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ABSTRACTS

ROOT KNOT NEMATODES: NEW INSIGHTS INTO PARASITISM SUCCESS. **Abad, P.** Institut Sophia Agrobiotech, UMR, INRA - CNRS - UNS, 400 route des Chappes, F-06903, Sophia-Antipolis, France.

The root-knot nematode *Meloidogyne incognita* is a widespread and polyphagous obligate asexual endoparasite of plants that causes serious and growing problems to agriculture. This lifestyle implies dramatic changes of plant cells into specialized feeding sites, which are induced by secreted proteins by the nematode, so-called parasitism effectors. An integrated approach of molecular techniques has been used to functionally characterize nematode parasitism proteins. The complete genome sequence of *M. incognita* revealed that the assembled sequence consists of homologous but divergent segment pairs that might represent former alleles in this species. Based on comparative genomics, we identified in root-knot nematode genomes a set of genes preserved during the evolution of plant-parasitic nematodes, only shared with organisms having a plant-parasitic lifestyle. These genes represent new targets to develop new methods to control plant-parasitic nematodes but harmless for the environment and consumer health. Another interesting feature of the *M. incognita* species is the paradox between its apomictic mode of reproduction and its potential host range encompassing more than 3,000 plant species. The adaptation of *M. incognita* to its environment (e.g., reproduction on resistant hosts) raises questions about genome plasticity leading to genetic variation and adaptive evolution. We reasoned that epigenetic mechanisms might in part be responsible for the generation of phenotypic variants that provide material for rapid adaptation. Thus, *Meloidogyne* spp. constitutes a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and hosts, in relation with parasitic success.

PLANT-PARASITIC NEMATODE THREATS TO GLOBAL FOOD SECURITY. **Abd-Elgawad, M.M.M.** Phytopathology Department, National Research Center, EI-Tahrir St., Dokki 12622, Giza, Egypt.

Plant-parasitic nematodes constitute a major constraint to agriculture. Estimates of their crop-loss are important for establishing research, extension, and budget priority. Regulatory policy action, pesticide impact assessment, resource allocation, and program prioritisation are usually contingent upon crop loss data. Recent questionnaire results of this study for world-wide estimates of crop losses due to plant-parasitic nematodes are presented herein. For the 20 life-sustaining crops, an average worldwide crop loss due to nematodes of 12.6% is estimated which equalled $215.77 billion annual yield loss. The other crops that represent a miscellaneous group important for food or export value have an average annual yield loss of 14.45% which equalled $142.47 billion. Losses for the total 40 crops average 13.5%. So, worldwide crop losses due to nematodes on 37 crops are estimated at $358.24 billion annually based on the 2010-2013 production figures and prices. These figures are staggering, and the real figure, when all crops throughout the world are considered, probably exceeds such estimations. Our current estimates far exceed previous ones probably because of challenging issues such as reduced number of effective nematicides available and limitation in their use due to environmental issues, increased adoption of intensive agriculture, climate change, occurrence of resistance-breaking pathotypes on economically important crops, and potential introduction of quarantine-nematodes. Basic and applied nematological research should be more oriented to provide better management of plant-parasitic nematodes in an economically and environmentally beneficial manner.

NEMATOLOGY EDUCATION AND TRAINING IN EGYPT AND NEIGHBOURING COUNTRIES. **Abd-Elgawad, M.M.M.** Phytopathology Department, National Research Center, EI-Tahrir St., Dokki 12622, Giza, Egypt.

During the second half of the twentieth century, pioneer nematologists from Egypt and neighbouring countries, taught nematology at universities in the Middle East for under and post-graduate students and few of them worked in the agricultural service institutions and governmental bodies for research, training and extension. In the last two decades, most Middle Eastern nematologists were taught by those pioneers or their students and graduated from local universities due to the high cost of education abroad. General Nematology courses are taught for undergraduates, but more specialized courses like Nematode Morphology, Nematode Physiology, Nematode Taxonomy, Nematode Management, Nematode Ecology, and Insect Nematology are taught for post-graduate students. In Egypt, infrastructure and training programs of nematology are based on three sectors namely: Egyptian universities (especially faculties of agriculture), the Agricultural Research Centre of the Ministry of Agriculture and Land Reclamation, and some institutes of the Ministry of Scientific Research. Similar governmental bodies are found in neighbour countries for nematode teaching, research and/or training programs. Currently, the numbers of Middle Eastern nematologists are almost enough to enhance the level of nematode education and training in their countries, but several drawbacks hinder the advance of applied nematology. These include lack of coordination and cooperation between specialists, insufficient awareness of nematode diseases and control especially by small scale farmers, limited financial resources, shortage in scientific instruments and equipment, and low activity of related agricultural

extension. The allocation of higher funding rates are proposed and assigned for specific nematological activities/sections to solve such problems.

MICOFLORA ON ROOT-KNOT NEMATODES IN SOIL WITH DIFFERENT LEVELS OF SUPPRESSIVENESS. **Adam[1], M., A. Westphal[2], J. Hallmann[1] and H. Heuer[1].** [1]Institute for Epidemiology and Pathogen Diagnostics , Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany.

Infective stages of plant-parasitic nematodes dwell through the soil matrix before entering host plant tissues. Attachment of specific nematode antagonists on the nematode during this process has been documented. There is a lack of information of attachment of microbes, which are not known as parasites of nematodes. In a greenhouse experiment of three soils with *Meloidogyne hapla*, numbers of galls, eggmasses and eggs were lower in non-treated portions than the heated equivalents. The hypothesis of a biotic cause of this suppression was supported by the differences of the microbial communities and concomitant differences in level of suppressiveness among the soils. In baiting studies with second-stage juveniles (J2) of *M. halpa*, differences in PCR-DGGE of the fungal ITS or bacterial 16S rRNA genes were detected in DNA isolated from soil-free J2 after incubation in the soils. Especially in fungal DGGE fingerprints, many fungal species were detected on the J2 that were below detection limit in the surrounding soil. On J2 from all three soils, species related to the genera *Davidiella* and *Rhizophydium* were detected. J2 baited in the most suppressive soil were specifically associated with the genera *Eurotium*, *Ganoderma*, and *Cylindrocarpon*. Among the 20 operational taxonomic units of bacteria specific for J2 in suppressive soil six were closely related to infectious species like *Shigella* sp.; most frequent were *Malikia spinosa* and *Rothia amarae*. In conclusion, a diverse microflora adhered to *M. hapla* J2 that may interfere with female fecundity of the nematode. Exploiting functions of these microbes may be possible.

COMPARISON OF LIFE HISTORY TRAITS OF THE ENTOMOPATHOGENIC NEMATODES *STEINERNEMA FELTIAE* AND *STEINERNEMA RIOBRAVE*. **Addis[1, 3], T., A. Teshome[2], O. Strauch[3] and R.-U Ehlers[3].** [1]Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Hermann-Rodewald-Str.4, 24118 Kiel, Germany; [2]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium; [3]e∼nema, GmbH, Klausdorfer Str. 28-36, D-24223 Schwentinental, Germany.

Life history traits (LHT) of entomopathogenic nematodes *Steinernema feltiae* and *Steinernema riobrave* were assessed at 25°C using a hanging drop technique. The LHTs were studied using 5x, 10x and 20x 10^9 cells ml^{-1} of *Xenorhabdus bovienii* and *Xenorhabdus cabanillasii* for *S. feltiae* and *S. riobrave,* respectively, in semi-fluid nematode growth gelrite. The results indicated that increased food density had a significant positive influence on offspring production on both, *S. feltiae* and *S. riobrave*. Highest offspring production was recorded at bacterial food densities of 20 x10^9 cells ml^{-1} with 813/female for *S. feltiae* and 1,913/female for *S. riobrave*. Significant positive correlations of R = 0.90 and 0.76 were found between body length and offspring production for *S. feltiae* and *S. riobrave*, respectively. The lowest intrinsic rate of natural increase (r_m) (1.1 days) was recorded for *S. feltiae* and the highest (1.4) for *S. riobrave*. A population doubling time (PDT) =0.6 days was recorded for *S. feltiae* and 0.5 days for *S. riobrave*. The life span of female nematodes, which ended the day following initiation of *endotokia matricida*, was not significantly different among the different bacterial food densities in both species. Significant differences in offspring production and population growth rate were assessed when comparing *S. feltiae* with *S. riobrave*. This result will be used to further investigate the optimal bacterial food density for mass production in bioreactors for maximum dauer juvenile recovery, synchronised population development and final yields of *S. feltiae* and *riobrave*.

IMPROVING PRODUCTIVITY OF *CITRULLUS LANATUS* SUBSP. *MUCOSOSPERMUS* (EGUSI MELON) THROUGH IDENTIFICATION AND USE OF LOCAL NEMATODE RESISTANT VARIETIES. **Affokpon[1, 2], A., H.K. Baimey[3], E.G. Achigan-Dako[2], C. Tossou[1], J.N. Lokossou[1] and A.H. Bokonon-Ganta[2].** [1]Institut National des Recherches Agricoles du Bénin, Bénin; [2]Université d'Abomey-Calavi, Bénin; [3]Université de Parakou, Bénin.

Citrullus lanatus subsp. *mucosospermus* F. (egusi melon, Cucurbitaceae) is a traditional fruit vegetable with important nutritional, economic and socio-cultural values in West Africa. However, root-knot nematodes (*Meloidogyne* spp.) are a major production constraint, and a reason for drastic reduction of the egusi melon cultivated areas. To develop strategies for sustainable root-knot nematode management in egusi melon production systems, eight egusi landraces from Benin were assessed for resistance or tolerance to root-knot nematodes in naturally infested fields. Results indicated that responses to root-knot nematode attacks differ significantly among egusi landraces. The egusi landrace BEN 1-3-7 showed the greatest potential in nematode suppression with a final nematode population density of 236 nematodes per 25 g roots. In contrast, the landrace BEN 1-5-13 had the highest final nematode population density (13929 nematodes / 25 g root), with the highest average hulled grain yield (528 kg/ha), suggesting a good tolerance of this landrace to root-knot nematodes. Root galling varied significantly between egusi landraces, with an average gall index of 3.25 over 10. Correlation analysis indicated, in some cases, a negative correlation between galling index and seed weight, and between nematode densities and seed weight.

The results provide evidence of genetic variability among West African landraces of egusi melon for reaction to root-knot nematodes.

POTENTIAL OF MALDI-TOF MASS SPECTROMETRY AS A DETECTION TOOL TO IDENTIFY PLANT-PARASITIC NEMATODES. **Ahmad[1], F., O.O. Babalola[1] and Hui-Fen Wu[2,3,4].** [1]Department of Biological Sciences, Faculty of Agriculture, Science and Technology, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa; [2]Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan; [3]Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan; [4]Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, Kaohsiung, 80424, Taiwan.

Plant diseases caused by plant-parasitic nematodes substantially reduce economic crop production every year, resulting in massive economic losses throughout the world. Accurate identification of plant-parasitic nematodes is essential to plant pathogen diagnostics and, thus, plant disease management. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, is a sedentary endoparasite that retards growth and development of plants by attacking the root system, causing galling, stunting, and other adverse effects. We demonstrated the feasibility for the use of MALDI-TOF MS for rapid, direct and sensitive detection of adult root-knot nematode and their second stage juveniles (J2). We have proposed simple pretreatment protocols and have identified *M. incognita* with help of generated mass spectra of targeted samples. We also reported the differentiation between the adult and juvenile stages of the nematode based on mass spectrometric profiling, with the adult and juvenile stages exhibiting different marker peaks. However, common peaks to both stages were also found. The ability to record biomarker ions, in a broad m/z range, which are unique to and representative of individual nematode species, could form the basis of identification of nematodes by MALDI-TOF MS. However, more work needs to be done on other genera and species to know if identification between species of nematodes is possible.

NEMATICIDAL ACTIVITY OF POKEWEED EXTRACTS. **Ahmed[1], S., P. Gerard[2] and P. Agudelo[1].** [1]School of Agricultural, Forest, and Environmental Sciences, Clemson University. Clemson, South Carolina 29634, USA; [2]Department of Mathematics, Clemson University. Clemson, South Carolina 29634, USA.

The nematicidal activity of pokeweed (*Phytolacca americana*) extracts on five plant-parasitic nematodes species was investigated. Extracts of leaves, stem, and roots were evaluated *in vitro* for their toxicity to *Ditylenchus dipsaci*, *Pratylenchus penetrans*, *Apehlenchoides fragariae*, *Rotylenchulus reniformis*, and *Meloidogyne incognita*. Extracts from the different plant parts obtained using different solvents (water, ethanol, methanol, and acetone) were also compared. The calculated LD50 for the extracts was used to determine the range of concentrations to be used in subsequent greenhouse studies. We report the results of greenhouse studies with root-knot and reniform nematode on soybean plants. Seedlings of soybean cv. Hutcheson were inoculated with 3,000 reniform nematodes or 2,000 root-knot nematode juveniles, one week after planting. One week after inoculation, selected extracts of leaves, stems or roots were added at a dose of 300 μg per plant, with the nematicide oxamyl (24% Vydate, 0.6 μl per plant) as positive control and untreated plants as negative controls. Each experiment was repeated twice and 20 replicates per treatment were included. Six weeks after inoculation, galls and egg masses per plant were assessed for the root-knot nematode experiments and reproduction factor for the reniform nematode experiments. Water extracts from pokeweed stems and leaves showed significant reduction of nematode reproduction and provided the same control as Oxamyl. These results suggest that pokeweed extracts are potential useful nematicides for management of root-knot and reniform nematodes.

HOST STATUS OF THREE *LOLIUM* GRASS CULTIVARS TO SOUTH AFRICAN *MELOIDOGYNE* SPEICES. **Ahuja[1], P., H. Fourie[1] and A.P. Nyczepir[2].** [1]Unit of Environmental Sciences and Management, North-West University, Potchefstroom-2520, South Africa; [2]USDA-ARS, SE Fruit & Tree Nut Research Laboratory, USA.

Lolium grass is used extensively as a cover crop and for forage production in South Africa. The host suitability of two popular South African *Lolium* cultivars (Agrihilton & Raaigrass) and one (Jesup Max-Q) from the USA were evaluated for their host status to *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions. *Lolium* and tomato seedlings (cv. Rodade) were inoculated with ± 2000 second-stage juveniles of the two *Meloidogyne* spp. The parameters recorded 56 days after nematode inoculation were i) number of egg masses and ii) number of eggs and second-stage juveniles/root system. Substantial, but insignificant variation existed among the three *Lolium* cultivars with regard to the parameters recorded. For *M. incognita* all three *Lolium* cultivars had Rf values > 1, indicating susceptibility to these nematodes. Conversely, Rf values < 1 were recorded for the three *Lolium* cultivars when evaluated against *M. javanica*, indicating resistance to the latter species. None of the evaluated cultivars of *Lolium*, however, were immune to either *M. incognita* or *M. javanica*. The susceptible tomato cultivar Rodade, had significantly higher numbers of egg masses and eggs/root system compared to those recorded for the *Lolium* cultivars. Inclusion of these three *Lolium* cultivars in integrated nematode management systems where *M. javanica* causes problems can contribute towards managing this nematode pest effectively. However, in soils where *M. incognita* prevails none of these three *Lolium* cultivars is recommended to be cultivated.

POTENTIAL OF VETIVER (*CHRYSOPOGON ZIZANIOIDES*) GRASS ROOT EXUDATES AND EXTRACTS AS A TOOL TO MANAGE *MELOIDOGYNE*. **Ahuja, P., M.S.A. Pretorius and H. Fourie.** Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520.

In 2013 *Meloidogyne* has been listed as the number one plant-parasitic nematode genus that damage agri- and horticultural crops worldwide. The withdrawal of synthetic nematicides from world markets necessitated studies aimed at evaluating various plant-derived products with nematicidal/nematostatic characteristics. Various concentrations of crude Vetiver root exudates and extracts, respectively, were evaluated for their effects on the motility of *Meloidogyne javanica* J2 as well as the reproduction of this pest in laboratory and greenhouse experiments. The 50, 75 and 100% root exudate concentrations reduced J2 motility significantly ($p \leq 0.05$) from 48 h after the onset of the trial compared to the tap water control. For root extracts, from 53 to 94% of the J2 were non-motile for the 25, 50, 75 and 100% product concentrations, respectively, compared to that of 35% for the tap-water control at termination of the trial (94h sampling interval). *In vivo* reproduction data showed no significant differences for the number of egg masses as well as eggs and second-stage juveniles (J2)/root system 56 days after J2 that were exposed to the Vetiver root exudates were inoculated on roots of tomato seedlings. Although Vetiver root exudates inhibited the motility of J2, they were able to recover when removed from it and thus reproduced in tomato roots. A similar assay is currently in process for the root extract concentrations. Follow-up studies will focus on identification and refining of the chemicals that adversely affected J2 motility. Field testing of such products are also envisioned since this may render an alternative and environmentally safe product for future use to minimise damage inflicted by *Meloidogyne* in crops.

INTRASPECIFIC VARIABILITY AND GENETIC STRUCTURE IN FRENCH *GLOBODERA TABACUM* POPULATIONS. **Alenda, C., J. Montarry and E. Grenier.** INRA, UMR IGEPP, Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France.

The dispersal abilities and the population genetic structure of nematodes living in soils are poorly known. In the present study, we have pursued these issues in the tobacco cyst nematode, *Globodera tabacum*, which is responsible for significant yield reductions in the USA, France, Spain and Italy. In this study, ten microsatellites markers were used to analyse the patterns of its genetic structure in France and to compare the genetic diversity observed to some European and American populations. Large heterozygote deficiencies were observed at most loci. The limited active dispersal ability of larvae in soil, which favours mating between (half) siblings or a genetic structure at a sub-population scale, could be responsible for this pattern. Bayesian genetic assignments revealed two distinct genetic groups that matched with the geographic repartition of two agricultural cooperative societies. Very high F_{ST} values were observed between the overall pairs of French populations and analysis of partitioning of molecular variability showed that an important part of the genetic variability was observed among agricultural cooperative societies (34%) and among populations (39%), which support limited gene flows among the TCN populations.

PLANT PARASITIC NEMATODES COMMUNITIES ASSOCIATED WITH OLIVE TREES IN MOROCCO. **Ali[1], N., E Chapuis[1], J. Tavoillot[1], M. Aït Hamza[2], A. El Mousadik[2], A. El Oulkadi[3], G. Besnard[4], A. El Bakkali[5], A. Moukhli[3], B. Khadari[6], C. El Modafar[7], M. Ater[8] and T. Mateille[1].** [1]IRD, UMR CBGP, Montferrier sur Lez, France; [2]UIZ-FST, Agadir, Morocco; [3]INRA, Marrakech, Morocco; [4]CNRS, UMR EDB, Toulouse, France; [5]INRA, Meknes, Morocco; [6]INRA, UMR AGAP, Montpellier, France; [7]UCAM-FSTG, Marrakech, Morocco; [8]UAE-FST, Tetouan, Morocco.

Plant-parasitic nematodes significantly contribute to economic losses in the ten top olive producing countries in the world, especially in the Mediterranean basin (Spain, Italy, Greece, Tunisia, and Morocco). Diversity and structures of plant-parasitic nematode communities respond to evolutionary, environmental and anthropogenic forces. Instead of controlling the main pathogenic nematode species as usual, one of the innovative strategies to control plant-parasitic nematodes would be to manage diversity in communities in order to lead them to be less pathogenic. The present study aims at understanding the contribution of olive domestication and human impacts on the plant-parasitic nematode communities by analyzing the diversity of plant-parasitic nematodes in cultivated olive compared to wild olive in Morocco. Thus, 220 samples were collected in 2012 in several sites with cultivated and feral olive trees (i.e. wild olive resulting from cultivated olive) in the olive production areas located all along the Atlas foothills, as well as on wild olive Morphobiometric observations revealed a significant diversity of plant-parasitic nematodes, belonging to 12 families and 28 genera. Our results showed the presence of genera *Pratylenchus* and *Meloidogyne* in all sampled systems that are known harmful taxa for agriculture especially in nurseries. These two genera were more abundant in the cropped systems. Principal component analysis revealed no significant effect of olive systems on the diversity of PPN in communities but on the PPN community patterns Other factors such as olive genotype, soil physic-chemical characteristics, geo-climatic characteristics, associated plants with olive trees will also be discussed as major factors affecting the plant-parasitic nematode community patterns.

EFFECT OF THE MECHANICAL WOUNDING OF GREEN BEAN ROOTS ON JOINT INFECTION WITH *MELOIDOGYNE JAVANICA* AND *MACROPHOMINA PHASEOLINA*. **Al-Nadhari, S.** King Saud University, Yemen.

The effect of the mechanical wounding of the roots of green beans, *Phaseolus vulgaris*, on the development and severity of the disease complex caused by *Meloidogyne javanica* and *Macrophomina phaseolina* was investigated in the greenhouse (25 ± 2°C). Five treatments with five replicates each were used in a randomized complete block design. The treatments included: 1) control, 2) *M. javanica*, 3) *M. phaseolina*, 4) *M. javanica* + *M. phaseolina* without root wounding, 5) *M. javanica* + *M. phaseolina* with root wounding. Root wounding was done by piercing (twinge) the root tips with very fine needle just prior to inoculation by the nematode and fungi. Results showed that root piercing has increased the severity of the disease complex. Plant growth decreased and gall and charcoal –root indices increased. Nematode reproduction also has increased. For example: plant weight has reduced ($P \leq 0.05$) almost 50%, compared to the control, where root galling and disease index were increased. *M. javanica* + *M. phaseolina* plus root wounding gave the highest number of eggs/g roots and the highest fungus colonization of the roots, compared to the same treatment, but without root wounding.

DETECTION OF SUBSTANCES RELATED TO THE RESISTANCE OF COTTON GENOTYPE TX 25 TO *MELOIDOGYNE INCOGNITA* RACE 3. **Alves[1], G.C.S., S. da Costa Santos[2], P.H. Ferri[2], V.H.S. Barbosa[2], M.R. da Rocha[2].** [1]Instituto Federal Goiano, Rodovia Geraldo Silva Nascimento Km 2,5, CEP 75790-000 – Urutaí, Goiás, Brazil; [2]Universidade Federal de Goiás, Escola de Agronomia, Caixa Postal 131, CEP 74690-900, Goiânia, Goiás, Brazil.

Plants produce a wide range of secondary compounds that are part of the arsenal against pathogens. These substances may be pre or post formed, or may exist in the plant regardless of the presence of the pathogen. The purpose of this study was to detect the biochemical activity in resistant wild cotton genotype TX 25, as compared to a susceptible cultivar, to *Meloidogyne incognita* race 3. Plants were grown under greenhouse conditions and each plant was inoculated with 5,000 eggs and J2. At 8, 24 and 35 days after inoculation (DAI) root extracts were prepared for detection and quantification of total phenols, total flavonoids, analysis of phenols by high performance liquid chromatography (HPLC) and metabolite quantification by nuclear magnetic resonance of hydrogen (RMN). Total phenols were higher at 35 DAI on both resistant and susceptible genotypes. Flavonoid glycosides and gossypol derivatives were quantified by HPLC. A flavonoid glycoside was considered one of the secondary compounds related to the resistance because it was higher on TX 25 in all evaluations. In NMR it was found that sugars also confer an initial protection to genotype TX 25.

PLANT PARASITIC NEMATODES: A SERIOUS THREAT TO AGRICULTURAL CROPS IN THE KINGDOM OF SAUDI ARABIA. **Al-Yahya, F.A.** Department of Plant Protection, College of Agriculture and Food Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Saudi Arabia.

Meloidogyne spp were first reported on tomato in 1957 in the Eastern region of Saudi Arabia. Since then many other plant parasitic nematodes have been reported causing serious losses to many economically important agricultural crops. Until now several efforts have been made for the eradication of these nematodes both in the greenhouse and in the field, but success has not yet been achieved. For successful and target-oriented control of theses nematodes, a comprehensive nematological survey must be carried out to identify the various plant-parasitic nematodes based on their prevalence and economic importance. Thus we have recorded a total of 30 nematode genera from various regions of the kingdom. These genera have been classified into three groups. The first group includes the genera of the highest prevalence and economic impact (e.g. *Meloidogyne* spp., *Heterodera*, *Pratylenchus* and *Tylenchulus*). The second group represents the genera with relatively medium importance and prevalence (e.g. *Rotylenchulus*, *Helicotylenchus*, *Rotylenchus* and *Tylenchorhynchus*.) while the third group includes the nematodes of least prevalent and economic importance. We believe this information would be of great importance to lay out meaningful control strategies.

NEMATODE INTERCEPTIONS IN INTERNATIONAL TRADE OF PLANTS FOR PLANTING. **Anthoine[1], G., B. Niere[2], L. den Nijs[3], T. Prior[4], L. Pylypenco[5] and N. Viaene[6].** [1]ANSES, 7 rue Jean Dixméras, 49044 Angers Cedex 01, France; [2]JKI-Plant Health Institute, Messeweg 11/12, 38104 Braunsweig, Germany; [3]NPPO-NRC, Geertjesweg 15, 6700 HC Wageningen, Netherlands; [4]FERA, 02FAO1/5, Sand Hutton, YO41 1LZ, York, United Kingdom; [5]Instutite of Plant Protection-dept of Plant Quarantine, 33 Vasilkovskaya Str., 03022 Kiev 22, Ukraine; [6]ILVO-Plant-Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium.

Trade of plants for planting and ornamentals in Europe has increased dramatically in the last few decades. For instance, numbers of *Ficus* imported to the Netherlands has increased from 1.6 million in 2006 to 3.8 million in 2010. Traded plants originate from different countries of which some play a major role at present (e.g. China). According to phytosanitary regulations (ISPM no 1 and the EU council directive 2000/29/EC) plants shall be free of harmful organisms or treated in such a way that no harmful organisms shall be present in order to prevent their introduction, spread and establishment. Absence of harmful organisms has to be checked by the importing country and when interceptions occur, appropriate measures should be taken. The latter is difficult to define. The findings should be officially notified by the European Union (EU) countries to the EU-commission via EUROPHYT. In this presentation we show what some countries (France, UK, Belgium, Germany,

Netherlands and Ukraine) have intercepted during the last decade. We will show the diversity of the nematode pressure in the framework of global trade and that different approaches in the countries on how to deal with inspections and treatment might be reflected in the data. A Dutch survey on import of 21 different countries over 3 years showed that around 20% of the samples contained EU-quarantine nematodes. Other nematodes of phytosanitary significance (based on PRA quick scan) were found in 11% of the samples. We will discuss what this data mean in terms of threats towards the importing countries.

KNOCKING-DOWN *MELOIDOGYNE INCOGNITA* PROTEASES BY PLANT-DELIVERED DSRNA HAS NEGATIVE PLEIOTROPIC EFFECT ON NEMATODE VIGOR. **Antonino-de-Souza Jr[1,2], J.D., R.R. Coelho[1,2], I.T. Lourenço[1,2], R.R. Fragoso[3], A.A.B. Viana[4], L.L. P. Macedo[2], M.C.M. Silva[2], R.M.D.G. Carneiro[2], G. Engler[5], J. de Almeida-Engler[5] and M.F. Grossi-de-Sa[2,4].** [1]Graduate Program in Biology Molecular, Universidade de Brasília, Brasília, Distrito Federal, Brazil; [2] Embrapa Recursos Genéticos e Biotecnologia, Brasília, Distrito Federal, Brazil; [3] Embrapa Cerrados, Planaltina, Ditrito Federal, Brazil; [4]Graduate Program in Genomic Sciences and Biotechnology, Universidade Católica de Brasília, Brasília, Distrito Federal, Brazil; [5]Institut National de la Recherche Agronomique, UMR 1355 ISA/Centre National de la Recherche Scientifique, UMR 7254 ISA/Université de Nice-Sophia Antipolis, UMR ISA, Sophia-Antipolis, France.

