, and the order Tylenchida represents the most diverse and successful group. In addition to the *Meloidogyne hapla* genome we previously sequenced, we have recently completed the genomes of *Pratylenchus coffeae*, and *Radopholus similis*, and are in progress on five additional species: *Ditylenchus dipsaci*, *Helicotylenchus multicinctus*, *Hoplolaimus columbus*, *P. penetrans*, and *P. thornei*. These species represent a diverse spectrum of migratory endo-parasites. In combination with the complete *M. hapla* and *M. incognita* genomes, these species represent a core to perform comparative genomic studies across the Tylenchida. The *M. hapla* genome contains 14,454 protein-coding genes, 5800 fewer than the free-living nematode, *Caenorhabditis elegans*. The genomes of *P. coffeae* and *R. similis* have approximately 6500 gene models. Extensive annotation has revealed that these species do share many genes in common, but that there are also genes that appear to be *M. hapla* specific. We are building on that observation to perform pan-order comparisons. We hypothesise that there may be a core Tylenchid genome, and acquiring these sequences provides solid data to identify it. We are examining the role of HGT, gene family expansion/contraction, and chromosomal organisation in the progression from migratory to sedentary endo-parasitism. In combination with other recently completed genomes including *Pristionchus pacificus*, and numerous animal-parasitic species, these data provide an important platform for comparative genomics across the Phylum Nematoda.

S23–P10

Cell biology of effectors: subcellular localisations of effectors from the potato cyst nematode *Globodera pallida*

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The potato cyst nematode *Globodera pallida* induces complex changes in its host and effectors secreted from the pharyngeal gland cells are thought to be important in these processes. The completion of the *G. pallida* genome sequencing project has allowed identification of the full effector complement of this species. However, many effectors are pioneer sequences with no similarity to other sequences in databases. Elucidating the functions of these effectors is a major challenge. Cell biology tools can be used for functional analysis of effectors in many ways. We have begun a large scale analysis of the subcellular localisation of effectors fused to fluorescent reporter proteins. Effectors have been identified that localise to the cytoplasm, endoplasmic reticulum, nucleus, nucleolus, peroxisomes and various other vesicles. Fluorescent markers of various subcellular structures have been used to confirm these localisations. Cell biology tools are also being used to confirm interactions between nematode effectors and host proteins identified in yeast two hybrid screens.

S24 – Potential of soil amendments and plant extracts to control plant-parasitic nematodes

Convenor: Wim Wesemael

S24–T1

Anaerobic soil disinfestation, a sustainable method to reduce populations of *Xiphinema index*?

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Grapevine fan leaf virus which is transmitted by dagger nematodes (*Xiphinema*) is a notorious virus disease in almost all grapevine-growing areas. Eliminating the vector could be one way to prevent virus transmission. So far chemical nematicides, which are dangerous from an environmental point of view, are often applied for this purpose. Anaerobic Soil Disinfestation (ASD), which is based on the activity and metabolism of already present soil microbial populations, might be a more sustainable alternative. After incorporation of organic material into soil and covering it with oxygen impermeable plastic, soil microbes fermenting the material create anaerobic conditions and produce several nematotoxic compounds such as ammonia, nitrous acid, hydrogen sulphide and volatile fatty acids.

Within this study, for the first time, we measured the effects of ASD on *Xiphinema index* in 11 l buckets filled with a mixture of 8 l of soil and different sources of processed organic material (Herbie®) or dried leaves of nettle (*Urtica dioica*)) applied at 0.01, 0.1 and 1% (V/V). Buckets were sealed with a lid and activity and vitality of nematodes was measured after 14 d and 28 d, respectively. In addition, at day 1, 5 and 7 we also measured the concentrations of several gasses within the buckets. Depending on the amounts of applied organic material, both the product of Herbie® and dried leaves of nettle led to anaerobic conditions and the formation of high concentrations of nitrous oxide, hydrogen sulphide and also methane. Strong nematostatic and nematicidal effects were seen after 14 and 28 d, respectively. Thus, at 1% organic material all individuals of *Xiphinema index* were killed. In our opinion, ASD might be an interesting alternative to manage populations of *Xiphinema index* in viticulture.

S24–T2

Efficacy of several organic and microbial fertilisers against root-knot nematodes (*Meloidogyne* spp.) in organic agriculture

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This study was conducted to determine the efficacy of materials such as organic and microbial fertilisers and some plant extracts in terms of their nematicidal effects against root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) as a biocontrol method in organic tomato (cv. Gökçe) production. Treatments consisted of the positive control treatment (with nematodes) (1), chemical control treatment using Fenamiphos (Nemacur®) (2), garlic extracts (NemGuard®) (3), biological control preparations containing the spores of *Paecilomyces lilacinus* strain 251 (Bioact®) (4), sesame oil (Nemax®) (5), organic fertiliser (Nemaflash®) (6), the extracts of *Quillaja saponaria* as nematicide (QLAgri®) (7), *Thymus* extracts (Bionem®) (8), Neem oil extracts (NeemAzal®) (9), and combinations of some of these materials such as (Nemax+QLAgri) (10), (Bioact+QLAgri) (11), (Nemax+Bioact) (12). Trials were conducted according to a randomised block design with four replicates during the autumn (August—January) of two successive years (2010 and 2011). Yield, galling index scale and population densities of the second-stage juveniles of *M. incognita* and *M. javanica* were determined to evaluate the effects of the treatments. In the first year (2010) the use of Bioact+QLAgri (37.83%) and Nemax+QLAgri (36.87%), in the second year (2011) Nemax (10.84%) and NemGuard (9.62%) reduced the root galling caused by *M. incognita* and *M. javanica* compared to the control plants. In terms of tomato yield, in the first year (2010) the use of Nemaflash (27.50%) and Nemax+Bioact (21.19%), in the second year (2011) Bioact+QLAgri (23.67%) and Nemax+Bioact (14.29%) increased yield significantly. It is concluded that the sesame oil (Nemax) alone or combined with other materials could be an effective control method against *M. incognita* and *M. javanica* in organic tomato production.