Meloidogyne incognita causes major damage and yield losses in numerous important crops worldwide. Examination of the *M. incognita* genome revealed a substantial number of proteases belonging to five different catalytic classes. Several reports indicate that *M. incognita* proteases could play essential roles in nematode parasitism, besides their function in digestion of giant cell contents. Nevertheless, the precise roles of these proteins during parasitism are still unknown, making them interesting targets for gene silencing to address protein function. We have knocked-down an aspartic (*Mi-asp-1*), a serine (*Mi-ser-1*) and a cysteine protease (*Mi-cpl-1*) by RNAi interference to investigate the function of these enzymes during the plant-nematode interaction. Tobacco lines expressing dsRNA for *Mi-ser-1* (dsSER), *Mi-cpl-1* (dsCPL) and for the three genes together (dsFusion) were generated. Interestingly, nematodes that infected plants expressing dsRNA for proteases produced a reduced number of eggs. In addition, nematode progeny matured in dsSER plants had reduced success in egg hatching, while progeny resulting from dsCPL and dsFusion plants were less successful to infect wild-type host plants. Quantitative PCR analysis confirmed a reduction in transcripts for *Mi-cpl-1* and *Mi-ser-1* proteases. Our data suggest that these proteases are involved in different processes during nematode development, like nutrition, reproduction and embryogenesis. A better understanding of nematode proteases and their possible role during a host plant-nematode interaction might help to develop novel strategies for phytonematode control.

NATURALLY OCCURRING ENDOPHYTES ASSOCIATED WITH WHEAT AS BIOCONTROL AGENTS AGAINST CEREAL CYST NEMATODES. **Ashrafi[1,2,3], S., W. Maier[2], G. Erginbas-Orakci[1], M. Finckh[3], and A. Dababat [1].** [1]CIMMYT-Turkey, P.K. 39 Emek 06511, Ankara, Turkey; [2]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, 38104 Braunschweig, Germany; [3]University of Kassel, Faculty of Organic Agricultural Sciences, Ecological Plant Protection, Nordbahnhofstr. 1 a, 37213 Witzenhausen, Germany.

Cyst nematodes (*Heterodera* and *Globodera* spp.) are distributed worldwide and have a great impact on crops. Within the *Heterodera avenae* complex, *H. avenae*, *H. filipjevi* and *H. latipons* are the most important cereal cyst nematode species (CCNs) which can cause significant economic losses. Despite the difficulties in controlling of cyst nematodes, biological control can be applied alone or in integrated management approaches to reduce the damages of nematode. Preliminary results obtained from a field experiment in Turkey indicated that there might be suppressive activities, potentially caused by fungi or bacteria, which can affect the nematode populations of CCNs. Therefore the present study is conducted with the following aims: i) isolation and identification of naturally occurring endophytic and parasitic fungi and bacteria from wheat and *Heterodera filipjevi* ii) evaluation of the biocontrol effects of the isolated fungal and bacterial species on *H. filipjevi*. To this aim a total of 175 bacteria and fungi associated with wheat and cysts of *H. filipjevi* were isolated from nematode infested fields. Isolates were purified and *in vivo* screening experiments will be conducted to evaluate their biocontrol potential towards the nematode. The total number of cysts extracted from both soil and the root system was counted and plant growth parameters including fresh root and shoot weight as well as plant height were measured. Preliminary results indicated that a total of 19 isolates including ten fungal and nine bacterial isolates are promising to be used in further detailed studies.

PATHOGENICITY AND MANAGEMENT OF *MELOIDOGYNE INCOGNITA* ON *PITTOSPORUM TOBIRA* IN FLORIDA. **Baidoo[1], R., R.H. Stamps[2], and W.T. Crow[1].** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611; [2]MidFlorida REC, University of Florida, Apopka, FL 32703, USA.

Pittosporum tobira is grown primarily as a cut foliage crop in Florida. However, many *P. tobira* farms have been abandoned owing to severe root-knot nematode infestation and lack of effective management tactics. Hence, research is underway by the University of Florida to investigate the pathogenicity of root-knot nematode *Meloidogyne incognita* on *P. tobira* and to identify pesticides or bio-pesticides that could be used to manage these nematodes on perennial cut foliage crops, including *P. tobira*. This research includes on-farm, small plot, and greenhouse experiments. Pesticide and

biopesticide treatments evaluated include commercial formulations of spirotetramat, furfural, and *Paecilomyces lilacinus* strain 251. Treatment applications were made during the spring and fall seasons according to manufacturer's specifications. Efficacy was evaluated based on J2/100cm^3 of soil, J2/g of root, and crop yield (kg/plot) or plant growth (canopy volume/plant). Greenhouse results reveal that *M. incognita* causes significant growth reduction in *P. tobira*. It was also found that, *M. incognita* infection increases incidence and severity of leaf spot disease caused by *Cercospora* sp. on this crop. On-farm and small plot trial results indicate that furfural and *P. lilacinus* were effective in reducing population density of *M. incognita* on *P. tobira*.

SURVIVAL AND INFECTIVITY OF THREE ENTOMOPATHOGENIC NEMATODES SPECIES ON *TRINERVITERMES OCCIDENTALIS* (ISOPTERA: TERMITIDAE) AS AFFECTED BY PESTICIDES, SOIL TEMPERATURE AND MOISTURE. **Baimey[1], H., L. Zadji[1], L. Afouda[1], M. Moens[2] and W. Decraemer[2, 3].** [1]University of Parakou. BP 123. Parakou, Route de l'Okpara, Benin; [2]Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium; [3]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium.

Experiments were conducted to studythe survival of four Beninese isolates of entomopathogenic nematode (*Heterorhabditis indica* Ayogbe1, *H. sonorensis* Azohoue2, *H. sonorensis* Ze2, and *Steinernema* sp. Bembereke) in aqueous solutions of three pesticides i.e. fipronil (50g/l), sulfur (80%) and glyphosphate (41%). Secondly to test the infectivity of the isolates following fipronil treatment on the last instars *Galleria mellonella*. Thirdly to test the effect of temperature on the virulence of the EPN isolates applied on the termite *Trinervitermes occidentalis* and lastly to test the effect of soil moisture content on termites. The time at which 50% nematodes survived (ST_{50}) after a pesticide treatment varied with nematode isolate from 1.38h (*H. indica* in sulfur at 2-fold rd) to 15.53h (*H. indica* in glyphosate at 0.5 fold rd). The higher the pesticide dose, the lower the ST_{50} value for each of the nematode isolates. The increase of both exposure time and fipronil concentration had negative impact on the efficacy of tested EPN isolates on *G. mellonella*. Termite mortality differed significantly between temperatures and soil moisture content for all tested EPN isolates. The greater the temperature at which termites and nematodes were exposed, the lower termite mortality with Azohoue2 giving highest mortality values at 27°C (91%), 30°C (69%), 33°C (67%) and 35°C (65%). However, the effect of soil moisture content on the EPN efficacy did not vary with the IJ concentration.

CURRENT TRENDS IN PHYLOGENY AND CLASSIFICATION: OBSERVATIONS FROM A MORPHOLOGICAL PERSPECTIVE. **Baldwin, J.G. and T. Pereira.** Department of Nematology, University of California, Riverside, California 92521, USA.

Challenges in phylogeny/classification are little changed from those articulated >25 years ago in ''Vistas of Nematology,'' but new priorities and tools point to major advances. These include increasingly rigorous molecular phylogenies using multiple genes, broader taxon representation, greater computer power and increasing analytical sophistication. Molecular-based phylogenies often point to errors in traditional classifications and raise related questions of morphological homology that are increasingly addressed with emerging microscopy tools supporting comparative digital imaging, analysis and reconstructions. Furthermore, genomics underlying phenotypic expression and evolution is adding a new dimension to recognizing homology, phenotypic plasticity and mechanisms/patterns of evolution. Often detailed morphological reconstructions first demonstrated with complex approaches can later be assessed with simpler techniques and broader taxon representation involving, for example, specific staining and confocal microscopy. Beyond phylogenetic classification increasing computational power and advances in digital microscopy/imaging have become important tools for assessing and vouchering biodiversity, including ''reverse taxonomy'' for efficient new species discovery. These tools have capability for improved global access to collections/metadata, vouchers and valuable rare specimens (e.g. types) that may include remote access to mounts on microscopes in distant collections. Twenty-five years ago challenges included how to approach inferring phylogeny, how to integrate traditional morphology with emerging molecular data, and how to express new findings in the inherently conservative process of classification. An overarching challenge was filling gaps in phylogenetic representation that demand progress toward globally inventorying and describing putatively more than 1,000,000 nematode species. These challenges remain, but emerging tools and techniques hold increasing promise toward further resolution.

THE TANGLED WEB WE WEAVE: HUMAN MEDIATED SPREAD OF NEMATODES VIA TRADE NETWORKS. **Banks[1,2,3,4], N.C., M.E. Hodda[1,2,3], S.K. Singh[1,3,5], D.R. Paini[1,2,3], K.L. Bayliss[4].** [1]Plant Biosecurity Cooperative Research Centre, Bruce, ACT, 2617, Australia; [2]CSIRO Biosecurity Flagship, Dutton Park 4102, Australia; [3]CSIRO Ecosystem Sciences, Canberra, ACT, 2601, Australia; [4]School of Veterinary and Life Sciences, Murdoch University, Murdoch 6150, Australia; [5]Graham Centre for Agricultural Innovation, Wagga Wagga, New South Wales, 2678, Australia.

Quarantine and hygiene are major measures for preventing damage from plant-parasitic nematodes, but there is little information on nematode spread, especially related to rapidly increasing trade associated with globalization. To investigate nematode movement associated with traded plant material, nematodes were sampled from markets of different sizes and types in several countries, together with the origins and destinations of the produce and volumes traded. Nematodes were

found consistently on various crops and associated soil. All trophic groups were represented. Markets differed in the distances produce travelled to get there, with some trading mostly local material, others trading mostly material from distant places, and others with a mix of the two. These patterns of movement can be analysed using network models and mapping software to suggest the speed, distance and amount of nematode spread using these pathways. This information will enhance biosecurity and quarantine through better targeting of surveillance and preventative measures.

RELATIONSHIPS BETWEEN THREE *MELOIDOGYNE SPECIES* ON SUGARCANE. **Barbosa[1], N.M.R., E.M.R. Pedrosa[1] and A. Chaves[2].** [1]Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, Recife/PE, 52171-900, Brazil; [2]Estação Experimental de cana-de-açúcar do Carpina, Rua Angela Cristina C. Pessoa de Luna, s/n, Carpina/PE, 55810-700, Brazil.

The root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* cause high losses on sugarcane fields around the world. Reports of *Meloidogyne enterolobii* parasiting the crop in north-eastern Brazil worried producers because of the fast dissemination and extreme severity of this species in guava fields. This study had as objectives comparing reproduction of *M. incognita, M. javanica* and *M. enterolobii* in association or individually on sugarcane variety RB 867515. This involved evaluating *M. enterolobii* penetration (3, 7, 10, 17, 24, 31, 38 and 45 days after inoculation), development and life cycle on the variety RB 98710, using *M. javanica* as the control. The last objective being) evaluating effect of *M. enterolobii* initial density (0, 20,000; 50,000 and 100,000 eggs per plant) in relation to *M. incognita* on RB 98710 at 120 days after inoculation. RB 867515 was a good host for *M. incognita* and *M. javanica*, but not for *M. enterolobii*, permitting free reproductions for the former two species. Esterase phenotypes presented quantitative variations in the ratios of the three species recovered at the end of the experiment, indicating *M. incognita* prevalence over the other species. At 45 days, *M. javanica* complete the life cycle in contrast to the low number of vermiform and swollen juveniles and no adult female of *M. enterolobii* found in roots of RB 98710. *Meloidogyne enterolobii* initial population did not affect the early development of RB 98710 120 days after inoculation, presenting a reproductive factor always lower than 1.

CELLULAR MECHANISMS IN PLANTS INFECTED BY CYST NEMATODES. **Baum, T.J.** Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, 50011, USA.

Cyst nematodes secrete effector proteins into host root cells, and these proteins are instrumental in the formation of feeding cells and mediating susceptibility through the suppression of plant defences. Consequently, the identification of effector proteins and their functions is of high importance. We have developed an effector identification method in which whole nematode glands are purified and subjected to RNA extraction for transcriptomic analyses. In addition to effector identification, the characterization of their functions in plant cells is of highest interest. In one example that we studied, a cyst nematode effector protein is phosphorylated by a cytoplasmic plant kinase, which results in translocation of the effector into the plant nucleus. There, the effector interacts with a plant transcription factor, likely resulting in gene expression alterations. In another example we discovered that as a result of cyst nematode infection, and probably effector functions, microRNAs change expression in the developing syncytium. In particular, the microRNA396 regulatory network has strong impact on cyst nematode parasitic success. This and other microRNAs that we have shown to change expression in the syncytium, thus, represent powerful molecular targets for cyst nematode effectors to modulate plant cell development. Ongoing functional characterizations of microRNAs as well as identification and characterization of nematode effectors promise to provide the mechanistic understanding required to devise novel control mechanisms.

OBSERVATIONS ON NEMATODE-SUPPRESSIVE SOILS LEADING TO IPM APPROACHES. **Becker, J.O.** Department of Nematology, University of California, Riverside, California 92521, USA.

Biological nematode population suppression has been recognized in a number of cyst nematode-infested fields. A common denominator is often that susceptible crops are grown for years in monoculture or in narrow rotations of the same or similar plants. We have shown that in a *Heterodera schachtii*-suppressive field on the Agricultural Operations, University of California, Riverside, the suppressive agent is the ascomycete *Dactylella oviparasitica*. It attacks primarily late juvenile stages of sedentary sugar beet cyst nematodes and the young females. However its population appears to decline rapidly after discontinuing the monoculture. The fungus then requires at least one or two nematode population cycles on the next host to build up again to a population suppressive level. This leaves beets or cruciferous crops at the seedling stage exposed to unmitigated attack of invasive second-stage juveniles. Preventing this early nematode-caused damage as well as subsequent secondary microbial attack of the nematode-weakened tissues is difficult to achieve with an augmentation of nematode-destroying microorganisms before or at seeding. Consequently, this approach is most effective in conjunction with other integrated pest control modules such as resistant or tolerant host crops, and nematode-resistant cover crops. We report here on the utility of seed coating with the biorational nematicide Avicta (a.i. abamectin) that provides early broad-spectum seedling protection followed by castration or destruction of the nematode females and their eggs by seed treatment-compatible hyperparasites.

SEED TREATMENTS AGAINST PLANT PARASITIC NEMATODES: WHEN A LITTLE GOES A LONG WAY. **Becker[1], J.O., and H.V. Morton[2].** [1]Department of Nematology, University of California, Riverside, CA 92521; [2]VIVA Inc., Greensboro, NC 27410.

Expensive hybrid and genetically modified seed make healthy seedling establishment an economic priority. The increasing popularity of multi-component seed treatments to deliver seed and seedling protection against a wide-range of pests and diseases has been primarily driven by a multitude of regulatory and economic factors that resulted in user-safe, cost-effective and environmentally sound products. Nematicides are the most recent addition to seed treatment cocktails, following long after fungicides and insecticides. The nematicidal seed treatment Avicta in particular has proven its efficacy in seedling protection of many crops against various plant parasitic nematodes. Avicta has also been shown to protect against secondary fungal diseases and accelerated root senescence by microorganisms that opportunistically invade nematode-weakened tissues. Furthermore, its active ingredient abamectin, comprised of natural metabolites of *Streptomyces avermectinius*, has no known antimicrobial activity. It therefore allows for the combination with compatible microbial control agents to increase nematicidal efficacy, to extend the protective duration and to provide a sustainable solution to the control of plant parasitic nematodes.

DETERMINATION OF THE EFFECT OF GROWING PARTIALLY RESISTANT POTATO VARIETIES ON THE POPULATION DENSITIES OF *GLOBODERA PALLIDA*. **Been[1], T., C. Schomaker[1] and N. Matkaris[2].** [1]Plant Research International, Plant Sciences Group, Wageningen University and Research Centre, P.O. Box 16, 6700 AA Wageningen, The Netherlands; [2]Nematology section, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent Belgium.

Twenty fields, where starch potatoes are grown every second year, were planted twice with the partially resistant potato cultivar Elles (RS = 25%) in 1993 and 1995. Four of these fields were resampled in 2012, to investigate the long-term effect of planting partially resistant potato cultivars on population densities of *Globodera pallida*. All fields were of 1 ha size, divided in plots of 10 x 16 meter (the latter in the direction of cultivation) and sampled to estimate population densities. The number of cysts (500 g soil)$^{-1}$ during this first period of 4 years did not decrease. In 2012, three fields showed a marked decrease and field four, where resistant cultivars were alternated with susceptible ones, a moderate decrease. Ultimately, cyst densities decreased from 690, 1007, 405 and 1150 to 169, 260, 57 and 761 cysts (500 g soil)$^{-1}$, respectively. The first use of Elles kept population densities either in check or caused them to decline and, therefore, was larger than the combined effect of natural decline and soil fumigation. Remarkable was the high natural decline (77%) observed in the non-host year of 1994. After the second crop, the nematode populations on two fields were more than twice as high as on the two other fields, probably due to the higher virulence of the local populations of the first two fields as estimated in a pot experiment. After 20 years, in all fields, population densities were comparable and had declined from 151, 110, 50 and 91 to 0.18, 0.10, 0.01 and 0.53 J2 (g soil)$^{-1}$, respectively.

EXPLORING THE CROP MICROBIOME FOR NEMATICIDAL ACTIVITY. **Beilinson, V., D. Tomso, S. Uknes, E. Ward and F.M. Leach.** AgBiome, 104 T.W. Alexander, Research Triangle Park, North Carolina 27709, USA.

Plant parasitic nematodes remain difficult to control for most crops. Chemical solutions face increasing regulatory pressure, and genetic resistance is often limited in scope and durability. AgBiome is leading an effort to discover new solutions for significant agricultural pests by conducting systematic screens for microbes with activity against plant parasitic nematodes. We have assembled a large collection of microbes from the microbiomes of major row crops and are characterizing isolates for activity against nematodes. We have also sequenced the genome of thousands of microbial isolates and are using sequence data to guide both strain isolation and active strain discovery. Plant microbiome communities are diverse, and we show that populations vary across plant-associated environmental compartments. Many of these communities, including those from endophyte and rhizosphere compartments, are likely to exert significant influence on nematode infestation. Screening efforts and new isolates for nematode control are presented as a component of an integrated biologicals product discovery pipeline.

NEMATODE COMMUNITY STRUCTURE IN CORN-BASED CROPPING SYSTEMS THEN (1995-1997) AND NOW (2012-2013). **Bender, B., and A. MacGuidwin.** Department of Plant Pathology, University of Wisconsin, 1630 Linden Drive, Madison, Wisconsin 53705, USA.

Nematode community structure was studied in a long term trial of grain- and forage-based cropping systems in southern Wisconsin. Three systems were sampled during the corn phase in 1995-1997 and 2012-2013: 1) continuous corn with high pesticide and fertilizer inputs and chisel plow tillage, 2) corn grown with low inputs and rotary tillage in rotation with soybean/winter wheat, and red clover, and 3) corn grown with low inputs, manure, and rotary tillage in rotation with alfalfa for two years. A forage pasture system planted with a mixture of red clover, orchard grass, timothy, and brome that was rotationally grazed by cows was also sampled. Soil samples assayed for nematodes using a combination of sieving, centrifugation and incubation methods revealed 28 nematode families at the study site and nematode abundance ranging from

500 to 5000 nematodes per 100 cm^3 soil. Corn yield increased in all three systems from 1989 to 2013, but system 2 consistently produced less yield than systems 1 and 3. In 1997, Maturity Indices distinguished system 1 from system 3. Maturity Indices were lower in all corn based systems as compared to pasture for 1995-1997. Preliminary faunal analyses of the current data set suggest that nematode community structure has not become more different in the systems over time.

POPULATION DYNAMIC OF RING NEMATODE IN PEACH ORCHARD MANAGED WITH CASTOR BEAN CAKE AND MILLET CROP. **Bernardo[1], J.T., A.C. Krolow[2] and C.B. Gomes[2].** [1]PPGFS/Universidade Federal de Pelotas, Campus Universitário s/n C. P. 354, Pelotas/RS, Brazil; [2]Embrapa Clima Temperado, Cx Postal 403, Pelotas/RS, Brazil.

The Peach Tree Short Life syndrome associated with the ring nematode, *Criconemoides xenoplax* has been a problem in peach orchards in southern Brazil since the 1980s. Therefore, the influence of incorporating of castor bean cakes and soil cropping with black oat (*Avena strigosa*) and millet (*Pennisetum americanum*) was investigated in a peach orchard 'Rubimel' naturally infested by *C. xenoplax*. During the spring/summer and autumn/winter seasons, 20 kg of castor bean cakes were incorporated into the soil at level of 10kg castor bean cake/m^2 in plots with five peach trees. Just after the residue application, black oat or millet were seeded in the plots. Peach trees maintained without weeds were used as control. The experiment was carried out during 2012 and 2013 using a randomized design with six replications. Four months after the application of the treatments, soil samples were collected before and 30 days after plant incorporations for evaluation of *C. xenoplax* populations using regression analysis. Additionally, the chemical characteristics of peach fruits were also evaluated. Independent of the treatment, the *C. xenoplax* populations behaved similarly. In the spring-summer seasons the populations decreased in the soil and increased during the autumn-winter season. However, the nematode populations were lower in the pots where the plants received the residue associated with cover crops. Evaluating the fruit quality, there were no difference in the pH, total titratable acidity and ascorbic acid between the different treatments, but in the plots with castor bean cake and cover crops a significant reduction of total soluble solids was verified and it can possibly be explained by increased nitrogen from the castor bean cakes.

PAECILOMYCES LILACINUS (=*PURPUREOCILLIUM LILACINUM*) AS A NEMATODE BIOCONTROL AGENT. **S.D. Berry.** BASF Agricultural Specialities (Pty) Ltd., 91 Clifton Canyon Drive, Gillits, 3610, South Africa.

Nematodes are a constant threat to crop production worldwide. Various management strategies have been developed over the years, with most of the emphasis being on chemical nematicides and varietal resistance. However, with the demise of many of the traditional chemical solutions, the identification and management of these pests is becoming increasingly more important. In the last few years much interest and investment has gone into researching biological control of nematodes. A number of different biological control organisms have been researched and a few developed into commercial products (e.g. *Paecilomyces lilacinus, Bacillus firmus, Pochonia chlamydosporia, Pasteuria penetrans*). The aim of this talk will be to discuss the history, modes-of-action, nematode targets, crops, commercial products and efficacy of *Paecilomyces lilacinus* (also known as *Purpureocillium lilacinum*) as a nematode biocontrol agent.

RECENT METHODOLOGICAL AND THEORETICAL ADVANCES FOR SPECIES DELIMITATION IN CONTEMPORARY NEMATODE TAXONOMY. **Bert[1], W., D. Slos[1], T. Janssen[1], P. Fonderie[1], H. Steel[1] and W. Decraemer[1,2].** [1]Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Royal Belgian Institute of Natural Sciences, Department of Invertebrates, Vautierstraat 29, B-1000 Brussels, Belgium.

Only a small fraction of the estimated existing nematode species has been described. To grasp the overwhelming nematode diversity, some nematologists still have confidence in methods that date back to the 17th century, while others want to abandon species as fundamental entities of diversity. We will discuss the necessity and shortcomings of traditional and new approaches. It is obvious that nematode taxonomy is confronted with the challenge to fully incorporate new theory, methods and data from disciplines that study the origin, boundaries and evolution of species. These recent methodological advances hold promise for species delimitation methods that reflect ''true'' speciation events. However, these advanced methods have their limitations in daily taxonomic practise, and therefore it is evident that forwarding one best possible taxonomical method is currently impossible. We advocate the simultaneous use and efficient integration of slow integrative taxonomy and fast molecular operational taxonomical units. Examples of our own approaches, targeting a comprehensive and reliable description of nematode biodiversity, will be illustrated using case studies on free-living, plant-parasitic, and facultatively animal parasitic nematodes. These efforts are based on a combined acquisition of informative sequences, ecological and morphological data; and integrated in phylogenetic frameworks and supported by an appropriate databank. Finally, the link of our taxonomical work with other ongoing research projects will be highlighted.

IS INTROGRESSION BREEDING ANSWER TO NEMATODE RESISTANT SUGARCANE IN AUSTRALIA? **Bhuiyan[1], S.A., B.J. Croft[1], E. Wong[1], P. Jackson[2] and G.R. Stirling[3].** [1]Sugar Research Australia, Woodford, Australia; [2]CSIRO Townsville, Australia; [3]Biological Crop Protection, Brisbane, Australia.

Root knot (*Meloidogyne* javanica) and root lesion (*Pratylenchus zeae*) nematodes are causing in excess of A$82 million losses to the Australian sugar industry annually. No commercial sugarcane varieties are resistant to these nematodes. A

collaborative introgression program with Chinese institutes has used new sources of germplasm, *Erianthus arundinaceus* and *Saccharum spontaneum* clones to generate over 100 new families. In 2012, approximately 150 clones from different introgression families have been screened in a glasshouse for resistance to *M. javanica* and *P. zeae* .The initial nematode population used for inoculation of test clones (Pi) and final population recovered after 12 weeks (Pf), were used to determine the multiplication factor (MF) =Pf/Pi. For both nematodes, the lowest multiplication factors were observed on basic *E. arundinaceus* (MF = 3 - 7) and *S. spontaneum* (MF =1.8 - 6) clones, indicating moderate to high levels of resistance. Average levels of resistance tended to decrease with successive backcrosses between the wild species and commercial sugarcane. However, approximately 30% of backcross-three (BC3) populations of *E. arundinaceus* showed moderate resistance (MF<10) to root-knot and lesion nematodes compared to commercial varieties Q208 (MF 14 - 42), Q240 (MF 20 - 24) and Q135 (MF 24 - 44). For backcross-two (BC2) *S. spontaneum* populations, 5% and 30% of clones had moderate levels of resistance (MF<10) to lesion and root knot nematodes, respectively. Individual nematode-resistant clones will be further tested and may prove to be a useful source of resistance to nematodes for commercial production or as parents for further breeding.

THE RELEVANCE AND MODE-OF-ACTION OF FUNGAL ENDOPHYTES IN THE BIOCONTROL OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* IN TOMATO. **Bogner[1], C.W., G.M. Kariuki[2], M. Kuska[1], M. Selim[1], F.M.W. Grundler[1] and A. Schouten[1].** [1]Rheinische Friedrich-Wilhelms Universität Bonn, INRES-Molecular Phytomedicine, Karlrobert-Kreiten Strasse 13, 53115 Bonn, Germany; [2]Department of Agricultural Science and Technology, Kenyatta University, P.O Box 43844-00100 Nairobi, Kenya.

Vegetable production has become increasingly significant in the coastal humid tropics of East Africa, in which tomato (*Solanum lycopersicum*) is one of the most important crops. Plant parasitic nematodes, especially root-knot nematodes (*Meloidogyne* spp.) pose a major threat to tomato production. The use of habitat-adapted endophytic fungi may provide a safe, efficient, reliable and affordable approach to control root-knot nematodes. Tomato endophytes collected from Kenya were identified and screened for their biocontrol activities in the tomato cultivar Moneymaker, various local Kenyan varieties and AVRDC – World Vegetable Center accessions. In particular, *Trichoderma* and *Fusarium oxysporum* isolates significantly reduced root-knot nematode egg densities by 20 to 50% when compared to the non-inoculated control. Split-root experiments and synchronized infection studies have shown that the fungus initiates certain systemic plant defense responses that affect both penetration and overall development of the nematode. Genome array studies have further shown that a *F. oxysporum* endophyte can dramatically affect gene expression in roots and leaves. In order to gain a deeper understanding of the induced defence responses, leading to this reduction in nematode infection, specific tomato mutants that are impaired in particular defense pathways were analysed with respect to root-knot nematode colonization in the presence and absence of the beneficial endophytes. At the same time, the expression of several marker genes and the activity of certain enzymes, all involved in the various induced defence responses, are being monitored. In addition, the effects of fungal metabolites on *Meloidogyne incognita* and the initiation of plant defense responses are being studied.

HOST STATUS OF SELECTED SWEET POTATO CULTIVARS TO *MELOIDOGYNE ENTEROLOBII*. **Brito[1], J.A., S.J. Vau[2] and D.W. Dickson[2].** [1]Division of Plant Industry, DPI, Gainesville, FL 32614; [2]Dept. of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Meloidogyne enterolobii was first reported in Florida, USA in 2001 infecting ornamental plants and since then numerous other plant species have been identified as hosts. In 2013, *M. enterolobii* also was reported in North Carolina infecting cotton and soybean. This nematode is capable of overcoming root-knot nematode resistant genes in several important agricultural crops, namely *Mi*-1, *Mir1*, *N, Rk* and *Tabasco* genes in tomato, soybean, bell pepper, cowpea, and sweet pepper, respectively. Our objective was to determine the ability of *M. enterolobii* to reproduce on selected sweet potato cultivars: Beauregard, Covington, Evangeline, Hernandez, and LA 05-111. All these cultivars, except Beauregard and Covington, are moderately resistant or resistant to *M. incognita*. Tomato cv. Rutgers was used as a control. Plants were inoculated (5,000 eggs and J2/plant) and arranged in a completely randomized design in a greenhouse, with an average temperatures of 24.8 °C. Galls and egg masses/root system (0-5 scale), eggs/egg mass, eggs/g of root fresh weight (grfw), and reproduction factor were determined. *M. enterolobii* infected and reproduced on all the sweet potato cultivars. Cultivar LA 05-111 sustained less root galling and egg masses than the other cultivars ($P \leq 0.05$); however, no difference was observed in the number of eggs/grfw between cvs. LA 05-111 and Beauregard. Covington, Evangeline, and Hernandez reacted similarly regarding amount of root galling, egg masses and eggs/grfw. The number of eggs/egg mass, which ranged from 462.4 to 503.5, was similar among all cultivars. In summary, all cultivars were good hosts for *M. enterolobii*.

VELUM®– A EVOLUTIONARY NEMATICIDE FOR EFFICIENT CROP PRODUCTION. **Broeksma[1], A., K. Puetzkuhl[2], S. Lamprecht[2], H. Fuersch[2].** [1]Bayer CropScience, 27 Wrench Road, Isando, South Africa; [2]Bayer CropScience AG - Alfred Nobel Strasse 50 - D - 40789 Monheim am Rhein, Germany.

Velum®, common name fluopyram, is a novel nematicide from the chemical class of pyridinyl ethyl benzamides which is developed to control nematodes in a broad range of crops. Fluopyram is the first nematicide acting via Complex ll (SQR)

inhibition. Fluopyram selectively inhibits Complex ll of the mitochondrial respiratory chain in nematodes which leads to a fast and severe depletion of the nematodés cellular energy (ATP). Moreover, fluopyram is characterized by a very favourable toxicological and ecotoxicological profile. After the restriction or withdrawal of several nematicides, protection of crops from plant-parasitic nematodes has become a major challenge. Velum® exhibits an excellent level of efficacy at very low dose rates compared to currently available nematicides. Velum® has proven itself in a number of trials conducted in South Africa to be a highly effective nematicide controlling a number of different nematode species but most importantly *Meloidogyne* spp. (root-knot nematode). Multiple field trials have demonstrated that Velum® provides an outstanding long-lasting nematode control also under high infestation pressure. Velum® is planned to be commercialised in different formulation types enabling it to be used in multiple application techniques such as granular spreading, drenching, in-furrow spraying and seed treatments. Velum® will provide new innovation in a field which has proved troublesome over the past years. It will allow farmers to achieve good nematode control with the benefit of operator safety and reducing the potential for the poisoning of companion and wild animals.

STUDYING THE RESPONSES OF PEPPER GENOTYPES TO AVIRULENT AND VIRULENT *MELOIDOGYNE INCOGNITA* ISOLATES OCCURRING IN ISRAEL. **Brown Horowitz[1], S., P. Bucki[1] and Pharan Ilan[2].** [1]Department of Entomology and the Nematology and Chemistry units; ARO, the Volcani Center, Israel; [2]The Plant Science Institute; ARO, the Volcani Center, Israel.

Root-knot nematodes (*Meloidogyne* spp.) are a major pest of bell pepper (*Capsicum annuum* L.) in Israel and world-wide. While most sweet pepper cultivars are resistant to the root-knot nematode species *Meloidogyne javanica*, they are usually susceptible to the *Meloidogyne incognita*. In pepper, resistance to root-knot nematodes has been associated with at least 10 dominant genes, *N, Me1, Me2, Me3, Me4, Me5, Me6, Me7, Mech1 and Mech2* inducing different response pattern in root cells. In Israel, resistant cultivars are not always effective and the occurrence of virulent isolates is becoming a serious problem. Herein the behavior of natural virulent *Meloidogyne incognita* isolates from major pepper-growing areas in Israel, toward pepper resistance genes *Me1 and Me3* was studied for the first time. Thirty Meloidogyne populations from pepper producing areas were subjected to the North Carolina host differential test. While all isolates reproduced well on pepper, watermelon and tobacco differential reproduction on cotton plants was observed. Among the tested isolates several isolates reproduced well and induced galls on pepper plants carrying the *Me3* but not on *Me1* plants, while pepper plants carrying both alleles demonstrated remarkable resistance towards all tested isolates. Further molecular characterization of the tested isolates was conducted by detailed sequence analyses of internal transcribed spacers (ITSs) of nuclear ribosomal genes. While the North Carolina host differential test suggested the predominance of *M. incognita* race 2 among the collected isolates differences in their virulence and behavior along with sequences comparisons of the rDNA region indicating high variation among the isolates.

EFFICACY OF NIMITZ™, A NEW NEMATICIDE OF THE FLUOROALKENYL GROUP AGAINST THE DAMAGE CAUSED BY *MELOIDOGYNE* SPP. IN *PRUNUS* ORCHARDS. **Brown Horowitz[1], S., R. Ozalvo[1] and D. Karmon[2].** [1]Department of Entomology and the Nematology and Chemistry Units; ARO, the Volcani Center, Israel; [2]Makhteshim-Agan, Airport City, Golan Street, Israel.

Root-knot nematodes *Meloidogyne* spp., are obligate sedentary endoparasites that cause losses in yield and quality of peach, plum (*Prunus* spp.) and almonds [*Prunus dulcis* (Mill) Webb] crops. In Israel the two major prevalent root-knot nematode species are *Meloidogyne javanica* (Treub) Chitwood and *Meloidogyne incognita* (Kofoid & White) Chitwood. Recommended control practices include pre- and postplant nematicide application, resistance rootstocks and crop rotation when available. However, the small number of available nematicides and restrictions on the use of non-fumigant nematicides hinder effective nematode control. Herein, we investigated the efficacy of NIMITZ™ (MCW-2, fluensulfone 480EC), against root-knot nematodes on the highly susceptible common *Prunus* rootstock GF-677. For evaluating NIMITZ™ efficacy, the effect of five different concentrations in the range of 0.01-0.5 cm^3/plant, on *Meloidogyne* spp. development following soil inoculation at pre- and post plant was studied. Plants were harvested 120 days after inoculation and galling incidence, nematodes population per plant in soil, and numbers of eggs produced per gram of root was determined. NIMITZ™ demonstrated highly nematicidial activity when 0.5 cm^3/plant were applied at preplant, resulting in healthy root system with no observed galling and no nematode reproduction that was detected in the treated roots. Application of 0.05 cm^3/plant at pre- and post planting resulted in decrease in galling incidence although eggs production remained similar to the non-treated rootstocks. Our results indicate that NIMITZ™ has the potential to be used as a new non-fumigant nematicide against root-knot nematodes particularly as a pre plant treatment to ensure protection to the planting rootstocks.

COMMERCIAL EXAMPLES OF THE USE OF ENTOMOPATHOGENIC NEMATODES IN INTEGRATED PEST MANAGEMENT (IPM) PROGRAMS. **Brown, A.P. and G. Martin.** BASF Agricultural Specialities Ltd., Harwood Industrial Estate, Harwood Road, Littlehampton, West Sussex, BN17 7AU, UK.

Commercial use of macro-biological entomopathogenic nematode (EPN) pest control products are increasing. Driven by reduced availability of chemicals and a desire to use more environmentally sensitive pest control programs, this growth is

expected to continue. As a result, biological control agents play an increasingly important role as components within many Integrated Pest Management (IPM) programs. As with many biological control agents, products containing EPN often require different shipping and storage conditions compared to chemical plant protection products (PPP). Advantages EPN offer such as; zero re-entry interval , zero harvest interval , target specificity, compatibility with a range of chemical and biological plant protection products and the ability to help manage resistance are some of the benefits EPN can offer IPM programs. Most commonly implemented in protected or semi-protected cropping systems, protected ornamentals and vegetables along with high value crops such as herbs and soft fruit are considered the main uses of this technology. Entomopathogenic nematodes can however be used in a wide range of cropping situations including top fruit orchards, field-grown brassicas and sports turf. This presentation will discuss a number of case studies where EPN products are commonly used within IPM programs in commercial situations.

CHARACTERIZATION OF *MELOIDOGYNE* SPP. FROM UGANDA AND TANZANIA. **Carneiro[1], R.M.D.G., M.R.A. Almeida[1], J. Aldemiro Junior[1], V.S. Mattos[1,2], V.R. Correa[1,2], D. Coyne[3].** [1]EMBRAPA - Recursos Genéticos e Biotecnologia, C. P. 02372, 70849-979 Brasília, DF, Brazil; [2]Department. de Fitopatologia, Universidade de Brasília, 70910-900, Brasília, DF, Brazil; [3]International Institute of Tropical Agriculture (IITA) East Africa , P.O. Box 30772-00100, Nairobi, Kenya.

Urbanization has been rising in developing countries and peri-urban vegetable systems have become more intensively cultivated leading to increased impact of root-knot nematodes (*Meloidogyne* spp) on crop production. A limited ability to accurately identify these pathogens has resulted in the misinterpretation of constraints with high inputs of highly toxic chemical compounds. Considering the importance of root-knot nematodes in Africa, a survey was carried out in vegetable fields (tomato, eggplant, green pepper, cassava, sweet pepper, okra and carrot) in Tanzania and Uganda. Root-knot nematode. populations were characterized biochemically using esterase (Est) phenotypes. Forty three populations and seven esterase phenotypes were identified in Tanzania. *Meloidogyne javanica* (Est J3, Rm: 1.0, 1.25, 1.4) and *Meloidogyne incognita* (Est I2, Rm: 1.0, 1.1) were detected in 14 and 15 populations respectively and can be considered major species. Three populations of *Meloidogyne arenaria* (Est A2, Rm: 1.2, 1.3) and one population of *Meloidogyne enterolobii* (Est Ent2, Rm: 0.7, 0.9) were detected. Ten mixed populations were detected in different combinations: *Meloidogyne izalcoensis* (Est I4, Rm: 0.86, 0.96, 1.24, 1.30) and *M. incognita*, *M. javanica*, *Meloidogyne arenaria* and *Meloidogyne hapla* (Est H1, Rm: 1.1). Sixty two populations and five Est phenotypes were identified in Uganda. *Meloidogyne javanica* had three different phenotypes: Est J3, Est J2a (Rm: 1.0, 1.4), J2b (Rm: 1.0, 1.25) and were detected in 34 samples. *M. arenaria* (Est A2) was detected in 16 samples, *M. incognita* (Est I2) in two samples and mixed species in 10 samples. Some populations had their identification confirmed with SCAR markers.

HOST STATUS OF DIFFERENT VEGETABLES TO *MELOIDOGYNE ETHIOPICA*. **Carneiro[1,2], M.D.G., J.K.A. Mattos[1], F.R. Sousa[2], V.R. Correa[2,3], R.M.D.G. Carneiro[2].** [1]Fac. Agronomia e Veterinária, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil; [2]Embrapa Recursos Genéticos e Biotecnologia, 70849-979 Brasília, Distrito Federa, Brazil; [3]Dept. Fitopatologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federa, Brazil.

Meloidogyne ethiopica is a root-knot nematode with emerging potential in Brazil. There are few reports about the hostability of different vegetable crops to this species. The objectives of this study were: a) to assess the hostability of different vegetable varieties to *M. ethiopica* and study the virulence of this nematode to pepper 'Margarita', chilli 'Silver' and tomato 'Laura', all carrying resistance genes to *Meloidogyne* spp.*;* b) to suggest a rotation system for *M. ethiopica* in infested areas. The experiments were carried out in greenhouses with randomized blocks and 8 replications. The susceptible tomato 'Santa Clara VF 5600' was used as control. Plants were inoculated with 10,000 eggs and evaluated two to four months after inoculation based on gall index, egg mass index and the reproduction factor (RF). Cultivars showing RF $\geq$ 1 were considered hosts, RF <1 poor hosts and RF = 0 immune or non-host. The eggs from each plant were extracted using 1% NaOCl. Eggplant 'Embú', zucchini 'Menina Brasileira', squash 'Tetsukabuto', sprouts 'Chinesa Michihilli', sprouting broccoli 'Santana', cucumber 'Safira', cabbage 'Kirei', lettuce 'Elisa' and 'Veronica', arugula 'Folha Larga' and spinach 'Nova Zelândia' were all hosts. The following plants were poor hosts: tomato 'Laura', lettuce 'Americana Grandes Lagos' and 'Veneza Roxa' and pepper 'De Bico'. Chilli 'Silver' and peppers 'Margarita' and 'Magali R' were immune or non-hosts. In a crop rotation system, vegetables considered poor host and non-host can be alternated with vegetables considered hosts, allowing management of nematode population densities and ultimately reducing environmental and economic damages.

INFLUENCE OF BT MAIZE TO SOIL NEMATODE COMMUNITIES. **Čerevková[1], A., and L'. Cagáň[2].** [1]Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic; [2]Slovak Agricultural University, A. Hlinku 2, 94976 Nitra, Slovak Republic.

Soil nematode fauna, the proportions of trophic groups and selected ecological indices were studied to evaluate impact of ISO and Bt maize hybrids on nematode communities in the field in Slovakia - Central Europe. The field trial included two variants (ISO and Bt maize) in 10 repetitions in two growing periods 2012 and 2013. Forty nematode species were identified

associated with the two maize variants. Most of identified genera belonged to the orders Dorylaimida (11), Tylenchida (11) and Rhabditida (8). Mean nematode abundance in the ISO variant were 318 and in the Bt variant 270 individuals per 50g of soil, respectively. Dominant taxa in both variants were *Acrobeloides nanus, Aphelenchus avenae* and *Filenchus* spp. with incidence of 95-100%. Dominant trophic groups in both variants were bacterial feeders followed by fungal feeders and root-fungal feeders. There were little or no differences observed in ecological indices between ISO and Bt maize variants. Year of sampling significantly influenced nematode communities and the differences between the years were markedly higher compared to those between ISO and Bt maize variants.

INFLUENCE OF INSECTICIDES TO SOIL NEMATODE COMMUNITIES. **Čerevková[1], A., and L'. Cagáň[2].** [1]Institute of Parasitology SAS, Hlinkova 3, 040 01 Košice, Slovak Republic; [2]Slovak Agricultural University, A. Hlinku 2, 94976 Nitra, Slovak Republic.

Soil nematode communities were studied after application of insecticides in maize fields in Slovakia. Field trial included the variants with granular application with tefluthrin (199.5 g a.i./ha), granular application with clothianidin (110 g a.i./ha), seed treatment with clothianidin (1.25 μg a.i/seed) and control variant without application of insecticides. Almost 20 000 soil nematodes were observed and 9 orders, 33 genera and 37 species identified. *Acrobeloides nanus*, *Cephalobus persegnis*, *Eucephalobus striatus* and *Basiria gracilis* were the dominant species, accounting for 48 % of the number of nematodes. The mean abundance and species diversity index were significantly lower for the maize variant with granular application of clothianidin. Bacterial feeders were the dominant trophic group for all 4 variants. The population numbers of the different trophic groups were significantly different between variant with granular application of clothianidin and the control. The ΣMaturity index, Maturity index and Plant parasitic index did not show significant differences among the variants. The highest values of the Enrichment and Structure indices were observed in the first month of the investigation in all four variants. According to cluster analysis, nematode species population densities were strongly affected by the date of soil sample collection and by the variants used in the experiment.

HETERORHABDITIS INDICA ISOLATE CH_2 INFLUENCE CELLULAR IMMUNE RESPONSE OF *GALLERIA MELLONELLA*. **Chaubey, A.K., Istkhar and Suman.** Nematology Laboratory, Department of Zoology, Chaudhary Charan Singh University, Meerut-250004, India.

Cellular immune responses of insects comprises mainly of hemocytes which circulate into the hemolymph of insects. They are very vital and have wide responses against invaders. Changes in the number of hemocytes also have an important role in insect's immunity. The present study dealt with the influence of *Heterorhabditis indica* isolate CH_2 on total and differential hemocytes counts (THC and DHC) of *Galleria mellonella*. Based on the results of bioassay experiments, *G. mellonella* (40 larvae) along with controls (five replicates for each) were infected with 20 IJ/larva each in 300μl double distilled water in six well plates. Hemolymph was collected after each six hours post infection period for 24 hours. Total hemocytes counts was calculated and presented in ±SD using the descriptive analysis. Total hemocyte counts after 0, 6, 12, 18 and 24h in control was 7428 ± 159.5 (5640 - 9100), 5276 ± 214.7 (3940 - 9040), 8804 ± 337.7 (4980 - 18440), 3596 ± 545.5 (2500 - 5400) and 7972 ± 114.5 (3660 - 12620), respectively. The same parameters were recorded after 6, 12, 18 and 24h of post infection was recorded as 13536 ± 628 (5140 - 22840), 7540 ± 352.6 (3140 - 11220), 5824 ± 124.7 (3920 - 7200) and 7368 ± 352.3 (4880 - 13480) respectively. Differential hemocytes counts were identified as prohemocytes, plasmocytes, granulocytes, spherulocytes, fat droplets and oenocytoides that also showed dramatic changes in both the control and post infection periods of 0, 6, 12, 18 and 24h. The durational fluctuations in TLC and DLC might be due to the action of hemocytes against the toxins produced by the bacteria and *H. indica* isolate CH_2. The various immune cells of *G. mellonella* resist bacterial toxins initially that usually reaches the maximum value of resistance.

PYRACLOSTROBIN AS RESISTANCE INDUCER OF *MELOIDOGYNE INCOGNITA* IN SUGARCANE. **Chaves[1], A., D.E. Simões Neto[1] and E.M.R. Pedrosa[2].** [1]Estação Experimental de Cana-de-açúcar do Carpina, Rua Ângela Cristina C.P. de Luna s/n, Bairro Novo, Carpina/PE 55810-000, Brazil; [2]Universidade Federal Rural de Pernambuco. Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife/PE 52171-900, Brazil.

Resistance induction is a promising alternative for the integrated nematode management success, especially considering effects of nematode interactions with other pathogen or saprophyte organisms, minimizing the use of pesticides in agriculture. Considering the importance of *Meloidogyne incognita* for sugarcane around the word and the recent arrival of *Puccinia kuehnii* in Northeastern Brazil plantations, a experiment was carried out under greenhouse to evaluate penetration, development and completion of life cycle of *M. incognita* in the variety RB867515 in presence of the fungicide pyraclostrobin base. Management treatments consisted of Pyraclostrobin (0.5L/ha pc), Carbofuran (7.0L/ha pc), Pyraclostrobin (0.5L / ha pc) + Carbofuran (7.0 L / ha pc) and control (without Pyraclostrobin and/or Carbofuran), with eight replicates. Nematode development was evaluated at 3, 7, 10, 17, 24, 31, 38 and 45 days after nematode inoculation. At 38 days, *M. incognita* was able to complete life cycle in control plants. Sausage and ovoid swollen forms of *M. inconita* juveniles were found in plants treated with Comet, but adult forms were significantly reduced compared to the control, indicating a possible

induced resistance mechanism. Data for plant height and number of tillers were significantly higher in treatments with carbofuran and pyraclostrobin comparing to the control, 90 days after nematode inoculation.

IDENTIFICATION OF AN ANNEXIN-LIKE PARASITISM GENE FROM CEREAL CYST NEMATODE, *HETERODERA AVENAE*. **Chen, C.L., S.S. Liu, D. Yang, Q. Liu and H. Jian.** Key Laboratory of Plant Pathology of Ministry of Agriculture, Department of Plant Pathology, China Agricultural University, Beijing 100193, People's Republic Of China.

Parasitism genes encoding secreted effector proteins of plant parasitic nematodes play important roles in facilitating parasitism. An annexin-like gene was isolated from the cereal cyst nematode *Heterodera avenae* with high similarity to *annexin 2* which encodes a secreted protein of *Globodera pallida*. Southern blotting revealed that there are at least two homologies in *H. avenae*. This identified *Ha-annexin* encods a predicted 326 amino acid protein containing four conserved annexin domains. The protein has no N-terminal secretion signal peptide predicted by Signal P 4.0, similar to ANNEXIN 2 of *G. pallida*. However, *in situ* hybridization showed that *Ha-annexin* transcripts exclusively expressed in the sub-ventral gland cells of the pre-parasitic second-stage juveniles, which indicated that Ha-ANNEXIN is probably a secreted effector protein as *G. pallida* ANNEXIN 2. Quantitative real-time RT-PCR analysis confirmed that *Ha-annexin* was upregulated in the parasitic second-stage juveniles, coinciding with the time when feeding cell formation is initiated. Using transient expression assays in *Nicotiana benthamiana*, we found that Ha-ANNEXIN could suppress programmed cell death triggered by the pro-apoptotic mouse protein BAX. When transiently expressed in onion epidermal cells, Ha-ANNEXIN was localized in the whole cell. Together, these results suggest that *Ha-annexin* most likely encodes a secreted effector protein that may play important roles in suppressing plant defences during the early parasitic-stage process.

THE NEMATICIDAL EFFECTS OF 5-AMINOLEVULINIC ACID ON *MELOIDOGYNE INCOGNITA*. **Cheng[1], F., D. Zhang[1], Y. Liu[1], J. Wang[2], J.Cheng[1] and Z. Zhang[3].** [1]Hunan Plant Protection Institute, Changsha 410125, Hunan Province, Peoples Republic of China; [2]Hunan Agricultural Economy and Regional Planning Research Institute 410125, Hunan Province Peoples Republic of China; [3]Hunan Vegetable Institute, Changsha 410125, Hunan Province, Peoples Republic of China.

Preliminary tests were done on the nematicidal effects of 5-aminolevulinic acid (ALA) on four plant-parasitic nematodes. When treated with 3.0 mmol/L ALA, hatching inhibition on *M. incognita* and *H. glycine* eggs was 70.1% and 56.9%, respectively. The results of a greenhouse pot experiment showed that the effect of ALA on *M. incognita* control was significant, with a 81.7% control efficiency when using a 2.4 mmol/L ALA treatment. The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity levels of ALA-treated J2 of *M. incognita* were 1.78, 2.33, and 1.35 times those of the control, respectively while the malondialdehyde (MDA) content of ALA-treated J2 of *M. incognita* was 2.7 times that of the controls. ALA-treated J2 of *M. incognita* acethlcholinesterase (AChE) activity was not significantly different from that in the controls, but their porphyrin content was significantly higher than that in the controls ($p \leq 0.05$). The protoporphyrin IX (PpIX) content of J2 *M. incognita* treated by ALA was 0.169 μg/mg compared to only 0.016 μg/mg in the controls. At the same time, the metabolism of protein also has been affected and the total protein content of the treatment decreased 67.09% comparing to the control. It is suggested that ALA induces nematodes to accumulate PpIX and causes a porphyrin metabolism disorder in nematodes, thereby producing reactive oxygen species (ROS) that are not scavenged by cytochrome C oxidase and leading to oxidation of lipoproteins in the cell membrane. Consequently, the cellular structure is damaged, resulting in nematode death.

EFFICACY OF APPLICATION OF *CROTALARIA INTERMEDIA* AS A COVER CROP FOR MANAGEMENT OF ROOT-KNOT NEMATODES A CROPPING SYSTEM. **Cheruiyot[1], H.K., J. Ochuodho[1], P. Njira[2], E. Omami[1] and J. Mbogoh[1].** [1]University of Eldoret, P.O. Box 1125-30100, Eldoret, Kenya; [2]Moi University, P.O. Box 3100-30100, Eldoret, Kenya.

Root-knot nematodes (*Meloidogyne* spp.) are a serious biotic production constraint affecting vegetable production in Kenya. This study was aimed at assessing the efficacy of *Crotalaria intermedia* in suppressing root-knot nematode population. Two application methods were assessed namely preplant and soil amendment. The experiment was conducted under greenhouse conditions at 23°C. Pot experiments were used with inoculation at 3000 second stage juveniles (J2s). Preplant application involved an initial introduction of *Crotalaria* (for 4 weeks) before bringing in the vegetable component as the main crop. Soil amendment involved harvesting and shredding of the *Crotalaria* and introducing into the rhizosphere. This was done at equal weights per treatment (250 g per pot). The assessment was done on two vegetable types; *Solanum nigrum* and *Cleome gynadra*. The untreated and or unamended pots served as control. The experiment was replicated three times. Watering was done twice a day at equal volumes. Data on number of root galls, juveniles/volume of soils and percentage reduction of juveniles and root galls was collected. Analysis was done using GenStats (version 12) and means separated using Fishers LSD. The results showed that the two application methods gave a significant ($P < .001$) variation in nematode population suppression.

EVALUATING THE POTENTIAL OF OYSTER MUSHROOM COMPOST WASTE FOR PLANT-PARASITIC NEMATODE MANAGEMENT. **Ching, S., and K-H. Wang.** University of Hawaii Manoa, Department of Plant and Environmental Protection Sciences, Honolulu, Hawaii 96822, USA.