S24–T3

Diversity, pathogenicity and control of root-lesion nematodes, *Pratylenchus* spp., on potato

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Root-lesion nematodes (RLN), *Pratylenchus* spp., are serious pests causing damage in a wide range of cultivated plants, including potato, *Solanum tuberosum*. Although RLN are known to infect potato, little work has been done to assess the importance of these nematodes in this crop. The wide host range of RLN suggests that there are few chances for the control of these nematodes by crop rotation, enhancing the risk of an increase in the population densities. Therefore, it is crucial to evaluate the presence of RLN species in potato fields and to develop sustainable control strategies which are essential for nematode management. This work aims to increase knowledge about diversity, pathogenicity and control of RLN. Isolates, from different regions of potato production in Portugal, are being identified molecularly and differences in the reproductive fitness and pathogenic ability assessed. Furthermore, identification of genes encoding proteins related to the pathogenicity will be carried out by studying the transcriptome using high throughput large-scale parallel pyrosequencing. Finally, the effects of *Solanum sisymbriifolium* and *S. nigrum* extracts on mortality of RLN will be evaluated. Preliminary results show that the majority of the 38 potato root samples already collected have RLN.

S24–T4

The effect of fodder radish as a green manure crop on the population of *Meloidogyne chitwoodi* during the intercrop period

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Green manure crops are important to avoid erosion and leaching of nitrogen in surface water during the intercrop season in temperate agriculture. Therefore, their use is stimulated by local and European policy. However, the presence of green manure crops could facilitate population build-ups of plant-parasitic nematodes. Resistant cultivars of fodder radish (*Raphanus sativus*) against the root-knot nematode *Meloidogyne chitwoodi* have been developed. Apart from its resistance, fodder radish also contains glucosinolates which have a known nematicidal effect. Two cultivars and candivars of fodder radish from a breeding programme for resistance against *M. chitwoodi* were examined both in field and pot experiments. Resistance was found in the cultivars and candivars. In the field experiment the decrease of the *M. chitwoodi* population was similar under fodder radish and black fallow. The content of glucosinolates and their retention time in the soil were examined. In leaf and stem glucoraphenin (GRE) was prevalent, in the soil glucoraphasatin (GRH) was the most important. Four days after incorporation of fodder radish the concentration of GRH was highest, after 18 days this was GRE. Three weeks after incorporation almost no glucosinolates were found in the soil. Glucosinolates from the aerial parts of the plants were released gradually. Therefore, incorporation before the winter (frost) period could enhance beneficial effects. However, this was not shown in the field with an initially low population of *M. chitwoodi*.

WORKSHOP 3 – Quality assurance in nematology

Convenors: Loes den Nijs & Geraldine Antoine

Workshop 3–T1

Quality assurance in nematology

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Quality assurance is applied in many organisations, something we all have to deal with, hopefully resulting in accreditation of the organisation when relevant. In the Nematology field, laboratories struggle with the definition of the scope of quality system, procedures for test validation, characterisation of test performance, organisation of proficiency tests, ensuring the quality of results, maintaining competence of technical staff and many other points.

The aim of this workshop is to share the problems and solutions of various issues related to quality assurance, and to learn from each other saving much time in doing so.

All examples and personal experiences, e.g. presentations of results of proficiency tests, validation of extraction methods or new methods are possible and welcomed.

Workshop 3–T2

More than a decade of quality assurance in a nematology laboratory: the French experience

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Quality assurance has become obvious or even mandatory in laboratories involved in official analysis. A further step beyond quality assurance is accreditation according to an international standard ISO 17025.

The current French reference laboratory in nematology initiated the development of quality assurance system in the late 1990s and was accredited according to the ISO 17025 in 2002 for morphological and molecular techniques. Since this time, the scope of accreditation has increased year after year.

This presentation will focus on the main technical actions developed to fulfil quality assurance and accreditation requirements, such as traceability of analysis and operating procedures, metrology associated with nematode detection and identification, validation of methods, evaluation of technical staff competence and others. Examples of the choices made and the documentation associated will be presented.

Workshop 3–T3

Validation of the mistifier extraction method and morphological identification of *Ditylenchus dipsaci* from flower bulbs and onions

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The Plant Protection Service of The Netherlands is accredited against ISO-IEC 17025 for various methods. For nematology the mistifier extraction and identification of *Ditylenchus dipsaci* from flower bulbs and onions is the method under accreditation. Therefore validation of the mistifier extraction method was necessary.

This presentation will describe the validation procedure and the difficulties encountered during this process. To evaluate the different relevant performance criteria various experiments were carried out. Before the extraction experiments were started the nematode counting performance of the technicians was evaluated, because this might influence the results of the extraction data. In the experiments the performance of two types of mistifier apparatus was compared. Additionally the performance of the mistifier apparatuses was compared with the centrifugation flotation method for root material infested with *Meloidogyne chitwoodi*.

For *D. dipsaci* the extraction from several matrices with various numbers of nematodes was studied. Futhermore the effect of the length of the extraction period on the number of recovered *D. dipsaci* was investigated. By these experiments the performance criteria repeatability, reproducibility, analytical sensitivity and robustness could be determined.