Three experiments were conducted to determine the concentration of oyster mushroom (*Pleurotus ostreatus*) compost as soil amendment and its water extract to suppress root-knot nematode, *Meloidogyne incognita*. The first experiment examined mushroom compost using coffee grounds as substrate at 1, 0.5 and 0.25% (w/w) on basil *(Ocimum basilicum)* grown in greenhouse pots containing sand-soil mix. No amendment and 1% coffee ground were included as controls. Mushroom amendment increased basil root growth but also increased population densities of *M. incognita*. The second experiment examined mushroom compost water extract (MCWE) at 50%, 33% and 25% of mushroom compost to water (v/v) in petri dishes along with a water control incubated with *M. incognita*. Water extracts at 50%, 33% and 25% paralysed 55%, 47%, and 37% of the nematodes, respectively as compared to 11% in control ($P < 0.05$). However, most nematodes revived when washed with water one day after MCWE incubation, indicating a nematostatic effect. The third experiment compared mushroom compost amendment at 50% and 33% (w/w) in organic compost media using 100-cm^3 containers inoculated with 80 *M. incognita* larvae per cone. Unamended media was included as control. Nematodes were extracted from the media at 1, 4, 7, and 30 days after inoculation using the Baermann funnel, together with sieving and centrifugal flotation. Mushroom compost at 33% suppressed nematodes after 7 days of incubation. These experiments provided evidence that MCWE could paralyze plant-parasitic nematodes, and amending mushroom compost in organic media could allow oyster mushroom mycelium to establish and suppress *M. incognita* within 7 days.

DISTRIBUTION AND INCIDENCE OF ROOT-KNOT NEMATODES OCCURRING ON CASSAVA IN THE IMPORTANT CASSAVA-GROWING AREAS OF THAILAND. **Chinnasri[1], B., K. Jindapunnapat[1], S. Kwankuae[1] and D. Coyne[2].** [1]Department of Plant Pathology, Kasetsart University, Bangkok, 10900, Thailand; [2]International Institute of Tropical Agriculture (IITA), c/o *icipe*, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya.

Surveys on the distribution and disease incidence caused by root-knot nematodes (*Meloidogyne* spp.) on cassava in the important cassava-growing areas in Thailand were conducted during 2012-13. Three provinces in the northeast, four in the east, one in the North, and one in the central plain of Thailand were sampled during the study. A total of 332 soil samples were collected and processed to determine the presence of root-knot nematodes. The results showed incidence of 66%,although many samples contained low nematode densities (below 20 nematodes/250 g soil). The surveys also found no obvious above-ground symptoms to indicate nematode infection on cassava in the field. This was in contrast to a previous targeted study which dealt with a conspicuous cassava decline associated with severe root-knot nematode infestation in northeastern Thailand. In addition to root-knot nematodes, these surveys demonstrated that other plant-parasitic nematodes such a *Pratylenchus* spp. and *Helicotylenchus* spp. were found associated with cassava. *Meloidogyne incognita* was identified with molecular techniques. Populations collected will be cultured and used for varietal screening of cassava and efficacy studies.

PHYTOCHEMICALLY BASED NEMATODE CONTROL: OPPORTUNITIES AND CHALLENGES. **Chitwood, D.J., and S.L.F. Meyer.** Nematology Laboratory, United States Department of Agriculture, Agricultural Research Service, Building 010A, BARC-West, Beltsville, Maryland 20705, USA.

Plants and fungi manufacture numerous classes of chemicals toxic or otherwise antagonistic to nematodes. Because natural products are frequently less damaging to the environment and often have lower human toxicity than synthetic nematicides, phytochemicals can receive less intensive regulatory attention. This is one reason why many researchers have examined extracts of, or other preparations from, thousands of plant and fungus species for existence of nematode antagonism. Consequently, hundreds of structurally diverse chemical compounds responsible for this activity have been identified. Specific experimental or practical application methods for using nematode-antagonistic phytochemicals in agriculture include the incorporation of plant amendments to soil, use of cover crops that produce nematotoxic compounds, treatment of soils or plants with plant extracts or plant-based formulations, or the direct application of compounds known to be nematotoxic. However, the actual agricultural utilization of phytochemically based nematode management agents has remained limited for several reasons, including expense and an unacceptable spectrum of biological activity which sometimes includes phytotoxicity. Additionally, these agents may not move in soils or may persist in a manner that is agriculturally effective yet environmentally unsafe. Specific materials and compounds examined at Beltsville will be utilized to illustrate these opportunities and challenges.

POSITIONING NEMATOLOGY WITHIN CROP PROTECTION AND FOOD SECURITY: HOW TO RAISE THE PROFILE OF NEMATOLOGY. **Ciancio, A.** Consiglio Nazionale delle Ricerche, Institute of Sustainable Plant Protection, Via G. Amendola 122/D, 70126 Bari, Italy.

Nematology originated in the nineteenth century as a monophyletic science stemming from zoology and taxonomy, and early studies concerned species description and classification. Subsequent development of nematological research in plant protection occurred in the 1950's, when industrialized agriculture evolved towards monocultures requiring effective

nematode management tools. The discipline evolution never stopped, and actual research work is integrated in world leading science endeavours. These are largely multidisciplinary, integrating different approaches and expertizes, ranging from ecology or natural sciences to molecular biology and genome research. Nematodes are everywhere in science, and this is indeed an important issue, producing a precious fallout for crop protection and security in food production. The FAO definition of food security clearly states that all people have the right to sufficient and safe food. The main goal is to satisfy, any dietary need and preference for a healthy life. Considering modern demography and worldwide differences, many challenges have yet to be afforded. Given their social, political or economic causes, nematological research may only partially contribute to reach this goal. However, actual levels of scientific knowledge, spanning from genome functioning to plant biology and ecosystem services are very significant. Nematological studies can constructively contribute to solve, at least in part, some of the food security problems affecting many communities. Fundamental conditions, at this regard, are the free access to education and scientific information for everyone, as well as the support of global efforts aiming at an independent production of knowledge through international cooperation.

NEXT GENERATION BIOCONTROL PRODUCTS AND THE PLANT RHIZOSPHERE: FROM SPECIALIZED NEMATODE PARASITES TO MULTITROPHIC RELATIONSHIPS. **Ciancio, A., L. Rosso, I. Pentimone and M. Colagiero.** Consiglio Nazionale delle Ricerche, Institute of Sustainable Plant Protection, Via G. Amendola 122/D, 70126 Bari, Italy.

Several research projects elucidated the role of soil microbial communities in sustainable phytonematode management. Some bionematicides are successfully commercialised, after encouraging laboratory and field assays. Their success as bionematicides will rely on factors like host specificity, soil or rhizosphere competition and parasitism transmission. Most of them, like the fungus *Pochonia chlamydosporia* or *Pasteuria* spp. bacteria, produce resting propagules, a property facilitating commercial formulations. For specialized parasites, epidemiological models as well as molecular detection allow estimation of their regulation activities. In the rhizosphere, multitrophic relationships involve many nematode regulating bacteria, often not yet described or difficult to identify, including *Bacillus* spp. and *Pseudomonas* spp. Safety for humans and other vertebrates, as well as formulation stability, are key factors for industrial exploitation. In temperate regions, specialized parasites follow the seasonal nematode population growth. As a consequence, an inundative approach is needed to induce a host local extinction or lower the nematode density peaks during early cropping times. For this reason, large numbers of viable propagules are needed from low cost fermentation facilities. For multitrophic organisms, a detailed knowledge is needed on their rhizosphere biology. Transcriptomic data on *P. chlamydosporia* endophytic on tomato showed that this fungus has a complex behaviour, including host growth promotion through the induction of plant resistance genes. Cultural practices as well as cropping systems may have a synergistic or detrimental influence on the nematode-antagonists dynamics. Market expectations and properties of industrial products released in the environment are also discussed.

PRE-PLANT TREATMENTS FOR THE PRODUCTION OF NEMATODE FREE SEED YAMS. **Claudius-Cole[1,2], A.O., D.L. Coyne[2], and L. Kenyon[3].** [1]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria; [2]International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320, Ibadan, Oyo State, Nigeria; [3]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK.

Various pre-treatments were evaluated in field experiments carried out at three sites in Nigeria. Setts, cut from yam tubers for the generation of seed yam material of preferred local cultivars, were treated before planting with a mixture of fungicide and insecticide, neem, hot water (HWT) (20 min @ 53°C) or wood ash (farmers practice) and compared with untreated setts. An additional experiment assessed the separate effects of the pesticides on seed yam production. Pesticide treated setts sprouted better than all other treatments and generally led to lower nematode damage of yam tubers compared with other treatments. Pesticide pre-treatment increased tuber yields over most treatments, depending on cultivar, but effectively double to those of the control. Pesticide and HWT setts produced the healthiest seed yams, and with fewer nematodes which stored significantly better. A combined insecticide and fungicide pre-plant sett treatment led to better sprouting and heavier tubers (15.7 kg/plot) than for either fungicide (13.2 kg) or insecticide (9.5 kg) alone or for wood ash (6.0 kg). Over two cropping cycles from seed through ware yam production, substantial benefits to yam productivity, in the region of 700%, were observed by using a simple and effective pre-treatment of setts that guarantees more, heavier and healthier seed yam tubers.

NEMATOLOGY TRAINING AND RESEARCH IN NIGERIA: CHALLENGES AND OPPORTUNITIES. **Claudius-Cole, A.O., and B. Fawole.** Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria.

This paper discusses the trends in nematology training and research in Nigeria. Nigeria is a large country with a population of 1.2 million students, out of over 160 million people, enrolled in universities and other higher institutions. There are about 120 federal, state and private universities in the country but only about a third offer degrees in agriculture. The number of students that apply for agricultural degrees is low compared to other courses like law or medicine. Few of the faculties of agriculture teach any Nematology at the undergraduate level. At the postgraduate level, only about 10% of the students that apply for crop protection offer to conduct research in Nematology. A high percentage of students taking postgraduate courses

in crop protection do not have basic knowledge of Nematology. Indeed, only few departments of Crop Protection teach Nematology as a stand-alone course. The challenges faced by scientists in Nematology include appropriate equipment, proper diagnosis, culturing nematodes for research and tedious data collection. The majority of the published papers are research on root-knot nematodes, with the current trend focussing on using non-chemical interventions of control. Opportunities for research and training in Nigeria, provided funding is available, are in the areas of nematode diagnostics, pest status of specific species on the current germplasm, evaluation of new nematicides and working with breeders to develop resistant cultivars. Scientists in Nematology should therefore seek research funds to attract, encourage and mentor post-graduate students.

EVALUATION OF TOMATO VARIETIES FOR RESISTANCE TO *MELOIDOGYNE* SPP. IN NIGERIA. **Claudius-Cole[1], A.O., J.L. Starr[2] and B. Fawole[1].** [1]Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria; [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas USA.

Ten tomato cultivars commonly grown in Nigeria in addition to new germplasm with resistance to *Meloidogyne incognita* were assessed for their reaction to local populations of *Meloidogyne* spp. The study evaluated populations of *Meloidogyne* collected from experimental field plots from University of Ibadan (UI), International Institute of Tropical Agriculture (IITA), Ijaye, Akobo, Federal University of Agriculture Abeokuta (FUNNAB) and Ilaro, (western Nigeria). Tomato plants were inoculated with galled roots obtained from each location in pot experiments laid out in a randomised complete block design with six replicates. Data on plant height, dry biomass, fruit weight, nematode population per plant, gall rating and reproductive factor were taken. Adult females and single egg masses were also collected from harvested galled roots and identified using perineal patterns and molecular techniques. The three local varieties, namely: Ibadan Local, Roma and Beske showed a typical susceptible reaction to root-knot nematodes. Galling was observed in all the tomato varieties assessed, although Small Fry had significantly fewer galls and lower reproductive factor compared to the other cultivars. Yield was significantly reduced in inoculated plants compared to their control in all cultivars except for Small Fry. Perineal patterns identified adult females as *M. incognita* or *Meloidogyne javanica*. However, molecular techniques further identified *Meloidogyne enterolobii*. Some isolates could not be identified with the techniques used. This is the first report of *M. enterolobii* in Nigeria. The presence *of M enterolobii* presents new challenges for the use of resistant tomato and other vegetables in Nigeria.

SCREENING OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR CONTROL OF *MELOIDOGYNE INCOGNITA* ON SOYBEAN. **Conrad[1], R., N. Labuschagne[1], H. Fourie[2] and R.A. Sikora[3].** [1]Department of Microbiology and Plant Pathology, University of Pretoria, Hillcrest 0083, South Africa; [2]Unit of Environmental Sciences and Management, North-West University, Potchefstroom 2531, South Africa; [3]Professor Emeritus, Institute of Crop Science and Resource Conservation, University of Bonn, Bonn 53115, Germany.

Meloidogyne incognita race 2 causes substantial yield losses on soybean in South Africa. Rhizobacteria previously isolated from virgin grassland were screened for their potential biological control activity. Selected isolates were first tested for compatibility with the nitrogen-fixing nodule-forming bacterium *Bradyrhizobium japonicum*, using a dual culture assay. Subsequently the bacterial isolates were screened for their ability to reduce *M. incognita* galling on soybean using a seedling bioassay. During the bioassay, bacterial cell suspensions (10 ml containing ca.10^8 CFUs per ml) were pipetted onto soybean seeds in a sterile medium in a greenhouse. At emergence of the first true leaves, each seedling was inoculated with 2000 *M. incognita* second-stage juveniles. Plants were assessed for galling after 19 days. The most promising isolate, tentatively identified as a *Paenibacillus* sp., reduced the number of galls per plant by 37% and per gram fresh root by 44% in comparison with the *M. incognita*-infected control. Shoot fresh biomass was increased by 46% while root fresh biomass was not significantly affected. This *Paenibacillus* strain was previously demonstrated to control root rot fungi on wheat. Further research is underway to evaluate this strain and other bacterial isolates on soybean cultivars with different levels of resistance to *M. incognita* in pot trials in the greenhouse. The modes of action of the bacteria are also being elucidated including their effect on the *in vitro* viability of second stage juveniles.

MELOIDOGYNE LUCI (NEMATODA: MELOIDOGYNIDAE), A ROOT-KNOT NEMATODE PARASITIZING DIFFERENT CROPS IN BRAZIL, CHILE AND IRAN. **Correa[1, 2], V.R., R.M.D.G. Carneiro[1], M.R.A. Almeida[1], A.C.M.M. Gomes[1], A.M. Deimi[3], P. Castagnone-Sereno[4] and G. Karssen[5].** [1]EMBRAPA - Recursos Genéticos e Biotecnologia, C. P. 02372, 70849-979 Brasilia, DF, Brazil; [2]Depart. de Fitopatologia, Universidade de Brasília, 70910-900, Brasília, DF, Brazil; [3]Depart. of Plant Protection, Fac. of Agriculture, Takestan Branch, Islamic Azad University, Takestan, Iran; [4]INRA-UMR1355, UNSA, CNRS-UMR7254, Institut Sophia Agrobiotech, BP167, Sophia Antipolis, France; [5]Plant Protection Service, WaNeCo collection, P.O. Box 9102, 6700 HC Wageningen, The Netherlands.

A root-knot nematode parasitizing vegetables, flowers and fruits in Brazil, Iran and Chile, was described as *Meloidogyne luci*. The female has an oval to squarish perineal pattern with a low to moderately high dorsal arc without shoulders, similar to *Meloidogyne ethiopica*. The female stylet is robust, 15-16 μm long; the distance from the dorsal pharyngeal gland orifice to the stylet base (DGO) is 3-4 μm. Males have a high, round head cap continuous with the body contour. The labial disc is

fused with the medial lips to form an elongated lip structure. The head region is not marked by incomplete annulations. Male stylet is robust, 20.8-23.0 μm long and has rounded knobs; the DGO is 2.5-4.5 μm. The stylet of juveniles (J2) is 12.0-13.5 μm long and the DGO is 2.3-3.3 μm. Biochemically, the esterase phenotype L3 (Rm: 1.05, 1.10, 1.25) is unique and the most useful character to differentiate *M. luci* from other *Meloidogyne* species. Reproduction is by mitotic parthenogenesis (2n = 42-46 chromosomes). In the North Carolina Host Range Test, this species reproduced on tomato cv. Rutgers, tobacco cv. NC95, pepper cv. California Wonder. No reproduction occurred on watermelon cv. Charleston Gray, cotton cv. Deltapine 61 and peanut cv. Florunner. In Neighbour-Joining analyses of ITS and D2-D3 rRNA sequences, populations of *M. luci* from Brazil, Chile and Iran clustered together and were clearly separated from other *Meloidogyne* spp., thus confirming that all three populations are very similar and conspecific.

SUSTAINABLE INTENSIFICATION: THE IMPLICATIONS OF NEMATODES TO FOOD SECURITY IN SUB-SAHARAN AFRICA. **Coyne, D.** International Institute of Tropical Agriculture (IITA), Nairobi, Kenya.

In a region of immense biophysical diversity, potential and hope, crop productivity in sub-Saharan Africa (SSA) is currently failing to keep pace with demand. A stark situation, as population growth gathers pace, urban populations balloon and production systems are dominated by subsistence agriculture. Continuing 'agriculture-as-usual' is not an option with significant shifts in production styles urgently required. The intensification of smallholder agriculture will constitute a crucial component of any strategy towards this goal. A very diverse group of smallholders dominates SSA agriculture, however, with broad heterogeneity creating a potential multitude of pathways from the current low productivity base. A 'one-size-fits-all' will thus not work but will need to seriously consider the aspect of sustainability. Intensified cropping systems, among other aspects, create focused selection pressures for pest and disease emergence. Nematode pests are commonly overlooked and/or misdiagnosed across the globe. Nowhere is this more marked than in developing countries under resource limited conditions, resulting in the consequent neglect of nematode management. Consequently, not only are innovative IPM options required but greater capacity in nematology in particular is equally necessary. Attracting interest in nematology as well as other plant health aspects should be key within the overall strategy to increasing agricultural productivity. Heavy reliance on synthetic pesticides will be less of an option for SSA farmers, particularly for nematode pests, as many nematicides are no longer available. Overcoming production losses will require suitable management options, chemical or otherwise, strong capacity building, and stronger links between the agro-input industry (private) and public sectors.

NEMATOLOGY CAPACITY BUILDING IN AFRICA AT THE INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE. **Coyne, D.** International Institute of Tropical Agriculture (IITA), Nairobi, Kenya.

Across the developing world agricultural productivity is steadily rising, which is in stark contrast to the decreasing outputs from sub-Saharan Africa. Consequently, there is need for a focus on improving crop productivity on this continent. As part of the measures required to address this challenge, improved pest and disease management will be crucial. This includes nematode pests, a regularly overlooked problem. Addressing the issue of nematode pests requires not only innovative integrated pest management options for their management but also a paradigm shift in raising awareness and providing training in nematology. This will require substantial investment in training and capacity building, as well as strengthening links between the public and the private sectors. Nematology *per se* is vastly under-represented across sub-Saharan Africa, with no expertise present in some countries even. At IITA harnessing interest and appeal in the discipline is being addressed through a number of routes. Integrating formal training into new projects with full financial support is a satisfying but a relatively rare occurrence. Other mechanisms involve provision of bench training for national technical staff, under-graduate and graduate students with limited resources. Use of workshops for highlighting nematology issues is also helpful and, where possible, using simple protocols to simplify the topic. In sub-Saharan Africa, as with other areas, retaining students and staff with skills, knowledge and interest in nematology is key to sustaining the science at the national level towards reducing the food security gaps.

PROSPECTING IN ESTABLISHED CEREAL CROPPING ECOSYSTEMS FOR MICROBIAL ENEMIES OF ROOT LESION NEMATODES. **Crampton, K., E. Adorada, C. Lisle and G. Ash.** Charles Sturt University, Panorama Ave, Bathurst NSW 2795, Australia.

Root lesion nematodes (*Pratylenchus* spp.) are responsible for millions of dollars in losses to the Australian grains industry each year. Current management strategies are not always practical, so development of alternative control measures would be of significant benefit. Microbial-based pesticides are one such alternative undergoing a revival of interest. The aim of this study was to isolate selected microbial enemies of plant parasitic nematodes, and evaluate their potential to control root lesion nematodes. Twenty-one farms across the Riverina, a district with a long cereal cropping history and known presence of *Pratylenchus* spp., were sampled. Three hundred and thirty one (331) isolates of *Paecilomyces*, *Trichoderma*, *Verticillium* and *Bacillus* were obtained following selective culturing, with *Trichoderma* being the most abundant. From these isolates, forty six (46) *Paecilomyces*, twelve (12) *Verticillium*, seven (7) *Trichoderma*, and ten (10) *Bacillus*, plus 13 additional *Bacillus* isolates from the Charles Sturt University Plant Pathology collection, were selected for assay against *Pratylenchus*

thornei in a greenhouse pot experiment. Seventy one (71) treatments were able to reduce the population number of nematodes compared to the untreated control, with a number of *Paecilomyces* isolates providing significant levels of control. Overall, our findings suggest that isolates obtained from established *Pratylenchus*-containing ecosystems are suitable for investigation as biocontrol agents.

SEROTONERGIC SIGNALLING IN *GLOBODERA PALLIDA*. **Crisford[1], A., E. Ludlow[1], J. Marvin[2], J. Kearn[1], V. O'Connor[1], P.E Urwin[2], C. Lilley[2] and L. Holden-Dye[1].** [1]Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK; [2]Centre for Plant Sciences, Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK.

The *Caenorhabditis elegans* gene *tph-1* encodes tryptophan hydroxylase the synthetic enzyme for the neurotransmitter 5-HT (serotonin). 5-HT is involved in food driven behaviours in *C. elegans* and implicated in stylet thrusting *in Globodera pallida*. Here we identifIed the *G. pallida* orthologue of *tph-1* and used *C. elegans* as a platform to show that it can function as a synthetic enzyme for 5-HT. To achieve this we tested the ability of *G. pallida tph-1* to rescue the aberrant feeding behaviour of the *C. elegans tph-1* mutant. In plant parasitic nematodes, 5-HT stimulates the activity of the stylet which is intimately involved in invasion of the host plant. To assay for 5-HT behaviour in plant parasitic nematodes we modified the electropharyngeogram recording that was established for *C. elegans* pharynx and recorded *G. pallida* electrical waveforms which are coincident with stylet thrusts. 5-HT stimulates stylet activity in a concentration dependent manner. We are currently investigating whether or not we can generate responses from host plant cues in this paradigm and will dovetail this with RNAi experiments for *tph-1* to delineate the role of 5-HT in the plant parasitic nematode host-dependent behaviour. In a further series of experiments we are investigating the functional roles of putative 5-HT receptors in stylet thrusting.

TREATMENT ZONE OF ABAMECTIN IN GOLF COURSE GREENS. **Crow[1], W.T., and L. Dant[2].** [1]University of Florida, PO Box 110620, Gainesville, Florida, 32611 USA; [2]Syngenta Crop Protection, 410 Swing Rd., Greensboro, North Carolina 27409 USA.

Abamectin has been recognized as an excellent nematicide for decades, but has only recently been used as a nematicide due to its poor movement in soil. Golf course greens offer a unique environment with several properties that make use of abamectin nematicide practical. These properties include plants with a shallow root system, sand-based growth media, and topical irrigation. In summer 2013, a golf green field trial studied effects of abamectin application on *Belonolaimus longicaudatus*, *Meloidogyne graminis*, and *Criconemoides ornatum* at two soil depth (0 to 5 and 5 to 10 cm-deep). Abamectin was applied five times at 14-day intervals. Nematode samples were collected before treatment, and after the second and fifth treatments. During the course of the trial, the majority of *B. longicaudatus* moved from the 0 to 5 cm to the 5 to 10 cm-depth, and no nematode differences among treatments were observed. The majority of *M. graminis* remained in the top 5 cm of soil throughout the trial, and abamectin reduced the population density of *M. graminis* in that portion of the soil profile. The majority of *C. ornatum* moved from the 5 to 10 cm to the 0 to 5 cm-depth during the trial, and population densities were reduced by abamectin at both depths. The results of this trial indicate that abamectin is an effective treatment against nematodes in the top 5 cm of golf green soil profile. These results also indicate that knowledge of seasonal distribution of target nematodes is important to maximize abamectin as a nematicide.

BIOMANAGEMENT STRATEGY TO CONTROL PHYTOPARASITIC NEMATODES OF EMERGING IMPORTANCE ON POTATO – A NEW THREAT TO EUROPE. **Cunha[1], M.J., O. Machado[1], R. Guilherme[1], I. Esteves[2], C. Vieira dos Santos[2], C. Maleita[3], I. Conceição[2], S. Chelinho[2], R. Francisco[2], I. Abrantes[2], M. Braga[3], A. Dias[3], S. Marceneiro[3] and H. Sousa[3].** [1]CERNAS, Department of Agronomic Sciences, High School of Agriculture, Bencanta, 3040-316 Coimbra, Portugal; [2]IMAR-CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal; [3]CIEPQPF, Department of Chemical Engineering, FCTUC, University of Coimbra, 3030-790 Coimbra, Portugal.

Diversity and pathogenicity of potato cyst nematodes (PCN), *Globodera* spp., occurring in Portuguese potato fields is well documented but the impact of other highly pathogenic species such as root-lesion nematodes (RLN), *Pratylenchus* spp., and root-knot nematodes (RKN), *Meloidogyne* spp., have been less studied. The wide RLN and RKN host range limits the use of crop rotation, enhancing the risk of an increase in the nematode population densities. Therefore, it is crucial to evaluate the present status of RLN and RKN species occurring in potato fields in order to develop sustainable control strategies. Studies on the efficacy and ecotoxicology of natural compounds obtained from agricultural residues/byproducts will allow the development of suitable natural delivery systems and the identification of new targets for nematode control, leading to improve crop protection systems. Results of the occurrence of RLN and RKN species in potato fields and of the effect of walnut hulls compounds on the nematode mobility will be presented. This project will provide information on the potential use of natural compounds as a biomanagement strategy to control phytoparasitic nematodes of emerging importance on potato and other crops. The information gathered will contribute to improve potato production and will generate recommendations for decision makers.

MANAGEMENT OPTIONS TO CONTROL CEREAL NEMATODES IN WHEAT UNDER RAINFED CONDITIONS. **Dababat[1], A.A., G. Erginbas-Orakci[1], H-J. Braun[2], H.I. Elekcioglu[3] and A. Morgonouv[1].** [1]CIMMYT, P.K. 39 Emek 06511 Ankara, Turkey; [2]CIMMYT Apdo. Postal 6-641, C.P. 06600, D.F. Mexico; [3]University of Cukurova, Faculty of Agriculture, Department of Plant Protection, 01330, Adana, Turkey.

Heterodera and *Pratylenchus* species are two generacausing significant yield losses to cereals around the world. Damage caused by these nematodes become enormous when they occur in a disease complex especially in areas where water stress exists. Breeding for resistance to both *Heterodera* and *Pratylenchus* spp. is still insufficient due to lack of expertise, recognition of these nematodes as a factor limiting wheat production potential, inappropriate breeding strategies, slow screening process, and limited funding. Breeding for resistance is becoming complicated and difficult when different species and pathotype coexist in nature. The Soil Borne Pathogens (SBP) program at the International Maize and Wheat Improvement Center addresses the use of integrated pest management options to manage *Heterodera* and *Pratylenchus* spp. including chemical treatments and biological control. The vision is to use resistant wheat germplasm in combination with other control methods to keep nematode damage below the threshold levels. Therefore, the SBP program successfully screened thousands of wheat germplasm to identify resistant source/s against *Heterodera* and *Pratylenchus* spp. As a result, more than 100 genotypes have been identified as resistant to *Heterodera* and *Pratylenchus* spp and genotyped to study the genetic background of the resistance sources through association mapping.

ROOT-KNOT NEMATODES PAN-GENOMICS REVEALS NOVEL TARGET GENES FOR SAFER AND MORE SPECIFIC CONTROL. **Danchin, E.G.J., M-J. Arguel, A. Campan-Fournier, L. Perfus-Barbeoch, M. Magliano, M-N. Rosso, M. Da Rocha and P. Abad.** Institut Sophia Agrobiotech, UMR, INRA - CNRS - UNS, 400 route des Chappes, F-06903, Sophia-Antipolis, France.

Plant-parasitic nematodes are annually worldwide responsible for more than $100 billion crop yield loss and those considered as causing most of the damages are root-knot nematodes. Nematicides were used to control these nematodes; however some of the nematicides are now banned from use because of their poor specificity and toxicity for the environment and human health. In the absence of sustainable alternative solutions, new control means, more specifically targeted against these nematodes and safe for the environment are needed. We identified and analysed 15,952 genes in root-knot nematode (*Meloidogyne* spp.) genomes that are conserved in various plant-target species while otherwise absent from the genomes of non-target species (such as those of chordates, plants, annelids, insect pollinators and molluscs). These genes are probably important for plant parasitism and their absence from non-target species make them interesting candidates for the development of more specific and safer control measures. Further bioinformatics pruning of this set of genes yielded 16 novel candidates that could be biologically tested. These novel genes have no characterized function and code for proteins without known conserved domain. Using RNA interference, we knocked down each of these 16 genes in the root-knot nematode *Meloidogyne incognita* and tested the effect on plant parasitism efficiency. Out of the 16 tested genes, 12 showed a significant and reproducible decrease in nematode infestation when silenced. They are thus particularly promising for the development of more specific and cleaner control methods.

DEVELOPMENT OF *GLOBODERA PALLIDA* IN THE TRAP CROP *SOLANUM SISYMBRIIFOLIUM*. **Dandurand[1], L.M., C.R. Brown[2] and P. Gajjar[1].** [1]University of Idaho, Moscow, Idaho 83844-2339, USA; [2]USDA-ARS, Vegetable and Forage Crops Research Unit, Prosser, WA, 99350, USA.

Globodera pallida (the pale cyst nematode) is a quarantine pest in the state of Idaho. Development of *G. pallida* life stages in roots of the potential trap crop *Solanum sisymbriifolium* (litchi tomato) was compared with development in potato. Pots with four-week-old litchi tomato or potato ('Russet Burbank') plants were inoculated at a rate of 5 eggs/g soil. After 2, 4, 6, 8, 10 and 16 weeks, roots were stained with acid fuschin, and nematode life stages were counted. The highest number of J2 larvae in potato roots was observed at 14 days post-inoculation (DPI), compared to litchi tomato where numbers of J2 larvae peaked at 42 DPI. Numbers of J3 larvae in potato roots were highest at 28 DPI, whereas few J3 larvae were ever observed in litchi tomato roots at these sampling times. Numbers of both J4/adult males and J4/adult females were highest in potato roots at 28 DPI. Numbers of J4/adult males in litchi tomato were highest at 42 DPI, whereas no J4/adult females were found in litchi tomato roots. In potato, egg production increased rapidly between 42 and 70 DPI. No eggs were produced in litchi tomato. The lack of nematode reproduction in roots of litchi tomato suggests that it may be an effective trap crop to help eradicate *G. pallida* in Idaho.

NEMALAN PRODUCTS AS ALTERNATIVE NEMATICIDAL AGENTS FOR THE CONTROL OF *MELOIDOGYNE* SPECIES IN TOMATO. **Daneel[1], M.S., H. Fourie[2], A. McLeod[3], F. Hiten[4] and W.P. Steyn[1].** [1]ARC-Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa; [2]North-West University, Unit for Environmental Sciences and Management, Private Bag X6001, Potchefstroom 2520, South Africa; [3]Stellenbosch.University, Department of Plant Pathology, Private Bag X1, Matieland 7602, South Africa; [4]Stellenbosch.University, Central Analytical Facilities, Mass Spectrometry Unit, Private Bag X1, Matieland 7602, South Africa.

Nemalan product combinations are organically produced by ZZ2 Natuur Boerdery for its nematicidal activity against nematode pests. These products are manufactured by anaerobic fermentation of lantana and garlic gloves with the addition of effective microorganisms (EM). *In vitro* laboratory and *in vivo* field assays were conducted to evaluate the efficacy of various Nemalan product combinations and dilutions against *Meloidogyne incognita* and *M. javanica*, respectively. For both species, second-stage juvenile motility was significantly ($P \leq 0.05$) inhibited after incubation in two dilutions (1:25 and 1:40) of NEM140911EM, Biolan290910, GN280812, NEM240712 and NEM 160212 for 72h at 26 °C. Immotility of juveniles was irreversible when they were transferred to sterilised tap water for 24h. Twenty six chemicals that were identified in Nemalan products were tested as three groups (plant volatiles, phenolic compounds and fermentation products) and in combination for juvenile motility evaluations showing a similar scenario. In field trials, an initial pre-plant application of NEM + EM @ 100 liter/ha followed by five bi-weekly applications of 30 liter/ha resulted in superior plant growth enhancement of tomato. Moreover, although no significant differences were evident root-knot nematode population levels were substantially lower compared to the untreated control until eight weeks after planting. The Nemalan treatments alone were, however, less effective in reducing root-knot nematode population levels than the combination of NEM + EM. Further research will be aimed at investigating other application methods and higher dosages to optimise the nematicidal effect of Nemalan products on root-knot nematodes.

BRASSICACEAE-BASED MANAGEMENT STRATEGIES TO REDUCE *MELOIDOGYNE* SPECIES DAMAGE IN CROPS. **Daneel[1], M.S., J. Lammers[2], H. Fourie[3], P. Ahuja[3] and W.P. Steyn[1].** [1]Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa; [2]Laboratory of Nematology, P.O. Box 8123, Wageningen University and Research Centre, Netherlands; [3]Unit for Environmental Sciences and Management, North west University, Private Bag X 6001, Potchefstroom 2520, South Africa.

Brassicaceae cover- and/or biofumigation crops qualify as a potential alternative to synthetically-derived nematicides for managing root-knot nematode pests in various crops. The host status of *Eruca sativa* (cv. Rocket Trio and Nemat), *Brassica juncea* (cv. Caliente and Fumigreen) and *Raphanus sativus* (cv. Doublet and Terranova) were determined for *Meloidogyne javanica* in greenhouse experiments while their efficacy as cover crop and/or biofumigant was evaluated in the field against mixed populations of *Meloidogyne incognita* and *Meloidogyne javanica*. Tomato seedlings (cv. Monica) were planted as a follow-up crop in these plots during November 2013. All cover crops in the greenhouse trial had a reproduction factor (Rf) < 1 and differed significantly ($P \leq 0.05$) from the positive standard (tomato cv. Rodade), Rf value of 5. Terranova and Rocket Trio had the lowest Rf values (0.005), while Caliente had the highest (0.04). *Meloidogyne* spp. numbers in root and soil samples from the field trial were low for all cover crops, with no significant differences ($P \geq 0.05$). However, for the tomato crop, significantly lower values for growth and vigour parameters as well as significantly higher nematode numbers in 30 g roots were recorded for plots where both *B. juncea* cultivars were grown compared to those where *E. sativa* and *R. sativus* crops were grown. Tomato shoot and fruit mass recorded from plots where *E. sativa* and *R. sativus* crops were initially cultivated were also substantially higher than those of the untreated control and both treatments where *B. juncea* crops were initially grown.

INTEGRATED SOLUTION PROPOSALS FOR NEMATODE CONTROL BASED ON THE CURRENT AND UPCOMING BAYER CROPSCIENCE PORTFOLIO. **Dauck, H.** Bayer Cropscience, Alfred-Nobel-Str. 50, 40789 Monheim am Rhein, Germany.

Many broad-spectrum fumigants for soil disinfection or highly effective chemical nematicides of the organophosphate or carbamate class are of toxicological or environmental concern, and will disappear or be severely limited in their use. Thus, the risk of crop damage in increasing and easy and effective nematode control is becoming more difficult for growers. Today there are rather few alternatives available to manage serious nematode pressure. A number of new chemical and biological products are developed by the crop protection industries which have better safety profiles, but are not quite as powerful as the old solutions. Thus, it is advisable to combine the use of the new products in an integrated approach, providing diversity by making use of their specific properties and strength. We will discuss examples for such integrated solution proposals based on the current and upcoming Bayer CropScience portfolio for nematode control.

FUNCTIONAL ANALYSES OF ROOT-KNOT AND CYST NEMATODE EFFECTORS. **Davis, E.L.** North Carolina State University, Dept. of Plant Pathology, Raleigh, NC 27695-7616 USA.

Root-knot and cyst nematodes secrete effector proteins from their esophageal gland cells through their stylets to modify plant cells for parasitism. A battery of different expressed parasitism genes that encode secreted nematode effector proteins have been isolated, and functional analyses of several novel root-knot and cyst nematode parasitism genes are presented. Expression of the *30C02* cyst nematode parasitism gene in the model plant species *Arabidopsis thaliana* did not alter visible plant phenotype but did increase susceptibility to cyst nematodes. Host-derived RNA interference (RNAi) in *Arabidopsis* targeted to *30C02* produced significant reductions in cyst nematode female numbers compared to control plants. The 30C02 effector protein interacted specifically with a plant beta 1,3 endoglucanase, and supporting nematode infection data suggest

that 30C02 interaction with this pathogenesis-related protein may modulate plant defense response to nematode attack. Expression of the cyst nematode *20E03* parasitism gene in *Arabidopsis* surprisingly provided increased plant tolerance to drought and salt stress. Expression of the root-knot nematode *8D05* parasitism gene accelerated shoot growth in *Arabidopsis* but did not influence root growth. Host-derived RNAi targeted to *8D05* significantly reduced Arabidopsis root gall number induced by root-knot nematodes. The 8D05 effector specifically interacted with plant major intrinsic proteins suggesting a role for 8D05 in modulating water and solute transport in nematode feeding cells. Expression of two additional novel root-knot nematode parasitism genes in *Arabidopsis* induced a reduction in successful root-knot nematode infection while host-derived RNAi targeted to these same two parasitism genes also significantly reduced gall number induced by root-knot nematodes.

EFFECT OF SOIL AMENDMENTS ON THE POPULATION DYNAMICS OF PLANT PARASITIC NEMATODES ASSOCIATED WITH ORGANIC OLIVE PRODUCTION. **Dawabah[1], A.A.M., and S.H. Al-Kahtani[2].** [1]Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Saudi Arabia; [2]Agricultural Economics Department, College of Food and Agriculture Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Saudi Arabia.

An olive (*Olea europaea* L.) orchard was selected in Al-Jouf region, north Saudi Arabia to investigate the effect of five different compost soil amendments on the population dynamics of plant-parasitic nematodes associated with olive under an organic farming system. Six treatments (five composts + non-treated control) with nine replicates (trees) for each treatment were established in a randomized complete block design. Rhizosphere soil samples were collected from all the tested trees, at four intervals. Plant-parasitic nematodes were extracted using a modified sugar flotation procedure, identified and counted. Results showed that the nematode populations consisting mostly of *Helicotylenchus digonicus*, *Helicotylenchus pseudorobustus* and *Pratylenchus vulnus* tended to decrease ($P \leq 0.05$) in March and September. The nematode population peaked in June 2011, after a period of mild atmospheric temperature. However, plant parasitic nematode numbers increased ($P \leq 0.05$) in all the non-control treatment with the only exception of compost 5 treatment. These increases were found to be proportional to the percentage of the sheep manure content in the compost itself. The highest numbers of plant-parasitic nematodes were detected in the soil of the trees amended with compost 1 (plant wastes + 5% sheep manure), then gradually as the percentage of sheep manure increased. It could be concluded that a compost containing plant wastes plus 40% sheep manure could reduce, or at least maintain the plant-parasitic nematode populations at their lowest levels in the rhizosphere of organically grown olive trees.

BIODIVERSITY AND CONNECTIVITY OF MARINE NEMATODES ASSOCIATED WITH ALGAE FROM TWO TROPICAL BEACHES. **de Oliveira[1,3], D.A.S., G.A.P. dos Santos[2], S. Derycke[3], T. Moens[3], W. Decraemer[1,4].** [1]Department of Biology, Ghent University, Ghent, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Department of Biology, Federal University of Pernambuco, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE, Brazil; [3]Department of Biology, Marine Biology section, Ghent University, Krijgslaan 281, S8, 9000 Ghent, Belgium; [4]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium.

Seaweed beds are highly productive ecosystems in marine coastal areas and have been exploited for industrial usage. Overexploitation has drastically affected the associated organisms including economically important fishery in tropical beaches. Seaweed beds harbor a high diversity of epifauna, including nematodes which play a key role in marine ecosystems and processes. Therefore, investigating possible recovery of the nematofauna from areas affected by seaweed harvesting is highly relevant. The relationship between the nematofauna from seaweeds and surrounding sediment is still poorly understood. Moreover, the dispersal capacity of the nematodes present on seaweeds is not well established. In this study we aimed 1) to describe and compare the structure of the nematode communities of the seaweeds and sediment from beaches along the eastern Brazilian coastline, and 2) to study connectivity among seaweed beds using COI sequences of a new species of *Paracanthonchus*, the dominant species of the epifauna.. Our results suggest that a) the nematofauna found in the seaweeds is very distinct from the one in the sediment, and suggests that the sediment does not act as a source for recovery of the seaweed bed nematofauna, and b) the two beaches which are more than 900 km apart, are genetically homogeneous (pairwise Fst = 0.02, $p>0.05$). These results suggest that connectivity between seaweed beds is sufficient to prevent genetic structuring, even at very large geographic distances.

NEMATICIDE DISCOVERY - OPPORTUNITIES AND CHALLENGES. **Desaeger, J.** DuPont Crop Protection, Stine-Haskell, 1090 Elkton Rd, Newark, DE 19711, US.

Agricultural challenges over the next 40 years are daunting – increase demand for agricultural products from global population growth and demand for fuel and feed; pressure on non-renewable resources, importance of water quality; increasing regulatory requirements reflecting an increased concern about safety of food supply, worker safety and impact on the environment. Economic losses to agriculture worldwide due to damage caused by plant parasitic nematodes are usually estimated to be around US$ 100 billion. Nematode control is becoming increasingly more difficult for growers all over the

world. Many nematicides have been banned in recent years due to intrinsic toxicity and environmental concerns. Other management options, such as crop rotation, have become more limited due to increased pressure on arable land. New nematode control options will be critical to secure global food production in the future and will very likely need to integrate several approaches, including chemistry, resistant cultivars and biological and cultural approaches. One of the great challenges nowadays is the need to offer growers effective solutions for nematode control, while at the same time recognizing and safeguarding societal and environmental needs. For decades nematicide discovery was low on the industry priority list, but more recently a new wave of nematode control research has emerged across the crop protection industry. At DuPont we are strongly committed to this opportunity and excited to be part of a new emerging era of nematode control.

STIMULATION HATCHING AGENTS OF *SOLANUM SISYMBRIIFOLIUM* EXUDATE EXTRACTS ON *GLOBODERA*. **Dias[1], A.M., S. Perpétuo[1], M.J. Cunha[3], I. Abrantes[1], T. Batista[2] and I. Conceição[1].** [1]IMAR-CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal; [2]Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal; [3]CERNAS, Department of Agronomic Sciences, High School of Agriculture, Bencanta, 3040-316 Coimbra, Portugal.

Potato cyst nematodes (PCN), *Globodera* spp., are one of the most difficult crop pests to control. The management of these nematodes has been achieved with the use of plant resistance, crop rotation and other cultural practices or pesticides. However, the control measures available are insufficient, not sustainable and environmental unfriendly. The identification of biological compounds from trap crops can be an alternative for the management of phytoparasitic nematodes. *Solanum sisymbriifolium*, a trap crop, seemed an ideal method for the control of PCN. The main goal of this study was to evaluate the hatching effects of root exudate extracts of *S. sisymbriifolium* (cv. Sis 6001), with 1, 2 and 3 months, on *Globodera*. The root exudate extracts were obtained from the soil, where plants had grown. The soil was placed in water, during 24 h in the dark, and the exudates collected in the water were filtered, concentrated and lyophilized. Laboratory assays, with four replicates and 15 cysts/replicate/treatment, were performed with five concentrations of each extract (0.4, 0.2, 0.1, 0.05 and 0.0025 mg/mL), using *Solanum. tuberosum* (cv. Désirée) root exudates and water as controls. The hatching of second-stage juveniles was evaluated during 30 days and the higher % of hatching for *Globodera pallida* was from 2 month old plants and for *Globodera rostochiensis* was from 1 month old plants, but with similar values obtained from 2 months old plants.

HATCHING AGENTS OF *SOLANUM SISYMBRIIFOLIUM* EXTRACTS ON *GLOBODERA*. **Dias[1], A.M., S. Perpétuo[1], M. J. Cunha[3], I. Abrantes[1], T. Batista[2] and I. Conceição[1].** [1]IMAR-CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal; [2]Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal; [3]CERNAS, Department of Agronomic Sciences, High School of Agriculture, Bencanta, 3040-316 Coimbra, Portugal.

Potato cyst nematodes (PCN), *Globodera* spp., are quarantine organisms with economic consequences and *Solanum sisymbriifolium* has been successfully used to control them. Plant extracts have nematicidal and/or nematostatic effects and these natural compounds could contribute to the management of PCN. In this study, the hatching effects of root exudates, root and aerial part extracts of *S. sisymbriifolium* (cv. Sis 6001), with one month, on PCN was evaluated to find out in which part of the plant the stimulation hatching substances are located and to identify the phytochemicals involved in these interactions. The root exudate extracts were obtained from the soil, where plants had grown, placed in water during 24 h, in the dark, and the plant extracts from dried and pulverized root and aerial parts. These extracts were dissolved in water and incubated in a water bath at 100 °C, during 60 min. The samples (exudates, root and aerial parts) were filtered, concentrated and lyophilized. Laboratory assays, with four replicates and 15 cysts of *G. pallida* or *G. rostochiensis*/replicate/treatment, were performed with five concentrations of each extract (0.4, 0.2, 0.1, 0.05 and 0.0025 mg/mL), using *S. tuberosum* (cv. Désirée) root exudates and water as controls. The hatching of second-stage juveniles was evaluated during 30 days. The hatching of the exudates extracts was higher, for all concentrations and for both species, suggesting that stimulating hatching compounds are more concentrated on the root exudates than in the plant extracts.

TERTIARY STRUCTURES OF ROOT-KNOT NEMATODE EFFECTORS INFORM FUNCTION. **DiGennaro[1], P., B. Bobay[2], and D. McK. Bird[1, 3].** [1]Department of Plant Pathology, NCSU, Raleigh, North Carolina 27695, USA; [2]Department of Molecular and Structural Biochemistry, NCSU, Raleigh North Carolina 27695, USA; [3]Bioinformatics Research Center, NCSU, Raleigh, North Carolina 27695, USA.

Plants employ suites of peptides to regulate organogenesis via cell-to-cell communication. Our interrogation of the available root-knot nematode genomes revealed families of genes encoding secreted peptides with high sequence similarity to the major regulatory plant peptides, notably CLE and CEP. We generated more than seven billion *Medicago* and *Meloidogyne hapla* ESTs to profile the expression of CLE and CEP, and we used root assays to establish bioactivity *in planta*. To examine a role for these peptides as *bona fide* elicitor/effectors, we first solved the tertiary structures of seven plant and nematode encoded CLE and CEP to high resolution. This established that, despite their small size (12 – 15aa), as mature peptides they have well defined structures. Comparative analyses of the CLE structures using NMR-based molecular

dynamics simulations revealed a common, central core motif of 7 residues, which presumably is responsible for general receptor recognition. Outside this core, different family members sample distinct conformational space that specifies receptor binding preference. We postulate that *Meloidogyne* spp. encoded CLE and CEP are delivered directly to the apoplast to target trans-membrane receptors. We have modelled these interactions using homology threaded receptor structures to obtain binding coordinates and free energies. Whether these nematode ligands function as mimics of developmental effectors, or as competitive inhibitors of plant ligands, remains to be definitively established. A likely function of plant peptides is to convert the analog signal presented by the classic secondary metabolite hormones, into a digital, cellular response. Presumably, root-knot nematodes exploit this machinery for the formation of discrete giant cells.

VARIETAL AND TECHNICAL INNOVATIONS FOR THE SUSTAINABLE AND INTEGRATED MANAGEMENT OF ROOT-KNOT NEMATODES. **Djian-Caporalino[1], C., M. Navarrete[2], A. Palloix[3], T. Mateille[4], A. Lefevre[5], A. Barbary[1], A. Fazari[1], N. Marteu[1], M. Chapuis[2], M. Tchamitchian[2], A. Dufils[2], A. Sage-Palloix[3], J. Tavoillot[4], L. Pares[5], H. Védie[6], C. Goillon[7], C. Taussig[7], S. Risso[8], R. Lanza[8], P. Castagnone-Sereno[1].** [1]INRA PACA, UMR1355 INRA/UNSA/CNRS, Institut Sophia Agrobiotech, F-06903 Sophia Antipolis, France; [2]INRA PACA, UR0767, Écodéveloppement, F- 84914 Avignon Cedex 9, France; [3]INRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France; [4]IRD, UMR CBGP, Campus de Baillarguet, CS30016, F-34988 Montferrier-sur-Lez Cedex, France; [5]INRA, UE0411 Sciences pour l'Action et le Développement, Domaine Expérimental de Alénya Roussillon, F-66200 Alenya, France; [6]GRAB, Groupe de Recherche en Agriculture Biologique, F- 84 911 Avignon Cedex 9, France; [7]APREL, Association Provençale de Recherche et d'Expérimentation Légumière, F- 13210 Saint-Rémy de Provence, France; [8]Chambre d'agriculture des Alpes Maritimes, F-06200 Nice, France.

The current restrictions on the use of chemical nematicides have contributed to increased root-knot nematodes (*Meloidogyne* spp.) problems in horticultural crops. In this context, plant resistance (*R*) appears as the most effective method of control, but the restricted number of cultivated vegetable species with root-knot nematode *R*-genes available (tomato, pepper), and the possible occurrence of virulent nematodes able to reproduce on *R*-plants may constitute a severe threat to this control strategy. To increase the sustainability of the protection, a scientific challenge exists in building cropping strategies based on the combination of genetic resistance with cultivation practices. Our projects aimed at assessing such innovative strategies in a multi-site device in research stations and commercial farms. First, we looked at three components of crop protection, i.e., *R*-efficiency, *R*-durability, and sustainability of rotating cultivation. Overall, a constant hierarchy of management strategies was observed, with Pyramyding > Alternating > Mixture of *R*-genes > Sequential use of a single *R*-gene introgressed in a susceptible background. We are currently analysing (1) the impact of agronomic practices on the parasite pressure in the soil and on ecological diversity including other nematode species, (2) the linkage between reduction of *Meloidogyne* populations in the soil and increase of *R*-genes durability, and (3) the sustainability assessment of such varietal and technical innovations. Multidisciplinary approaches were combined to foster synergistic and long-term goals. The diversity of partners and associated forces brought all the complementary expertise needed for answering specific short-term questions as well as generic mid- to long-term expectations.

OCCURRENCE, DISTRIBUTION, AND IMPACT OF NEMATODES IN SOYBEAN FIELDS IN THE SOUTHERN UNITED STATES. **Donald[1], P., K. Lawrence[1], T. Kirkpatrick[2], B. Kemerait[3], J. Bond[4], D. Herschman[5], C. Overstreet[6], A. Wrather[7], G. Lawrence[8], S. Koenning[9], P. Adugelo[10] and C. Canaday[11].** [1]Entomology & Plant Pathology, Auburn University, Auburn, Alabama, USA; [2]Southwest Research & Extension Center, University of Arkansas, Hope, Arkansas, USA; [3]Plant Pathology, University of Georgia, Tifton, Georgia, USA; [4]Plant Pathology Southern Illinois University, Carbondale, Illinois; [5]University of Kentucky Research & Education Center, University of Kentucky, Princeton, Kentucky, USA; [6]Plant Pathology and Crop Physiology Louisiana State University, Baton Rouge, Louisiana, USA; [7]Plant Sciences University of Missouri, Missouri, USA; [8]Biochemistry, Molecular Biology, Entomology & Plant Pathology Mississippi State University, Mississippi State, Mississippi, USA; [9]Plant Pathology North Carolina State University, Raleigh, North Carolina, USA; [10]School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, South Carolina, USA; [11]Entomologyu & Plant Pathology, University of Tennessee. Jackson, Tennessee, USA.

Changing crop management practices throughout the southern soybean production area stimulated a survey of plant-parasitic nematodes present in soybean production fields with an eye toward the consequences of common crop hosts in the potential management strategies. Soil samples were collected from each state (150/state) over a two-year period. Data collected included genera of plant-parasitic nematodes, soil texture, and GPS. Root-knot, reniform and soybean cyst nematodes were identified as the most potentially damaging nematodes present in these fields. Distribution of these three nematode genera was not uniform between states nor within states. Soil texture data showed distribution of these nematodes across multiple soil types but higher levels in certain soil types indicating certain soil types were more conducive to higher reproduction. Microplot and field studies aimed at these three genera showed soybean varietal differences in yield as well as nematode reproduction.

PLANT ESSENCES AS TOOL FOR MANAGEMENT OF THE NORTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE HAPLA*). **Douda[1], O., M. Zouhar[2], E. Nováková[2], R. Pavela[1].** [1]Crop Research Institute, Division of Plant Health, Drnovská 507, 161 06 Praha 6 – Ruzyně, Czech Republic; [2]Czech University of Life Sciences, Faculty of Agrobiology, Food and Natural Resources, Department of Plant Protection, Kamýcká 129, 165 21 Praha 6 – Suchdol, Czech Republic.

The root-knot nematode, *Meloidogyne hapla* is a prominent root vegetable pest in the Czech Republic. Effects of plant essences from *Pongamia pinnata*, *Tagetes erecta* and powder botanical pesticide Presto for *M. hapla* management on carrot (*Daucus carota*) were tested. Tests were conducted on container-grown carrots maintained under greenhouse conditions. The delivery method involved absorbing the tested essences in perlite particles placed in a substrate in the case of plant essences. The results showed a statistically significant reduction ($P \geq 0.05$) of gall numbers of the carrots when treated by Presto. Similar results were achieved with *Pongamia pinnata*, *Tagetes erecta* essences. However evaluation of fresh and dry root weights showed certain signs of phytotoxicity caused by tested substances. Based on these results the management of *M. hapla* using tested treatments seems to be possible, however further research is desirable to determine the appropriate dosage needed for efficient and most appropriate delivery method of these substances under field conditions.

EFFECT OF HYDROGEN CYANIDE TREATMENT ON *BURSAPHELENCHUS XYLOPHILUS* MORTALITY IN WOOD. **Douda[1], O., M. Zouhar[2], M. Maňasová[2], M. Dlouhý[3], J. Lišková[3] and P. Ryšánek[2].** [1]Crop Research Institute Prague, Division of Plant Health, Drnovská 507, 161 06 Prague 6, Czech Republic; [2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Plant Protection, Czech Republic; [3]Lučební závody Draslovka a.s. Kolín.

The Pine Wood Nematode (*Bursaphelenchus xylophilus*) is one of the economically most important quarantine nematodes in Asia and Europe. *Bursaphelenchus xylophilus* originates from North America and its introduction into Japan, China, Korea and Portugal led to devastation of the local conifers from the genus *Pinus*. Direct protection of pine trees in affected countries is expensive and difficult. As wood transported within the international trade plays major role in *B. xylophilus* spreading into new areas, attention should be given to wood treatment. With methyl bromide phasing out new chemicals should be investigated as an alternative. Gaseous hydrogen cyanide (HCN) seems to be promising as a methyl bromide alternative. For that reason the main aim of this study was testing of HCN potential as a nematode killing agent for wood treatment. Data considering HCN concentration in gas chamber during test fumigations and inside treated wooden blocks were also acquired. Results show overall good efficiency of HCN treatment on *B. xylophilus* mortality, total mortality was achieved after 8 hours lasting fumigation with the target HCN concentration of 12.30 $g \cdot m^{-3}$.

GLOBAL METRICS TO MEASURE THE IMPACTS OF NEMATODES: THEN WHAT? **Duncan, L.** University of Florida, IFAS, Citrus Research and Education Center 700 Experiment Station Rd., Lake Alfred, FL 33850, USA.

Understanding the economic importance of plant parasitic nematodes is necessary for the rational allocation of research and management resources by public or private organizations and growers. This talk reviews some of the key methodological contributions to crop loss assessment in nematology and highlights some previous estimates of regional and worldwide damage to various crops caused by plant parasitic nematodes. Deterministic and stochastic algorithms that employ loss assessment to forecast optimum management systems, and methods by which these models are being used for site-specific management of nematodes are also described. Advances in multivariate analytical methods have improved the capacity to identify biotic and abiotic properties that modulate relationships between nematodes and hosts. As the effects of key environmental properties becomes apparent, it will be possible to improve the efficiency of site-specific IPM and to ask new questions, such as how soils can be modified to mitigate losses to plant parasitic nematodes, or how to conserve and materially enhance the services of agriculturally beneficial nematodes such as those that are pathogens of insect pests.

NEMATODES: INDICATORS OF METAL POLLUTION IN THE WONDERFONTEIN CAVE (SOUTH AFRICA). **du Preez[1], G., H. Fourie[1], P. Theron[1], and A. Swart[2].** [1]North-West University, Unit for Environmental Sciences and Management, Private Bag X 6001, Potchefstroom 2520, South Africa; [2]ARC-Plant Protection Research Institute (PPRI), Biosystematics Division, Private Bag X134, Pretoria 0001, South Africa.

Subterranean ecosystems (caves) are highly sensitive to anthropogenic influences, however, are mostly overlooked in the application of environmental management practices. Fortunately, nematode community structures can be analysed in order to serve as ecosystem health indicators. The Wonderfontein Cave, located in the Witwatersrand Basin mining area, suffer an influx of water from the highly polluted Wonderfontein Spruit. The objectives of this study were i) to analyse the nematode community structures present within soil, sediment, water, and guano samples from nine selected sites along a transect within the cave ii) and to relate nematodal data to the concentration levels of selected metals observed. Standard methodology was applied for the extraction and morphological identification of nematodes; substrate samples were subjected to total acid digestion and metal concentrations were measured by using an ICP-MS. The faunal functional guild analysis was used to calculate nematode community structure and enrichment indices of all substrates. CANOCO's Principal Component

Analysis (PCA) was applied to study correlations between nematode trophic groups and selected metals. Preliminary results revealed that Chromium, Copper, Zinc, and Nickel concentrations, of especially sediment samples, were significantly higher than acceptable levels as reported by aquatic invertebrate quality guidelines. Nickel for example was elevated 65 times above the guideline's threshold effect concentration. The nematode faunal analysis revealed that only single substrates (sediment and soil) from five sites host both enriched and structured communities, while the PCA indicated that the structure of the Wonderfontein Cave nematode communities is influenced by metal concentrations.

SUPPRESSIVENESS OF ORGANIC AND CONVENTIONAL FARMING SOILS AGAINST *HETERODERA AVENAE*. **Eberlein[1], C., H. Heuer[2], J. Moos[3], H.M. Paulsen[3], and A. Westphal[1].** [1]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [3]Thünen-Institute of Organic Farming, Trenthorst 32, 23847 Westerau, Germany.

Heterodera avenae induces losses on cereal crops worldwide. The aim of this study was to test if biotic soil factors affect the development of female populations of *H. avenae* in soil of different locations, crop management and cropping sequence history. Soil samples from four fields were placed in root observation boxes of 1-L capacity either untreated or heat-treated (134°C, 10 min). Three of these were from current cereal fields, one from a field with a history of limited frequency of cereal in the rotation sequence served as a control. Each box was planted to six seeds of nematode-susceptible *Hordeum vulgare* 'Hanka' and inoculated with 10,000 J2 of *H. avenae*. With first appearance, nematode females visible on the root surface were counted weekly. Maximum female numbers were detected from the second week of observation. Thereafter, numbers remained at a similar level. At all monitoring times, the numbers of females in the untreated soil were less than those in heated soil, indicating that a biotic component negatively affected *H. avenae* females on the roots. The highest number of females was found in the control throughout the monitoring period. Soils from conventional or organic fields and from the cereal monoculture all had similar female numbers. These soils with some suppressive capacity had regular cropping of the host plant in common differentiating them from the control soil. This suggested that regular cropping of a host plant may increase suppressiveness. The cause of these differences in female populations warrants further investigation.

LIFE CYCLE OF *GLOBODERA ROSTOCHIENSIS* AND *GLOBODERA PALLIDA* ON EARLY POTATO CULTIVARS: TIMES ARE CHANGING. **Ebrahimi[1,2], N., N. Viaene[2,3] and M. Moens[1,2].** [1]Laboratory for Agrozoology, Ghent University, Coupure links 653, 9000 Ghent, Belgium; [2]Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium; [3]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium.

Potato cyst nematodes (PCN) are the most economically important nematode pests of potato. Early harvesting is part of a preventive management approach for very early potato varieties in Belgium. Tubers are harvested before June 20 because it is assumed that no cyst formation occurs by that date. However, this assumption might not be valid anymore because of climate changes, the availability of new cultivars (with new traits), and the increasing prevalence of *G. pallida*. Therefore, pot and field trials were conducted to verify the date when first cysts appear in Belgian growing conditions. The development of different populations of both species on 3 potato cultivars, *viz.* Eersteling (susceptible to PCN), Première (resistant to *G. rostochiensis*) and Ambassador (partially resistant to *G. pallida*) was monitored in a growth chamber with simulation of seasonal field temperatures. On Eersteling and Première, males, females and cysts of *G. pallida* were found 8, 9 and 11 weeks (May 15), respectively, after soil infestation with cysts, when the accumulated degree-days (DD) were 261, 322 and 450. No *G. pallida* females were observed on Ambassador. Males, females and cysts of *G. rostochiensis* were found 10, 10 and 12 weeks (May 22) after inoculation, respectively, corresponding with 292, 292 and 398 DD. Observations in two fields and in microplots under prevailing weather conditions in 2013 revealed that cysts of both *Globodera* spp. were formed on June 12. Our observations show that both species of *Globodera* develop earlier than was assumed based on previous data.

DEVELOPMENT AND VALIDATION OF A QUANTITATIVE ASSAY FOR VIABILITY TESTING OF POTATO CYST NEMATODES. **Ebrahimi[1,2], N., N. Viaene[2,3] and M. Moens[1,2].** [1]Laboratory for Agrozoology, Ghent University, Coupure links 653, 9000 Ghent, Belgium; [2]Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium; [3]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium.

The potato cyst nematodes (PCN) can be managed by a series of integrated management practices. However, knowledge of the survival of the nematodes in the field would make the evaluation of management strategies more accurate. This study aimed to develop and optimize a quantitative method to determine the number of viable eggs of PCN based on trehalose present in live eggs. Trehalose was extracted from cysts, a dilution series of eggs formed and quantified. Results showed that more trehalose was detected when cysts were crushed than when left intact. Reaction volumes needed to be adapted to the number of eggs because small reaction volumes hampered an accurate quantitative extraction of trehalose; optimally 11 eggs per μl reaction volume can be used. The detection limit was defined as 5 viable eggs. The trehalose method was compared with visual assessment and the hatching test, commonly used methods to determine cyst viability, by using cysts recovered

from soil after exposure to soil amendments (pig slurry, cattle slurry, farmyard manure, chitin compost, nitrogen fertilizer, wood chip compost alone and in combination with biochar). There was no difference between the visual assessment and the trehalose method. The visual assessment, however, is time consuming, requires expertise and could be subjective. Less viable eggs were measured with the hatching test than with the other methods. Hence, the viability assessment method based on trehalose seems to be a practical, sensitive, fast and cheap technique.

THE CHANGING SCOPE OF CROP PROTECTION. **Eckstein, K.E.** Bayer CropScience, P.O. Box 143, Isando, 1600, South Africa.

Consumers and food companies are demanding safer food which includes the requirement for crop protection products with a more favourable environmental profile, less active ingredient per ha and restriction on the Pre Harvest Intervals (PHI) of applications while increasing standards on food security and food quality. Historically nematicides have required considerable care in the application, storage, handling and transport of thereof. Furthermore the rates are high and are open for misuse. During the recent years Bayer CropScience has followed a WHO Class 1 phase out strategy resulting in the the global phase out of Temik® (aldicarb) and divestment of Mocap (ethoprophos) and Nemacur (fenamiphos). Bayer CropScience has made substantial investments in the Research and Development of nematicides and nematode suppressing compounds which are effective but at the same time meeting modern user, environmental and consumer requirements. The most recent development has been the introduction of Velum (fluopyram) as a nematicide in Zimbabwe. It not only offers superior control but also the benefit of a use rate of maximum 2 kg/ha product in comparison to 25 kg of aldicarb. Furthermore biological compounds in combination with chemical offers are becoming an important addition to integrated nematode control strategies. This includes the introducion of Votivo (*Bacillus firmus)* as the seed treatment in Poncho® Votivo® in the United States of America and South Africa. To ensure the supply of an increasing demand in safe and secure food Bayer CropScience is engaged in sustainable partnerships between the farming community, food companies and input providers such as seed and equipment companies.

HOW TO MAKE EPN USE MORE COST EFFECTIVE? **Ehlers, R.-U.** e~nema, GmbH, Klausdorfer Str. 28-36, D-24223 Schwentinental, Germany.

Biological control measures are usually higher in costs than conventional synthetic chemical products. Either biological control products find a niche to control pests for which synthetic compounds cannot provide a satisfying solution or they have to compete with chemical products. The production cost for EPN has shown a marked decrease over the last decade. Reduction of application density is one important parameter to reduce costs. Reduction in nematodes per ha can be achieved by improving application technology or use of more virulent strains or species. Important progress was made by exploiting economies-of-scale. With growing markets, production capacity has more than tripled during the last decade. Process stability is another important element to reduce production costs. Progress in production technology is reported. Other important factors are storage and transport cost. Nematode products are shipped at temperature of 4-10°C. To maintain temperature smaller batches are packed with ice and transported by courier services. Once larger quantities are handled, the use of cooled containers is possible and can help to reduce transport costs. Beneficial traits of EPN define biological limits of the products and breeding programmes can target, for instance, enhancement of storage or production potential or virulence. Thus a combination of technical and biological tools is available to improve competitiveness of EPN products.

ENTOMOPATHOGENIC NEMATODES IN BIOLOGICAL PLANT PROTECTION. **Ehlers, R.-U.** e~nema, GmbH, Klausdorfer Str. 28-36, D-24223 Schwentinental, Germany.

Biological control provides environmentally friendly and effective plant protection measures. Acceptance is steadily increasing as well as market share. Entomopathogenic nematodes (EPN) are an important element of biocontrol. In the past they have mainly been used in niche markets. During the recent years biotechnical production and downstream processing has improved and production capacities have more than tripled. Product quality has improved as well as transport logistics enabling provision of viable and effective material at the farmeŕs gate. Application technology and timing contributed to increase affectivity of EPN products. Today EPN are also applied in out-door markets. An overview will be given on major current and future EPN markets.

BIOLOGICAL CONTROL OF WESTERN CORN ROOTWORM LARVAE (*DIABROTICA VIRGIFERA VIRGIFERA)* WITH DIANEM® (*HETERORHABDITIS BACTERIOPHORA*). **Ehlers, R.-U.** e~nema, GmbH, Klausdorfer Str. 28-36, D-24223 Schwentinental, Germany.

The biocontrol product Dianem® contains entomopathogenic nematodes, *Heterorhabditis bacteriophora*. It is officially registered in Austria as a plant protection product to control larvae of the corn rootworm (*Diabrotica virgifera virgifera*). Field results from Hungary, Austria and Italy applying 2 x 10^9 nematodes/ha per ha obtained the same control as with chemical seed dressings with neonicotinoides or with application of granular insecticides containing the pyrethroid Tefluthrin. Adapted application technology has been developed to apply nematodes with 200 liters of water/ha to the seeds.

Although the insect larvae occur approximately a month later, the nematodes persist long enough to control the pest. Insects penetrate into the roots where they are not easily reached by insecticides, whereas nematodes follow the insects into the galleries and kill the larvae 2-3 days after infestation. Latest field results which have used the novel application technology will be presented. Since the seed treatment with neonicotinoides was banned by the European Commission in 2013, the correct use of the product Dianem® was demonstrated to more than 2000 farmers in Austria and nematodes will be used commercially for the first time on larger scale against this invasive maize pest.

USE OF FLUORESCENT DYES TO LOCALISE NEMATODES WITHIN ROOT TISSUE. **Ehwaeti[1], M.E.M. and V. Blok[2].** [1]Omar Al Moukhtar University, Elbedi, Libya; [2]The James Hutton Institute, Invergowire, Dundee, DD2 5DA, Scotland, UK.

Nematodes comprise a very small amount of root tissue once with the tissue and are difficult to locate, particularly in roots that have limited transparency such as potato. The aim of this work was to label juvenile nematodes with a fluorescent dye prior to root inoculation and then to examine the roots with a fluorescence microscope to identify their location with root tissues. The dyes PKH26 which fluoresces red and PKH67 which fluoresces green were used to label nematodes. Nematodes were soaked in diluted dyes for 2-4 hours prior to inoculating roots. Sprouts of three potato genotypes, Desiree, and JHI breeding lines 11415 and 12601, were placed in vermiculite for 2-3 weeks to allow the production of roots several centimetres in length. Desiree is susceptible to both *Globodera pallida* and *Globodera rostochiensis*. Breeding line 11415 has *H1* resistance to *G. rostochiensis* (Ro1) and *H3* quantitative resistance to *G. pallida*. Breeding line 12601 has *H3* quantitative resistance to *G. pallida,* but is susceptible to *G. rostochiensis*. Roots were then cleaned and transferred to petri plates containing 20ml of 20% w/v Pluronic F-127 gel. The medium was then allowed to solidify. Roots were observed with a fluorescent microscope for up to 14 days to identify where nematodes were within the root tissue. Root pieces (0.7cm) containing nematodes identified by their fluorescence, were excised using a scalpel and immediately fixed. The fixed root sections were then embedded in wax. The wax blocks were sectioned, sections were collected on microscope slides and then wax removed. These sections were observed with the fluorescence microscope. Several examples of nematodes within root tissue were observed.

COMPARISONS OF NEMATODE ASSAY LABS EFFICIENCIES AND RECOMMENDATIONS FROM TEN NEMATODE ASSAY LABS UTILIZING SAMPLES FROM TURFGRASS. **Eisenback[1], J.D. and Paula Agudelo[2].** [1]Virginia Tech, Department PPWS, Blacksburg, Virginia 24061 USA; [2]Clemson University, Clemson, South Carolina 29634 USA.

Nematode advisory programs access the damage caused by plant-parasitic nematodes to diverse crops including turfgrass. The objectives of this study were to determine the efficiency of ten nematode assay labs and to compare the recommendations for their management. Three locations were selected including the Virginia Tech Turfgrass Research Center, Augustine Golf Course in Stafford, Virginia and the Philadelphia Country Club in Gladwyne, Pennsylvania. Nematode assay laboratories from ten states, Alabama, Florida, Georgia, Louisiana, Massachusetts, Michigan, Mississippi, North Carolina, South Carolina, and Virginia processed soil from each of three sites of the bentgrass greens. Soil was thoroughly mixed and divided into 30 samples of 500 ml each and three samples from each site were mailed to each lab. They were processed using standard operating protocols and recommendations were based on the results. South Carolina reported the highest (567) counts from the Virginia Tech Turfgrass Research Center and Louisiana reported the lowest (13). Only Alabama and North Carolina suggested a possible problem caused by root-knot nematodes, even though South Carolina, and Florida found higher numbers. Total plant-parasitic nematodes extracted from the Augustine Golf Course by Michigan were the highest (317) and the lowest from Louisiana (13). The highest total nematode counts for Philadelphia Country Club were reported by Massachusetts and Michigan (14,239, 11,800, respectively), and the lowest by Mississippi and North Carolina (849, 260, respectively). These nematodes were suspected to be damaging by Alabama, Massachusetts, Michigan, South Carolina, and Virginia but not Florida, Georgia, Louisiana, Massachusetts, and North Carolina. These results show that nematode counts vary from state to state, as well as recommendations regarding their potential damage to turfgrass.

HETERODERA SCHACHTI: TRANSCRIPTOME AND EFFECTOROME. **Elashry[1], A., S. Habash[1], N. Ahmadinejad[2], H. Schoof[2] and F.M.W. Grundler[1].** [1]INRES Molecular Phytomedicine, University Bonn, Karlrobert-Kreiten-Str.13, 53115 Bonn, Germany; [2]INRES Crop Bioinformatics, University Bonn, Katzenburgweg2, 53115 Bonn, Germany.

The beet cyst nematode *Heterodera schachtii* is a biotrophic sedentary nematode, which depends on a multinucleate, syncytial feeding structure. The nematode secretes effector proteins into a specifically selected host cell to induce and maintain the syncytium. We analysed the *H. schachtii* transcriptome to identify its secretome and functionally annotate it using the presence of signal peptide and the absence of transmembrane domain as filters. In this way, we identified nearly 500 putative secretory proteins specific to plant-parasitic nematodes (P-PSP). Among them, we found 24 novel candidates supported by previously known *H. schachtii* ESTs. However, these ESTs had not been identified before due to their truncated sequences. We also used the Biana Interlog Prediction server (BIPs) to identify proteins in *Arabidopsis thaliana* which putatively interact with our P-PSP. In this second approach we identified 40 putatively interacting proteins. Some of them

may play a role in pathways of metabolites that are enriched, and/or genes that are differentially expressed in *A. thaliana* after infection with *H. schachtii*. In order to further characterize candidate effector genes, we analyzed their expression level and localization during different stages of parasitism by qPCR and *in situ* hybridization, respectively. In this way we identified a number of pioneer effectors and putative interacting proteins within the host plant. A detailed analysis of the effectorome will contribute to further understand the molecular processes involved in feeding site formation and to develop novel managing systems for the control of *H. schachtii*.

DOES SOIL WATER POTENTIAL CAUSE ALLOPATRY AMONG CLOSELY RELATED STEINERNEMATID SPECIES IN FLORIDA? **El-Borai[1,2], F., N. Killiny[1] and L. Duncan[1].** [1]University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, Florida 33850, USA; [2]Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

A geospatial survey of soil properties and native entomopathogenic nematodes (EPNs) in Florida citrus orchards revealed allopatric spatial patterns between some of the dominant species. Redundancy analysis showed that soil properties related to soil water potential explained significant variability in the EPN community structure. We studied behavioral and physiological responses to soil water potential of two closely related, allopatric *Steinernema glaseri*-group species. *Steinernema diaprepesi* is a dominant EPN in deep, well drained soils of the elevated central ridge region, whereas *Steinernema* sp. has been detected only off the central ridge in orchards with shallow ground water depth. In laboratory studies we monitored the survival of both species in a variety of soils, each at water potentials ranging from saturated to permanent wilting point. The persistence of *Steinernema* sp. was greater in saturated soil than in well-drained soil, whereas *S. diaprepesi* persisted better in well-drained compared to saturated soil. Thus, the behaviours of these species in controlled assays were congruent with those that could result in the observed spatial patterns in Florida. Comparative proteomic analysis revealed differences in protein expression by each species in well-drained and saturated soil. Detected proteins are being identified by LC-MS-MS to understand mechanisms of habitat adaptation. Our data suggest the plausibility of engineering soil moisture conditions in ways that favour more desirable (effective) EPN species. Conversely, these particular behavioural traits are likely to be useful in guiding the selection of EPN species for use in different ecoregions.

STUDIES ON THE CEREAL NEMATODES, *HETERODERA* SPP. AND *PRATYLENCHUS* SPP. IN TURKEY. **Elekcioglu[1], I.H., M. Imren[2], H. Toktay[3], E. Yavuzosmanoglu-Sahin[4], E.B. Kasapoglu[1] and A. Dababad[5].** [1]Cukurova University, Faculty of Agriculture, Department of Plant Protection, Adana, Turkey; [2]Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection Bolu, Turkey; [3]University of Nigde, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey; [4]Karamanoglu Mehmetbey University, Vocational School, Department of Plant and Animal Production, Karaman, Turkey; [5]CIMMYT (International Maize and Wheat Improvement Centre), Wheat Improvement Program, Ankara, Turkey.

Cereal cyst nematodes and root lesion nematodes are economically important in wheat production systems worldwide. *Heterodera filipjevi* is widely distributed in the Central Anatolia and East Anatolia whereas *H. avenae* and *H. latipons* were found in the eastern Mediterranean and south-eastern Anatolia region of Turkey. *Pratylenchus thornei* and *P. neglectus* are the predominant species of root-lesion nematodes in central, east and south-eastern Anatolia. Cereal cyst nematodes and root lesion nematodes cause yield losses between 5-50%. The effect of temperatures on the incubation duration of cysts of *H. avenae* was investigated under *in vitro* conditions. A number of variables were assessed to optimize *P. thornei* screening including initial nematode density, inoculation volume, soil type, tube size, reference cultivars, harvest time and watering regime. Growth room experiments showed clear differentiation between the resistance and susceptible cultivars after 9 weeks in sandy growth medium (70:29:1 sand, field soil and organic matter) with small tubes (15x100 mm) inoculated with 400 cultured *P. thornei*/plant. All *H. avenae* populations collected from south-eastern Anatolia were found to belong to Ha1 group Ha21 pathotype. The efficiency of some sources of resistance (*Cre*R, *Cre*1, *Cre*2, *Cre*3, *Cre*7 and *Cre*8) in wheat against several *H. avenae*, *H. filipjevi* and *H. latipons* population was investigated *in-vitro* to resemble climate conditions in Turkey. *Cre*1, *Cre*3 and *Cre*7 genes induced resistance against both *H. avenae* and *H. latipons* populations. Plants with *Cre*R and *Cre*8 genes revealed resistance against *H. filipjevi*. However, no gene was found to show resistance against all three nematode species together.

STUDIES ON THE CITRUS NEMATODE, *TYLENCHULUS SEMIPENETRANS*, COBB IN THE EAST MEDITERRANEAN REGION OF TURKEY. **Elekcioğlu[1], I.H., E.B. Kasapoğlu[1], H. Toktay[2] and M. Imren[3].** [1]University of Çukurova, Faculty of Agriculture, Department of Plant Protection, 01360, Adana Turkey; [2]University of Nigde, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey; [3]Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection, Bolu, Turkey.

Turkey is one of the most important citrus-producing countries in the Mediterranean region. The citrus nematode (*Tylenchulus semipenetrans*) was found an important parasite of citrus in this region and 90 % of citrus orchards were infested with this parasite. Population densities were mostly above economic threshold. Mediterranean biotype was found

mainly in this region. Population dynamics of citrus nematode were studied in different citrus orchards in Adana condition, population reached the minimum level on December - January and the maximum level on July - August. Nematicide treatment resulted increase in orange yield (0.9 %), and the following year it reached 9.2 % although it was not statistically significant. Sour orange (*Citrus aurantium* L.) known susceptible to this pest is commonly used as rootstocks in this region. To determine the resistance of citrus rootstocks to *T. semipenetrans* different rootstocks were investigated in the collection orchard of University of Cukurova, Faculty of Agriculture and roots and soil from 34 sour orange clones (Tuzcu clones), *C. obovoidea, C. ampullaceae, C. sulcata, C. taiwanica, C. volkameriana*; *Poncirus trifoliata,* Troyer citrange, Carrizo citrange, Kleopatra mandarin and Yuzu rootstocks were examined. Citrus nematodes were found in different densities in all samples taken from roots and rhizosphere of the different rootstocks and were also found in the roots of *Poncirus trifoliata,* Carrizo sitrange and Troyer citrange which are known as resistant or tolerant, from previous studies. In order to reach more definitive conclusions on this subject, all of these rootstocks should be screened under controlled conditions.

NEMATODES ASSOCIATED WITH GREENHOUSE BANANA PRODUCTION IN TURKEY. **Elekcioğlu, I.H., G. Yoraz and E.B. Kasapoğlu.** University of Çukurova, Faculty of Agriculture, Department of Plant Protection, 01360, Sarıçam, Adana Turkey.

Commercial banana production is well established in the subtropical regions of Turkey. The adoption of banana production under protection has increased production and productivity. In Anamur and Bozyazı, protected cultivation is common, with open cultivation widely used in Alanya and Gazipasa. In 2008, the production areas increased to 40 000 da and production increased to 200 000 tons, with accompanying high quality fruit. Root-knot nematodes and *Helicotylenchus* spp. cause economic losses in banana production. These nematodes cause damages by feeding directly on roots and also by inducing soil-borne diseases. Population dynamics of nematodes were examined in Bozyazı district of Mersin province in a greenhouse between 2011 and 2013. During this period, soil samples were collected each month, and the nematodes extracted. Plant parasitic nematodes were identified to species level and population densities determined. *Helicotylenchus multicinctus, Helicotylenchus dihystera, Meloidogyne incognita* and *Meloidogyne javanica* were identified, with *H. multicinctus* the most common. In general, the population density of *H. multicinctus* declined in December and January and increased in March. *Helicotylenchus dihystera* was not common in the study area with root-knot nematodes also not observed in high numbers.

VBC-90017: A NEW BIORATIONAL NEMATICIDE FOR THE CONTROL OF PLANT-PARASITIC NEMATODES. **Eldridge, R.** Valent BioSciences Corporation, 870 Technology Way, Libertyville, IL 60048, USA.

VBC-90017 is a new, novel, biorational nematicide under development by Valent Bio-Sciences Corporation. VBC-90017 has a number of user friendly features including a liquid EC formulation, application flexibility, and an excellent toxicity profile. Initial field efficacy studies show nematode gall reduction, population suppression and subsequent positive plant responses in potato, tomato, cucumber and tobacco. Field development trials conducted on a range of crops, and the unique features and benefits of this new material are discussed.

ROOT-LESION NEMATODES, *PRATYLENCHUS* SPP., PARASITIZING POTATO IN PORTUGAL. **Esteves[1], I., C. Maleita[2] and I. Abrantes[1].** [1]IMAR-CMA, Department of Life Sciences, FCTUC, University of Coimbra, 3004-517 Coimbra, Portugal; [2]CIEPQPF, Chemical Engineering Department, FCTUC, University of Coimbra, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal.

Root-lesion nematodes *Pratylenchus* spp., are widespread and economically important parasites of several cultivated plants, including potato. In Portugal, potato cyst nematodes, *Globodera* spp., are a frequent problem in main production areas and root-knot nematodes, *Meloidogyne* spp., have been found coexisting with potato cyst nematodes. Although the diversity and pathogenicity of potato cyst nematodes are well documented, little work has been done to assess the importance of *Pratylenchus* spp. to potato. This work aimed to assess the diversity of root-lesion and other plant-parasitic nematodes in potato crops. Sampling encompassed fields in northern, central and southern regions of Continental Portugal. Nematodes were extracted from 42 soil and root samples, using standard extraction techniques, identified to genus level and population densities quantified. The identification of Pratylenchus spp. was based on the diagnostic morphological characters, species specific primers and D2D3 28S rDNA sequencing. Root-lesion nematodes were detected in all regions, except in the south, where nematodes were only found in the soil. *Pratylenchus* spp. were identified in 83% and 79% of soil and root samples, respectively. Meloidogyne spp. and potato cyst nematodes were also found in roots infected with root-lesion nematodes. *Pratylenchus penetrans* was the most abundant species followed by *Pratylenchus neglectus, Pratylenchus crenatus* and *Pratylenchus thornei*. The information obtained on the ubiquity and diversity of root-lesion nematodes reinforced the need for a careful monitoring of these nematodes in potato crops. Further studies are being conducted to assess the pathogenicity of the Pratylenchus spp. on common commercial potato cultivars.

CHARACTERISATION OF NOVEL SECRETED PROTEINS AND THEIR POTENTIAL ROLE IN CYST NEMATODE PARASITISM. **Eves-van den Akker[1,2], S., C.J. Lilley[2], J.T. Jones[1] and P.E. Urwin[2].** [1]The James Hutton Institute, Invergowrie, DD2 5DA, Scotland; [2]University of Leeds, Leeds, LS2 9JT, England.

Plant parasitic nematodes comprise several groups; the most economically damaging of these are the sedentary endoparasites, causing losses to agriculture worth in excess of £75 billion per year. Sedentary endoparasitic nematodes are obligate biotrophs and modify host root tissue, using a suite of effector proteins, to create a feeding site that is their sole source of nutrition. Genes potentially involved in the feeding process of the potato cyst nematode, *Globodera pallida,* have been identified through analysis of life-stage specific transcriptome sequences mapped to its recently assembled draft genome. Differential expression analysis coupled with signal peptide and transmembrane prediction enabled identification of a range of novel, putatively secreted proteins. A role in cyst nematode feeding has been implicated for one gene family whose members were found to be highly diverse between individuals of the same population. *In situ* hybridisation was used to demonstrate expression of this gene family in the amphid sheath cells of parasitic stages. These cells service the amphids, structures that may be the origin of the feeding plug. Secretion of the encoded proteins has been confirmed using immunochemistry to localise them in host potato roots at the plant-nematode interface. Despite the variation in gene sequence between individual nematodes, *in planta* expression of double stranded RNA targeting a conserved region of the gene family results in approximately 80 % reduction in total nematode infection. Representatives of this gene family have been identified in other cyst nematode species but not in root-knot nematodes.

MELOIDOGYNE INCOGNITA INFECTED *CORCHORUS OLITORIUS*: DRAMATIC EFFECT OF DIFFERENT EXTRACTS OF *EUCALYPTUS OFFICINALIS*. **Fabiyi, O.** Department of Crop Protection, Faculty of Agriculture University of Ilorin, Ilorin, Nigeria.

This study investigated the nematicidal properties of various fractions from *Eucalyptus officinalis* obtained by different methods of extraction. The fractions were partially characterized using Fourier transform infra-red (FT-IR) and gas chromatography mass spectroscopy (GCMS) analysis. The constituent of *E. officinalis* as revealed by the GCMS result include eucalyptol (19.3%), α-pinene (3.8%), citronellol (4.2%), α-terpeneol (39.2%) and 1, 8-cineole (33.5%). The FT-IR spectral data obtained also supported the finding that 1,8 cineole and α terpeneol were the main constituents of the *E. officinalis* fractions. The fractions obtained from the methanol extract chromatographed on silica gel 100-120 mesh grade produced significantly ($p<0.05$) higher nematicidal activity with 40.7% mortality as against 23.6% recorded for the n-hexane partition of the aqueous extract. This was followed by dichloromethane partition of the residual aqueous extract and n-hexane crude extract filtered on aluminium oxide column which had 35.1% and 30.1% mortality respectively. In field trials, the chromatographic fraction of the methanol extract (MeOH/si-gel/cc_1 and MeOH/si-gel/cc_2) produced significantly ($p<0.05$) higher plant height, number of leaves and branches. *Corchorus olitorius* that were treated with synthetic nematicides and the chromatographic fractions were significantly taller and with more leaves per plant. No galling or *M. incognita* were observed on the treated plants in comparison with the untreated control. Few nematodes were recovered from the soil treated with the fractions. Biggest growth stimulation was observed in *C. olitorius* treated with high concentration methanol extract chromatographic fractions.

A NEW SPECIES OF *CAMPYLAIMUS* (DIPLOPELTIDAE) FROM THE DEEPEST NORTHWESTERN PART OF THE SEA OF JAPAN. **Fadeeva[1], N.P., V.V. Mordukhovich[1] and J.K. Zograf[2].** [1]Far Eastern Federal University, 27 Oktyabrskaya St., Vladivostok, 690600, Russia; [2]A.V. Zhirmunsky Institute of Marine Biology FEB RAS, 17 Paltchevsky St., Vladivostok, 690041, Russia.

Undescribed free-living marine nematodes were found during surveys of the biodiversity and community patterns of the meiobenthos in the deepest north-western part of the Sea of Japan. This is the first report of three *Campylaimus* species from this region. Overall, the genus of *Campylaimus* is characterized by a complex taxonomic history with a large number of synonyms and *species inquirendae*. The Sea of Japan material allowed us to investigate intra-specific variability, using both scanning electron microscopy and light microscopy and to discover amphidial fovea as diagnostic morphological characters. Three species that was previously undescribed were found, namely *Campylaimus orientalis, Campylaimus amphidialis* and *Campylaimus minutus*. They differ from other species of *Campylaimus* by the shape, length and position of the amphideal fovea, minute body size and shape of the spicule and gubernaculum. A report of the biogeography of the different species of *Campylaimus* is presented.

TAXONOMY AND BIOGEOGRAPHY OF *CURVOLAIMUS* WIESER, 1953 (ENOPLIDA) WITH DESCRIPTION OF NEW SPECIES. **Fadeeva[1], N.P., V.V. Mordukhovich[1] and J.K. Zograf[2].** [1]Far Eastern Federal University, 27 Oktyabrskaya St., Vladivostok, 690091, Russia; [2]A.V. Zhirmunsky Institute of Marine Biology FEB RAS, 17 Paltchevsky St., Vladivostok, 690041, Russia.

The deep-sea nematofauna remains very poorly known at both global and regional scales. Information on the deep-sea free-living nematode species of the northwestern Pacific remains extremely limited. Examination of material recently

collected by the German-Russian KuramBio (Kuril-Kamchatka Biodiversity Study) deep-sea expedition to the abyssal plain adjacent to the Kuril-Kamchatka Trench on board of RV *Sonne*, conducted in summer 2012, has revealed new species of the genus *Curvolaimus*. Currently, the genus *Curvolaimus* contains 5 species, mainly found in shallow waters. The morphology and morphometry of *Curvolaimus belogurovi* is examined using light and scanning electron microscopy. *C. belogurovi* can be easily distinguished from all known congeneric species in having a combination of the following features: greater body and spicules and spicule length to anal body diameter ratio. The photomicrographs of the new species by scanning electron microscopy (SEM) are presented with a detailed morphological description. A conception on the biogeography of this genus at species level is presented.

INTEGRATED NEMATODE MANAGEMENT IN URBAN AGRICULTURE. **Fernández[1], E., H. Gandarilla[2] and R. Cuadra[3].** [1]Instituto de Investigaciones de Sanidad Vegetal (INISAV), Calle 110 # 514 e/ 5taB y 5taF. Miramar, Playa. La Habana Cuba; [2]Laboratorio Central de Cuarentena Vegetal (LCCV), Calle Ayuntamiento e/San Pedro y Lombillo, Plaza, La Habana, Cuba; [3]Instituto de Investigaciones Fundamentales en Agricultura Tropical. Santiago de las Vegas, La Habana, Cuba.

For the last 15 years Cuba has developed low input production system for different food crops in urban and peri-urban areas. Some insects, mites, fungi, bacterium, virus and nematodes are important pests under these conditions. Cuban research institutions have studied the host range and distribution of plant parasitic nematodes associated with the main crops produced in urban and peri-urban environments. These studies are included, in the official Integrated Nematode Management program. Different plant-parasitic nematodes including root-knot, burrowing, lesion and spiral nematodes are associated with coffee, vegetable, tuber root and fruit crops. Management alternatives that are less harsh environmentally have been incorporated in various different production systems. The most important management alternatives are the use of healthy plant material, soil tillage, crop rotation, use of trap crops, organic matter, biofumigation and, biological control agents (strains of *Trichoderma virides and Trichoderma harzianum, Bacillus thuringiensis, Pochonya chlamydosphoria*). The Integrated Nematode Management system includes also the use of good agricultural practices that had been improved by training technicians and producers.

NEMATODE ECOLOGY AND SOIL HEALTH. **Ferris, H.** Department of Entomology and Nematology, University of California, Davis, CA 95616, USA.

A healthy soil provides desired ecosystem services. It has interacting physical, chemical and biological components. The productivity of systems often has been developed through focus management of chemical and physical components with minimal consideration of the biological component, which has resulted in unhealthy soils. Once conditions are such that a desired function is not performed by any of the contributing species, the soil is no longer healthy relative to that function. Biodiversity increases the amplitude of each function and consequently the health of the soil. Nematodes are both direct contributors and indicators of the contributions of other organisms to soil health. Nematode assemblages indicate three attributes of the biological component of the soil: the nature of ecosystem services available (faunal analysis); the magnitude of the services (metabolic footprints); and the complementarity of services across microhabitats (functional diversity). Functional guilds are comprised of species that contribute similarly to an ecosystem service. For example, nematodes in decomposition food web channels can be assigned to functional guilds based on the nature of their prey (bacteria or fungi) and life course characteristics. Their species diversity can be partitioned into the diversity of guilds and within-guild diversity. Diversity of guilds provides a measure of continuity of ecological services as conditions change; within-guild species diversity ensures that the service is provided across differences in the physical and chemical nature of the habitat. Current and anticipated advances in molecular techniques for determination of nematode abundance, diversity and function will facilitate application of bioindicator-based measures of soil health.

NEMATODE BIOSECURITY IN AMENITY TURF AND AGRICULTURE. **Fleming[1], T., K. Entwistle[2], M. McClure[3], A.G. Maule[1], T. Martin[1,4], M. Hainon-McDowell[4] and C.C. Fleming[1,4].** [1]Molecular Bioscience-Parasitology, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, UK; [2]The Turf Disease Centre, Waverley Cottage, Sherfield Road, Bramley, Hampshire RG26 5AG, UK; [3]Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA; [4]Agri-Food and Biosciences Institute, Belfast, UK.

Plant parasitic nematodes are significant pests across a range of economically important sectors. The importance of effective biosecurity and the threat from emerging nematode pests are of increasing concern to nematologists and of real significance to plant health policy makers across many countries. Serious levels of nematode damage in golf greens and sports pitches have been linked to the movement and use of contaminated construction and planting materials and often heralds the emergence of similar problems in agriculture. The recent discovery on an Irish golf course of *Anguina pacificae*, an aggressive turf-grass pest previously only identified in Northern California, raises the debate on whether current international biosecurity methods are adequate and/or adhered to. Additionally, results from a survey assessing the distribution and occurrence of the nematodes present in Northern Irish agricultural land will be presented. These data will highlight the

emergence of nematode pests such as *Meloidogyne minor* and indicate factors which may be important in driving climate induced changes in nematode populations.

ENTOMOPATHOGENIC NEMATODES IN OLIVE ORCHARDS IN THE REGION OF CABORCA, SONORA, MEXICO. **Flores-Lara, Y., B. Arias-Tobin, B. Rivera-Orduño, E.P. Delgado-Quintanar.** Grupo disciplinario de Biotecnología agrícola y Ambiental, Universidad de Sonora Unidad Regional Norte Caborca, Caborca, Sonora, México.

Entomopathogenic nematodes (EPNs) are lethal to insects while safe for plants and animals. *Heterorhabditis sonorensis* was recently identified in asparagus fields in the region of Caborca, Sonora, Mexico, but there are no further reports of EPNs in other crops or regions of the Sonora state. Our main goal in this study was to determine the presence of EPNs in olive orchards in the Caborca region. We identified and characterized EPN isolates as well as other environmental factors such as soil environment and vegetation. Eight samples per site were taken from a total of 12 olive orchards in the region. Each of these eight samples was the result of 5 sub-samples, mixed, and placed containers at sampling. In the laboratory, the EPNs were extracted using the bait method. The weeds found at each sampling site were identified. Chemical and physical soil characteristics such as pH, organic matter and texture were also determined. Caborca, Sonora is one of the most important agriculture regions in Mexico, providing mass quantities of asparagus as well as olives for export. Both crops however are currently experiencing soil pest problems that have been limiting production factors. The use of integrated pest management control including the application of native EPNs would be a key factor in the successful control of these pest problems.

EFFECTS OF VERMICOMPOST ON BENEFICIAL NEMATODES, *RHABDITIS* SPP. **Foley, D., S.P. Marahatta and J.-H. Lau.** Kauai Community College, University of Hawaii, Hawaii, USA.

Rhabditis spp. are beneficial bacterivorous nematodes that play an important role in nutrient cycling. High numbers of *Rhabditis* nematodes both encourage and are an indicator of particularly nutrient-rich soil environments. Vermicompost, a soil enhancer, is widely used for its micronutrient qualities and known for contributing to the beneficial soil microorganism habitat, especially by organic farmers. Results, however, on how the addition of vermicompost directly benefits soils have been anecdotal in nature, lacking scientific field trials. Can vermicompost be used to create a soil habitat conducive to enhancing the number of *Rhabditis* nematodes? A laboratory experiment was conducted to find out how different quantities of vermicompost on crop planted soils of Hawaii affect *Rhabditis* nematode populations. Vermicompost was mixed to field soil at rates of 0%, 0.5%, 1.0%, and 2.0% and kept in a controlled environment. After one week, nematodes were extracted using the Baermann Funnel technique and identified. *Rhabditis* nematodes were counted under an inverted microscope and results were analyzed. Compared to other treatments, soil with a 1% application of vermicompost enhanced the number of *Rhabditis* ($P < 0.05$), while differences between treatments of 0%, 0.5%, and 2% were non-significant ($P > 0.05$) on *Rhabditis* numbers. By incorporating 1% vermicompost into crop planted soil, farmers can encourage *Rhabditis* nematode populations and improve soil health.

MOLECULAR BASIS OF PINEWOOD NEMATODE PATHOGENICITY: TRANSCRIPTOMIC AND PROTEOMIC STUDIES. **Fonseca[1], L., J. Cardoso[1], F. Leisico[2], P. Gomes[2], M. Pinheiro[2], I. Abrantes[1] and C. Egas[2].** [1]IMAR-CMA, Department of Life Sciences, University of Coimbra, 3004-517 Coimbra, Portugal; [2]Advanced Services Unit, Biocant, 3060-197, Cantanhede, Portugal.

During the last years, progress has been made to understand the pathogenicity of the pinewood nematode *Bursaphelenchus xylophilus*. The main objectives of this study were to identify gene(s)-encoding *B. xylophilus* pathogenicity related proteins and to detect specific pinewood nematode proteins. The transcriptomic studies by pyrosequencing revealed the expression of putative pathogenicity genes: cellulases, expansin, pectate lyase, VAPs, calreticulin, annexin, ubiquitin, chitinase, SK1 and RING genes. In addition, the transcriptomes of different developmental stages grown on fungus and pine trees and *Bursaphelencus mucronatus* on fungus were obtained. Differences on oxidative detoxification, phenolic compound degradation, signalling pathways and host molecule recognition were detected in the *B. xylophilus* developmental stages grown on fungus and pine. Comparing with *B. mucronatus*, four functional gene groups were identified only in pinewood nematodes: G protein coupled receptors (GPCR), fatty acid metabolism, response to stimuli and nervous system genes. These gene products may improve pinewood nematode survival and propagation inside the tree as compared to non-pathogenic species. Protein profiles of *B. xylophilus* grown on fungus and pine and of *B. mucronatus* on fungus were also analysed by two-dimensional gel electrophoresis. The majority of protein spots were present in all protein extracts, with slight variations in the intensity corresponding to proteins that could be differentially expressed in both species in different environmental conditions. Further studies are being conducted by liquid chromatography – mass spectrometry analysis. This study expanded the knowledge on the nature and function of gene(s) encoding of the pinewood nematode pathogenicity related proteins which could be targeted for control strategies.

THE STATUS, MILESTONES AND CHALLENGES OF NEMATOLOGY RESEARCH IN SOUTH AFRICA. **Fourie[1], H., S. Berry[2], R. Knoetze[3], A. Swart[4], M. Marais[4], A.P. Malan[5] and S. Steenkamp[6].** [1]North-West University, Unit for Environmental Sciences and Management, Private Bag X6001(Internal Box 264), Potchefstroom 2520, South Africa; [2]BASF

Agricultural Specialities (PTY) Ltd, PO Box 15132, Ashwood, 3605, KwaZulu Natal, South Africa; [3]Directorate Inspection Services, Department of Agriculture, Forestry and Fisheries, Private Bag X5015, Stellenbosch 7599, South Africa; [4]Nematology Unit, Biosystematics Programme, Agricultural Research Council-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa; [5]Stellenbosch University, Department of Conservation Ecology and Entomology, Privaatsak X1, Matieland 7602, Stellenbosch, South Africa; [6]Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa.

Nematology research in South Africa expanded substantially since its inception in the 1900's. Milestones achieved are the establishment of the local nematology society, numerous publications on terrestrial phyto- and aquatic nematodes and also human and animal parasites, collaborative international networking (e.g., quarantine diagnostic protocols for *Ditylenchus destructor, Ditylenchus dipsaci, Aphelenchoides ritzemabosi, Aphelenchoides fragariae* and *Aphelenchoides besseyi*), increased use of the adapted NaOCl extraction technique, identification of *Aphelenchoides arachidis* in groundnut kernels and *Meloidogyne enterolobii* in guava and vegetable roots, description of new speciels including five new EPN and three *Globodera* spp., novel research on controlling false codling moths using EPN's in citrus, registration of *Heterorhabditis bacteriophoa* for commercial use and use of improved molecular and morphological techniques to identify nematodes. Furthermore, the National Collection of Nematodes is now digitized with 200 000 specimens listed. Major challenges facing nematologists include innovative solutions to manage phytonematodes, the conducive effect of current cropping systems to nematode pest build-ups, limited/ lack of genetic host plant resistance sources and the devastating effects of seed-leaf and –gall nematodes on grass-seed production and *Ditylenchus africanus* on the dwindling groundnut industry. The role and effect of non-parasitic nematodes as bio-indicators of soil quality receives priority, while nematologists are also involved in Plant Health Clinics. Challenges remaining include optimising the effect of biological as well as cover crops as management options, implementation of conservation agriculture and its effect on existing nematode assemblages and effects of climate change on nematodes. Nematology research is growing with leaps and bounds and should be funded accordingly by decision makers and administrators.

NEMATOLOGY TRAINING AND EDUCATION IN SOUTH AFRICA: STATUS, OPPORTUNITIES AND FUTURE PROSPECTS. **Fourie, H. and A. Mc Donald.** Unit for Environmental Sciences and Management, North-West University, Private Bag X6001 (Internal Box 264), Potchefstroom 2520, South Africa

Since the inception of plant nematology research in South Africa, training of students and/or interested persons has received priority. At present, Nematology training and education are done both formally and informally at graduate and postgraduate levels by several local universities and institutions. These include the North-West University (North West Province), University of Stellenbosch (Western Cape Province), University of Pretoria (Gauteng Province), University of KwaZulu-Natal (KwaZulu-Natal Province), University of the Free State (Free State Province) and University of Limpopo (Limpopo Province) that are located strategically across the country. Formal training at these universities is generally aimed at local and international students at Honours-, Masters- and PhD levels with progressively more emphasis on execution of multi-disciplinary projects. Plant and/or Insect Nematology are, however, also presented to graduate students at selected universities. Collaboration with international institutions manifests in training postgraduate students by means of internationally-funded projects and initiatives such as EUMAINE. Another formal training intervention is accredited Nematology short courses that are presented by the North West University, University of KwaZulu-Natal and University of Stellenbosch, being attended by non-graduates, graduates, as well as post-graduates. Informal training is aimed at in-house education of personnel at various institutions where Nematology research and diagnostic laboratories exist. Endeavours to raise awareness about nematodes is, on the other hand, continuously undertaken at various platforms to ensure that e.g. commercial and developing farmers, prisoners and school children are exposed to the importance and impact of nematodes on crop production and soil quality.

EFFECTS OF FURFURAL AND OTHER SUGARCANE BASED TEST PRODUCTS ON *MELOIDOGYNE* SPECIES. **Fourie[1], H., W.J. van Aardt[1], C. Venter[1] and L.R. Tiedt[2].** [1]Unit for Environmental Sciences and Management, North-West University, Private Bag X 6001 (Internal Box 264), Potchefstroom 2520, South Africa; [2]Laboratory for Electron Microscopy, North-West University, Private Bag X 6001, Potchefstroom 2520, South Africa.

Limited knowledge about the mode of action of furfural-based products, such as MultiGuard Protect®, Crop Guard® and other sugarcane-based test products exist. Second-stage juveniles (J2) of *Meloidogyne javanica* and *Meloidogyne incognita*, respectively, were suspended in 2 ml-aliquots of various concentrations of these products. Data on J2 motility, mortality, ultrastructure and specific oxygen consumption rate were subsequently recorded *in vitro*. Moreover, *M. javanica* J2 that were exposed for 96h to different concentrations of one of the test products were inoculated on roots of tomato seedlings (cv. Rodade) in a greenhouse. Untreated controls, consisting of sterilised tap water were included in all trials and resulted in a significant ($P \geq 0.05$) number of motile J2 (92 to 97 %) being recorded in laboratory trials. Conversely, significantly higher numbers of J2 were immotile after suspension in the different products, especially 48h onwards. Staining of immotile J2 with a Trypan blue solution resulted in < 10% being identified as dead after suspension in different product concentrations for 96h.

Scanning electron microscopy results indicated that lateral line structures of J2 suspended in the highest Crop Guard® concentrations had a ''sunken'' appearance. Ultimately, $\dot{M}O_2$ measurements confirmed that the respiratory physiology of J2 was adversely affected after suspension in Crop Guard®. Reproduction of *M. javanica* on the susceptible tomato cultivar Rodade resulted in significantly lower numbers of eggs and J2 being obtained for the two highest concentrations of the particular test products compared to those for the lowest concentrations. Results demonstrated that two of the test products as well as MultiGuard Protect® and Crop Guard® had an adverse effect on root-knot nematode J2 used in these experiments.

HOST STATUS OF FRUIT PLANTS TO *MELOIDOGYNE ENTEROLOBII*. **Freitas[1, 4], V.M., V.R. Correa[1,2], M.D.G. Carneiro[1], J.G. Silva[1], C.B. Gomes[3], J.K. Mattos[4], L. Somavilla[3] and R.M.D.G. Carneiro[1].** [1]Embrapa - Recursos Genéticos e Biotecnologia, C. P. 02372, 70849-979 Brasília, DF, Brazil; [2]Depart. de Fitopatologia, Universidade de Brasília, 70910-900, Brasília, DF, Brazil; [3]Embrapa Clima Temperado, C.P. 403, 96001-970 Pelotas RS, Brazil; [4]Fac. Agronomia e Veterinária, Universidade de Brasília, 70910-900, Brasília, DF, Brazil.

Meloidogyne enterolobii (= *M. mayaguensis*) has been reported in various states of Brazil causing severe damage in commercial guava plantations. The host suitability of nineteen fruit species of economic importance in Brazil were evaluated against *M. enterolobii*. Plantlets of different species grown in plastic bags were inoculated with 10,000 eggs of *M. enterolobii* per plant. The plants were evaluated for resistance to *M. enterolobii* three to eight months after inoculation, considering the fresh root weight plants, gall index, egg mass index and the nematode reproduction factor (RF = final population/initial population). Ten banana (*Musa* spp.), six barbados cherry (*Malpighia* spp.), one fig fruit (*Ficus carica*), two grape (*Vitis* spp.) and six melon (*Curcumis melo*) genotypes were considered susceptible to *M. enterolobii*, as well as one papaya (*Carica papaya*) genotype (RF>1). Açaí (*Euterpe oleraceae*), atemoya (*Annona cherimola*), avocado (*Persea americana*), cashew nut (*Anacardium occidentale*), citrus (*Citrus* spp.), coconut (*Cocos nucifera*), grape rootstocks, jabuticaba (*Plinia trunciflora*), mango (*Mangifera indica*), mulberry fruit (*Morus alba*), passion fruit (*Pasiflora* spp.), sapodilla (*Manilkara zapota*), soursop (*Annona muricata*), starfruit (*Averrhoa carambola),* olive (*Olea europaea*) and strawberry (*Fragaria x ananass*) were considered as non-hosts or poor hosts to the nematode. These species may be planted in crop rotation in areas infested by *M. enterolobii*.

HYPERSPECTRAL SENSOR TECHNIQUES AND POPULATION MODELLING OF *HETERODERA SCHACHTII* FOR ASSESSING THE SPATIO-TEMPORAL DYNAMICS OF NEMATODE INFESTATION IN SUGAR BEET VARIETIES UNDER FIELD CONDITIONS. **Fricke[1], B., K. Schmidt[2], M. Daub[3], H. Goldbach[1].** [1] INRES-Plant Nutrition, Faculty of Agriculture, Bonn University, Germany; [2] Nemaplot, Bonn, Germany; [3] Julius Kühn Institute, Plant Protection in Field Crops and Grassland, Elsdorf, Germany.

Crop losses in sugar beet due to *Heterodera schachtii* infestation are of major concern in many areas. Sustainable crop management provides resistant and tolerant varieties against pathogen infestation. The present study aims at monitoring the infestation process and to characterize specific phenotypic traits of different sugar beet cultivars related to nematode damage under field conditions. The application of hyperspectral sensor techniques allows non-invasive monitoring of the leaf canopy. Assessing hyperspectral signatures from canopy reflection is used to describe the stress response of the plants. A high spectral resolution, however, creates massive amounts of data. Thus, the classification of vegetation vitality by different spectral vegetation indices from ratio of selected wavebands is used to reduce the amount of sensor information. Choosing the most appropriate wavebands to correlate with plant physiological parameters may then facilitate data collection and handling. It bears, however, the risk of losing important information. In a novel approach, however, the entire spectral range of canopy reflection is considered. The classification by the Nemaplot® model is based on two steps. First, the model is fitted to the hyperspectral signature by transforming the wavelength information to specific numerical parameters. Second, these numeric parameters are interpreted with a discriminant analyses in order to classify the signature which correlates best with nematode infestation. The stress answer of sugar beets over the course of the day and across a season was sampled and data are presented where hyperspectral information is used to describe *Heterodera* population dynamics.

NEMATODES ASSOCIATED WITH EDAMAME, *GLYCINE MAX* L. (MERR.) IN ARKANSAS, UNITED STATES. **Fultz, J., and T. Kirkpatrick.** University of Arkansas, Fayetteville, Arakansas 72701, USA.

Edamame, also known as vegetable soybean, *Glycine max*, was introduced to the United States from Japan in 1890 and has been growing in popularity as a high-fibre, low-sugar snack in recent years. In 2012, American Vegetable Soybean and Edamame, Inc. established the first commercial processing plant in the United States near Mulberry, Arkansas. About 2,000 acres of edamame are now being grown annually in the state. Since edamame is harvested as an immature seed, management practices vary from those used for conventional soybean. Plant-parasitic nematodes, particularly *Meloidogyne incognita* (root-knot) and *Heterodera glycines* (soybean cyst) known to be wide-spread in Arkansas, are pests of concern. The objective of this study was to determine the nematodes associated with edamame in Arkansas fields, and to evaluate new breeding lines for resistance to these nematodes. Production fields of 2013 were surveyed immediately following harvest to determine the presence, identity, and relative population density of nematodes. Where they were detected, *Meloidogyne* spp. second stage

juveniles were collected and used to establish greenhouse populations on tomato and sequenced for further identification to species. Additionally, edamame lines developed by the Arkansas soybean breeding program were evaluated for host suitability to *M. incognita* and *H. glycines* in greenhouse trials. All lines were good hosts for *H. glycines* race 3, but *M. incognita*, race 3, reproduction was significantly lower in one of the lines. This work will aid producers and crop advisors in developing nematode management strategies for edamame production and provide insight on the potential risk of root-knot and soybean cyst nematodes to this emerging crop.

DEVELOPMENT AND ASSESSMENT OF MICROSATELLITE MARKERS FOR THE SPECIFIC IDENTIFICATION OF THE BEET CYST NEMATODE, *HETERODERA SCHACHTII*. **Gamel[1], S., A. Letort[1] and E. Grenier[2].** [1]French Agency for Food, Environmental and Occupational Health and Safety (Anses), Plant Health Laboratory, Nematology Unit, Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France; [2]INRA, UMR 1349 IGEPP, Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France.

Heterodera schachtii is known as the sugarbeet cyst nematode due to the major agronomic damage observed on sugarbeet culture. Other plant families can also be affected by this polyphagous nematode such as Brassicaceae (i.e. oilseed rape, cabbage) and Solanaceae (i.e. potato, tomato). Despite *H. schachtii*'s global presence, the species is regulated in many non-European countries. Consequently, imported crops have to be free from *H. schachtii*. In this context, the development of a reliable identification method sugarbeet cyst nematodes appears necessary both for improving agronomic strategies by preventing sugarbeet cultivation in contaminated fields and also for promoting agricultural trade of other crops. As *H. schachtii* belongs to the *Schachtii* group, the analytical method for a reliable identification of *H. schachtii* must be very specific. After sequencing numerous nematode populations and performing bioinformatic analysis, the study focused on the selection of specific and sensitive microsatellite markers for the identification the sugarbeet cyst nematode using conventional PCR. The findings showed that one primer set is specific enough when tested among 18 non target populations of *Globodera* and *Heterodera* genera of which 10 were from the *Schachtii* group (*Heterodera betae*, *Heterodera. glycines*, *Heterodera trifolii*, *Heterodera daverty* and *Heterodera ciceri*). The sensitivity of the test allowed the identification of a single juvenile of *H. schachtii*, making this method suitable for preplant analysis and import/export control.

PHASE-OUT OF METHYL BROMIDE AND NEMATODE MANAGEMENT: ACHIEVEMENTS AND FUTURE CHALLENGES. **Gamliel, A.** Institute of Agricultural Engineering, ARO, the Volcani Center, Bet Dagan 50250, Israel.

The phase-out of methyl bromide in Israel resulted in gradual reduction in methyl bromide use, until its total elimination in 2012. During the phaseout process, all the available and the registered soil fumigants were evaluated for the control of various soilborne pests and primarily nematodes. Additionally the combinations of fumigants were evaluated in order to enhance the performance of each fumigant alone. During these years few effective combinations, e.g. metham sodium and 1,3-dichloropropenfor the control of soilborne fungi and nematode were registered and are commercially applied. The quest for new fumigants yielded only dimethyl disulfide, which has been registered and is still in commercial use. The commercial application of soil solarization underwent a considerable boost following the implementation of the methyl bromide phaseout. Solarization combined with nematicide fumigants provided effective control of root knot, in the production of high cash crops, e.g. strawberries and peppers. Bottlenecks were identified during the phase out of methyl bromide; these are focused in four major areas: application problems, low efficacy with specific soilborne pests, the cost factor, and the human factor. From a technology-diffusion perspective, certain alternatives such as application of new nematicides, and their combination with solarization, although comparable to integrated pest management, are complex, often more expensive than the equivalent chemical control methods. The phaseout of methyl bromide resulted also in gradual development of nematodes, pathogens and weed populations in fields which has been treated repeatedly with methyl bromide. Root knot nematodes have spread over vast area during the last years and require additional chemical and nonchemical treatments in order to provide a platform for a healthy and productive crop. More pests are yet to emerge, and the challenges in their effective control become a more challenging task.

PASTEURIA NISHIZAWAE LIBRARY AND SCREENING THE BEST STRAIN FOR PRODUCT DEVELOPMENT. **Gao, X., M. Doroh, S. Griswold, T. Hewlett, C. Marshall, A. Perret-Gentil and A. Santos.** Syngenta Crop Protection, 12085 Research Dr., Suite 185, Alachua, Florida 32615, USA.

We are developing a *Pasteuria* library that plays a vital role in the new product development and improvement of product performance against plant parasitic nematodes at the Pasteuria Bioscience site of Syngenta. Presently 106 strains of *Pasteuria* from 13 different genera of nematodes are available. *Pasteuria nishizawae* strains from the soybean cyst nematode *Heterodera glycines*, were used as a model for developing screening techniques. Nematode samples were collected in crop growing seasons. *Pasteuria* spores on the nematode's cuticle or inside nematode bodies were examined under inverted microscopes (640×). *Pasteuria* spp. were isolated and cultured with the standard operating procedure. *In-vitro* spores were produced through fermentation. Strains were selected based on culture assays, 24-well infection bioassays and other screening experiments. Thirty four strains of *P. nishizawae* were tested against 10 selected soybean cyst

nematode-populations in the laboratory bioassays. Pn1, Pn3, Pn5, PnS38 and PnS46 were found to have high infection capacity. These isolates were tested in growth chamber experiments conducted in small containers. Each pot was filled to 2 cm from the top of the pot with sand. Three soybean seeds were sown per container, and one thousand *H. glycines* juveniles were inoculated per pot. Infected roots were stained with Red Food Colour for visualization of nematodes two weeks after inoculation. Results from cultural assays, laboratory bioassays and greenhouse tests indicated that Pn1, PnS79 and PnS84 were promising strains for new products. The screening systems established for testing *P. nishizawae* against *H. glycines* have been used for other *Pasteuria* spp. on other nematodes.

STEINERNEMA - XENORHABDUS SYMBIOSIS AND *YERSINIA PSEUDOTUBERCULOSIS*: BROTHERS IN ARMS OR HACKING SYSTEM? **Gengler[1,2], S., A. Laudisoit[3] and P. Wattiau[1].** [1]Veterinary & Agrochemical Research Centre, Brussels, Belgium; [2]Institute of Life Sciences, Université Catholique de Louvain-la-Neuve (UCL), Belgium; [3]School of Biological Sciences, University of Liverpool, United Kingdom.

The capacity of soil invertebrates to act as intermediary hosts was the starting question of our study and entomopathogenic nematodes (EPN) were investigated in this respect. We wondered whether notorious mammalian pathogens taxonomically related to *Xenorhabdus*, were able to ''hack'' the symbiotic relationship associating *Xenorhabdus* and *Steinernema* EPN. Our previous results showed that *Yersina pseudotuberculosis*, that causes gastroenteritis in mammals, can survive during several generations of the EPN life cycle *in vitro*. Genetic determinants that allowed *Y. pseudotuberculosis* to survive inside EPN are currently under study. The potential implication of the recently discovered type 6 secretion system components as well as yplA phospholipase involved in survival within the insect host is systematically investigated. Deletion mutants obtained so far show promising results since an yplA mutant is unable to survive inside EPN. At the same time, we are investigating whether *Y. pseudotuberculosis* really plays a symbiotic role in EPN or if it just hacks the system. To deal with this question we obtained monoxenic IJs hosting *Y. pseudotuberculosis*. Our first results show that the capacity of *Y. pseudotuberculosis* to survive infection cycles inside EPN lacking *Xenorhabdus* is substantially reduced. Moreover, multiplication of EPN inside the insect cadaver seems to be affected and emergence of IJs from the cadaver is delayed. This strongly suggests that *Y. pseudotuberculosis* cannot maintain in EPN in the absence of *Xenorhabdus*. These findings, if they turn out to have an environmental significance, may reveal an unexpected biotic reservoir explaining the long-term persistence and dissemination of pathogenic bacteria in the environment.

INTRASPECIES DIVERSITY IN THE ROOT KNOT NEMATODE *MELOIDOGYNE ETHIOPICA*. **Gerič Stare, B., P. Strajnar, S. Širca and G. Urek.** Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia.

The root-knot nematode *Meloidogyne ethiopica* are considered one of the most important emerging global plant pests. *Meloidogyne ethiopica* is considered a tropical, polyphagous pest that is able to parasitize at least 80 different host plants. However, this pest can survive winters in moderate temperate region with temperatures below freezing point. This species was reported from Africa (Tanzania, Kenya, Ethiopia, Mozambique, Zimbabwe, South Africa), South America (Brazil, Chile) and Europe (Slovenia, Greece, Turkey, Italy). We have compared populations of *M. ethiopica* from South Africa, Brazil and Europe (Slovenia, Greece, Turkey) and evaluated variability of biochemical markers used for identification (EST, MDH isozyme profiles) and several molecular markers (ITS, 18S and 28S rDNA and mtDNA) used in phylogenetic studies of genus *Meloidogyne*. The studied populations, determined as *M. ethiopica* based on morphological and biochemical characters, have displayed three different patterns of isozyme esterase. While some of *Meloidogyne* species have one species-specific esterase pattern, some species like *M. arenaria, M. incognita* and *M. exigua* are known to display several esterase patterns, and some patterns can be found in more than one species (e.g. A1). Sequences from our tested populations were combined with other *Meloidogyne* spp. sequences from public databases to assess phylogenetic relationship between different populations of *M. ethiopica* and closely related species. Great care should be taken when molecular marker is selected to study phylogenetic relationships or to confirm identification of Meloidogyne *species* as variability both at rDNA and mtDNA was observed in this and previous studies.

ANALYSIS OF ENDOPHYTIC FUNGI AND PLANT-PARASITIC NEMATODES FROM IRRIGATED AND UPLAND RICE ECOSYSTEMS IN KENYA. **Gheysen[1], G., T. Kyndt[1], F. Soraya de Carvalho[2], M. Höfte[2], W. Bert[3], T. Janssen[3], R.K. Mibey[4] and P.N. Njira[1,4].** [1]Department of Molecular Biotechnology, Ghent University, Coupure Links 653 B-9000, Ghent, Belgium; [2]Department of Crop Protection, Ghent University, Coupure Links 653 B-9000, Ghent, Belgium; [3]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000, Ghent, Belgium; [4]Moi University, P.O Box 3900-30100, Eldoret, Kenya.

Endophytic fungi are ubiquitously distributed in almost all plants species. They have been found to colonise single or multiple plant organs, often protecting their host plants from biotic as well as abiotic stresses. In the present study, the diversity of endophytic fungi and plant-parasitic nematodes from irrigated and upland rice ecosystems was analysed using DNA sequences and morphological characteristics. A total of 45 and 25 fungal species were identified from irrigated and upland ecosystems, respectively. In the irrigated ecosystem, a relatively high number of different fungi were isolated.

Epicoccum nigrum was the most dominant species in this ecosystem. Species richness was lower in the upland ecosystem, where *Fusarium oxysporum* isolates were extremely abundant. Nematodes were found only in the upland ecosystem, and were identified as *Pratylenchus zeae* and *Pratylenchus goodeyi*. Previous records showed *P. zeae* to be a rice pathogen, but *P. goodeyi* is described here for the first time in rice, a remarkable observation especially in coastal low altitude areas characterised by high temperatures. Preliminary results from greenhouse experiments indicate that *P. goodeyi* can indeed infect and multiply in rice. Based on available information from other crops studied, *Epicoccum nigrum* and *Talaromyces flavus* were selected and are currently tested for their biological activities against these lesion-nematodes.

DIVERSITY OF ENTOMOPHILIC NEMATODES AND THE THREAT OF CRYPTOGENIC INVASIVE SPECIES. **Giblin-Davis[1], R.M., N. Kanzaki[1, 2] and K.A. Davies[3].** [1]Fort Lauderdale Research and Education Center, University of Florida-IFAS, 3205 College Ave., Davie, Florida 33314, USA; [2]Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [3]Australian Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia.

Nematode fauna are often highly specialized for microniche utilization, *i.e*., many nematode species are highly habitat specific. For example, a transect survey of soil, litter, epiphyte and insect-associated nematode fauna in the same site yields different nematode community strata, and the communities do not necessarily overlap each other. Thus, the ''insect-associated nematode community'' can potentially be considered as a specific ''habitat'' of specialized and divergent entomophilic nematodes. Based on entomophilic nematode surveys conducted by the authors, correlation between the nematode assemblage pattern (*e.g*., feeding habitat groups) and the life history of their host/carrier insect (*e.g*., feeding resource, habitat preference and life cycle) was expected, and the number of entomophilic nematode species was estimated at somewhere between 40,000 and 500,000 species. Considering that the number of currently described species for the entire phylum is about 27,000, most of the entomophilic nematodes are hypothesized to be undescribed species. Biodiversity can be affected by many different kinds of environmental alternations. Here, we focused on the potential for cryptogenic invasive species being introduced by international trade as a threat to the diversity of entomophilic nematodes by introducing several contemporary examples.

GENETIC IMPROVEMENT AND BREEDING OF EPN: THE RACE FOR THE ''SUPER NEMATODE''. **Glazer, I.** Department of Nematology, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel.

Entomopathogenic nematodes (EPN) which are used as commercial biocontrol agents were targeted for genetic improvement in recent years. Most studies were aimed at improvement of traits which were believed to contribute to the efficacy of these organisms under field condition. That included, enhancement of heat and desiccation tolerance, host finding and resistance to nematicides. In these studies substantial enhancement of the selected trait was demonstrated. The contribution of these traits to nematode efficacy under field condition was not yet demonstrated. In many cases the enhanced trait was not stable and rapid degradation was observed following relaxation of the selection pressure. Furthermore, reduction of fitness can occur during multiple reproduction cycles in large scale liquid culture of the nematodes. Modern genetic and molecular tools such as RNA-seq, transcriptomics can be utilised to follow and understand the molecular basis of a beneficial trait. Molecular markers and QTL can also help to develop new strategies for genetic improvement of EPNs. New techniques for genome editing (TALENs and CRISPR-Cas9) may be able to modify important traits. In the presentation, approaches to address the challenges concerning development of the ''super nematode'' will be discussed.

CHARACTERIZATION OF *MELOIDOGYNE* SPECIES FROM IRRIGATED RICE IN SOUTHERN BRAZIL. **Gomes[1], C.B., R.R.D. Negretti[2], V.S. Mattos[3], L. Somavilla[2], R. Manica-Berto[2], D. Agostinetto[2] and R.M.D.G. Carneiro[3].** [1]Embrapa Clima Temperado, C.P. 403, 96001-970 Pelotas RS, Brazil; [2]PPGFS/Fac. Agronomia, Universidade Federal de Pelotas, C.P. 354, Pelotas RS, Brazil; [3]Embrapa Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-979, Brasília DF, Brazil.

Southern Brazil has the largest cultivated area of irrigated rice in the country. Nonetheless, rice production may be limited by several plant pathogens, including root-knot nematodes (*Meloidogyne* spp.). Considering the importance of root-knot nematodes in Brazil, a survey was carried out in irrigated rice fields in Rio Grande do Sul (RS) and Santa Catarina (SC) states. *Meloidogyne* populations were characterized biochemically by esterase (Est) and malate dehydrogenase (Mdh) phenotypes and morphologically by the female perineal patterns. Fifty five *Meloidogyne* spp. populations were detected in 48% of rice samples, and a total of five esterase phenotypes were identified. In Rio Grande do Sul state *Meloidogyne graminicola* (Est VS1, Rm: 0.70), *Meloidogyne* sp.2 (Est R2, Rm: 0.81, 0.91) and *Meloidogyne* sp.3 (Est VS1-2, Rm: 0.61, 0.70) were identified, which corresponded to 80, 40 and 10% of samples, respectively. In Santa Catarina state *M. graminicola, Meloidogyne javanica* (Est J3, Rm: 1.00, 1.20 and 1.35), *Meloidogyne* sp.1 (Est R1, Rm: 1.02), *Meloidogyne* sp.2 and *Meloidogyne* sp.3, accounted for 93.7, 12.5, 62.5, 12.5 and 6.2% of samples, respectively. *Meloidogyne javanica* showed a N1 Mdh phenotype (Rm: 1.0), while other populations exhibited a N1a (Rm: 1.4) phenotype. Morphology of female perineal patterns allowed identification only for *M. javanica* and *M. graminicola*. To further characterize these tree atypical

populations, sequencing and phylogenetic analyses of internal transcribed spacer-rRNA (ITS) and D2-D3 segment of 28S rRNA have been done. Future phylogenetic studies involving these atypical isolates will be performed.

USE OF NUTRITIONAL SUPPLEMENTS FOR THE MANAGEMENT OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) INFECTING POTATO. **Gondal[1], A.S., N. Javed[1], S.A. Khan[1] and M. Shahid[2].** [1]Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan; [2]Plant Pathology Research Institute, Faisalabad, Pakistan.

A wide range of synthetic chemicals has been discriminately used as the major control measure against plant pathogens. Environmental pollution, degradation, insecticide resistance development and other agronomic concerns have prompted scientists to seek alternative disease management strategies. Present in-vitro studies were conducted to evaluate the efficacy of nutritional supplements including micro-power, humic acid and plant protectors containing benzoic acid against root-knot nematode (*Meloidogyne incognita*) infection on susceptible potato cultivar. Each treatment applied as single or in combined form significantly reduced the number of galls and egg masses and promoted overall plant growth as compared to un-amended control. Application of aqueous solution of 4% plant protector + 4% micro-power + 2% humic acid enhanced the number of leaves, root and shoot developmentand tuber weight and decreased the root weight, with the minimum number of females, root galls and egg masses recorded. Nematodes fecundity was observed to be the highest for the control treatment resulting in poor plant growth and development of higher number of galls and egg masses. The significantly lower number of galls and egg masses and enhanced plant growth observed with the combined application of plant protector 4%, micro power 4% and humic acid 2% indicated this treatment to be superior.

EFFECTS OF LONG-TERM CORN-SOYBEAN CROP SEQUENCES ON THE NEMATODE COMMUNITY. **Grabau, Z.J. and S.Y. Chen.** University of Minnesota Department of Plant Pathology, Southern Research and Outreach Center, 35838 120th Street, Waseca, Minnesota 56093, USA.

The effects of long-term corn-soybean crop sequences on the nematode community were studied at a field site that was established in 1982 in Minnesota, USA. The crop sequences were: (i) five-year rotation between crops such that both crops are in years 1, 2, 3, 4, and 5 of monoculture every year; (ii) annual rotation with both crops planted each year; (iii) continuous monoculture of each crop; (iv) annual rotation between two cultivars, but crop monoculture of each crop. Since 1995, sequence IV was single-cultivar monoculture of each crop. Since 2010, soybean susceptible to soybean cyst nematode (*Heterodera glycines*) and *Bt* corn were used for crop sequences i, ii, and iii while monoculture of a *H. glycines*-resistant soybean cultivar or a non-*Bt* corn cultivar was used for sequence iv. Beginning in 2010, half of each plot was treated with granular nematicide. In 2013, the nematode community in each plot was assessed at planting, midseason, and harvest. Nematicide was effective against plant-parasitic nematodes, but also reduced fungivore population and shifted the nematode community to a lower ecological succession. Crop sequences strongly affected plant-parasitic nematodes, but also affected fungivores, bacterivores, nematode community diversity, maturity, and enrichment. Differences in the nematode community were most dramatic when comparing crops rather than length of monoculture within a crop. After dramatic change in the initial years of growing a crop, changes in the nematode community continued incrementally as years in monoculture increased. This study shows the agronomic and ecological impact of crop rotation.

DIRECT AND INDIRECT CONSEQUENCES OF *GLOBODERA PALLIDA* SELECTION BY RESISTANT POTATO GENOTYPES. **Grenier, E., S. Fournet, M.C. Kerlan, D. Eoche-Bosy and J. Montarry.** INRA, UMR IGEPP, Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France.

In this study, we have first explored the variability of resistance durability in different potato genotypes harboring the same resistance QTL but differing by their genetic background. The indirect consequences of the resistance adaptation in terms of local (*i.e.* genotype-specific) adaptation and cross-virulence were then also investigated. Following the virulence of the potato cyst nematode *Globodera pallida* in a long-term experimental evolution protocol, our results showed that nematode populations were able to adapt to the resistance of four potato genotypes carrying the QTL *GpaV* from *Solanum vernei,* and that the plant genetic background has an impact upon the durability of resistance. The pattern of local adaptation observed here revealed a trade-off between the adaptation to a resistant potato genotype and the adaptation to another resistant genotype differing in its genetic background. In terms of cross-virulence between potato genotypes derived from different resistance sources (*Solanum sparsipilum* and *Solanum spegazzinii*), we showed that the adaptation to resistance QTL $GpaV_{vrn}$ does not necessarily allow the adaptation to collinear *GpaV* loci. The results presented here will be useful for identifying durable strategies for resistance deployment. The virulent populations obtained from these experimental evolutions are actually used in order to identify, through a genome-scan approach, the genomic regions involved in that resistance breakdown.

DEVELOPMENTS IN THE IMPLEMENTATION OF ENTOMOPATHOGENIC NEMATODES IN INTEGRATED PEST MANAGEMENT SYSTEMS IN NORTH AMERICA. **Grewal, P.S.** Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA.

Entomopathogenic nematodes (EPNs) have emerged as excellent alternatives to chemical pesticides. They have been evaluated against nearly 200 insect pest species almost all of which can be controlled under field conditions. Broad host range

and ability to seek and kill insects in soil and in cryptic habitats such as plant roots and tree trunks, where most chemical pesticides fail to reach make EPNs especially attractive. Ease of application via standard pesticide spray equipment and via diverse irrigation systems has facilitated the adoption of EPNs in diverse ecosystems. Compatibility with numerous agrochemicals including insecticides, miticides, fungicides, herbicides, wetting agents, plant growth regulators, and spray adjuvants enhances their use in IPM. Some pesticides even enhance EPN pathogenicity via synergism. Although, EPNs are most widely used against soil-dwelling stages, applications against above ground pests have also been successful. Foliar applications in glasshouses have shown particular promise. Applications to insect galleries in tree trunks provide excellent control of wood boring insects. Slow release formulations and partially-desiccated EPN-infected cadavers hold promise in prophylactic EPN use particularly for delivery via growing media commonly used in greenhouses and potted plant industries. Successes have also been reported in the use of EPNs in traps designed to lure and kill cockroaches, houseflies, grasshoppers, molecrickets, cutworms, and weevils. EPNs have also shown promise for the control of human and animal pests including fleas, yellowjackets, ants, termites, lice, and ticks. This presentation will provide an overview of the progress in implementing the nematodes in integrated pest management systems in North America.

QUARANTINE NEMATODES DETECTED IN NINGBO PORT, CHINA. **Gu, J.** Technical Centre, Ningbo Entry-Exit Inspection and Quarantine Bureau, 9 Mayuan Road, Ningbo 315012, Zhejiang, China.

Since 2012, many seedlings or trees mainly from Japan and Italy were imported through Ningbo port, China. After laboratory examination and identification with morphological and molecular methods, many species on the Quarantine List of China were repeatedly found. Including *Meloidogyne mali*, *Meloidogyne camelliae*, *Pratylenchus japonicus*, *Pratylenchus hippeastri*, *Pratylenchus pseudopratensis*, *Trichodorus viruliferus*, *Trichodorus japonicus*, *Longidorus martin Xiphinema rivesi* and some more species still not identified. So Rooted plants in soil or growth medium could be an important pathway for the spread of nematodes. Phytosanitary treatment is necessary but problematics. Since morphological identification is not easy, we suggest that D2D3 region of 28S rDNA gene could be a DNA barcoding gene.

EFFICACY OF TWO ABAMECTIN FORMULATIONS ON *BELONOLAIMUS LONGICAUDATUS* IN GREENHOUSE AND FIELD TRIALS. **Gu, M. and W.T. Crow.** University of Florida, Gainesville, Florida 32611, USA.

Belonolaimus longicaudatus (sting nematode) is considered as one of the major destructive plant parasitic nematodes on golf course turf in Florida. However, since the cancellation of fenamiphos, effective management options for this nematode on golf courses are few. Our research evaluated two formulations of abamectin (Avid 0.15 EC and A12115I, an experimental 1.7% a.i. formulation) for ability to reduce population density of *B. longicaudatus* and to improve turf health. In separate trials Avid 0.15 EC at rates of 80, 40, and 20 g abamectin/ha reduced population density of *B. longicaudatu*s on turf in pots while A12115I at rates of 35 and 70 g abamectin/ha did not. In a field trial on *B. longicaudatus* infested turf, the two abamectin formulations applied every 14 days at the rate of 35 g a.i/ha with and without applications of the plant activator acibenzolar-S-methyl were compared to each other and to untreated plots. Both abamectin formulations improved turf percent green cover and the Avid 0.15 EC formulation was slightly better on some dates. Applications of acibenzolar-S-methyl enhanced percent green cover from both abamectin formulations compared with either abamectin formulation alone. These studies indicate that abamectin may be a useful nematode management tool for *B. longicaudatus* on turfgrasses. Additional greenhouse and field trials evaluating abamectin formulations and combinations with other compounds are currently underway.

MELOIDOGYNE INFECTIONS UNDER INFRARED SPECTROSCOPY (FTIR-ATR). **Guerra[2], M., N. Cubillán[3], A.M. Casassa[4], E. Portillo[1] and E. San-Blas[1].** [1] Instituto Venezolano de Investigaciones Científicas, Centro de Estudios Botánicos y Agroforestales, Laboratorio de Protección Vegetal, Av. 8 entre Calles 79 y 80, Maracaibo, Venezuela; [2]Unidad de Tecnología Láser y Optoelectrónica, Instituto Zuliano de Investigaciones Tecnológicas, Km11. Carretera Vía La Cañada, Maracaibo, Venezuela; [3]Laboratorio de Electrónica Molecular, Facultad Experimental de Ciencias, Universidad del Zulia, Maracaibo, Venezuela; [4]Instituto de Investigaciones Agronómicas, Facultad de Agronomía, Universidad del Zulia, Maracaibo, Venezuela.

Infrared spectroscopy is a technique developed to observe vibrational aspects of the functional groups from the molecules of a given sample. Basically, a sample is irradiated with different wavelengths and a detector capable to register the vibrational mode of the molecules, builds a spectrum. These spectra can be compared and differences nsee if one sample is different to another one. Infrared spectroscopy was used to compare infected or not infected tomato plants. Thirty tomato seeds were sown in pots (1 l) and after 30 days, 15 of them were inoculated with root-knot nematodes p. and the remainder were used as control plants. After 6 weeks, the plants were removed from the pots and washed clean of the soil. Root and leave samples were taken and let dry for 24 h at room temperature. The air dried samples were placed in a Fourier Transformed Infrared Spectrometer (FTIR) with an Attenuated Total Reflectance (ATR) accessory and the resulting spectra collected. The total data was pretreated to eliminate CO_2 and water interference, smoothed and a second derivative procedure was done. The main spectral contributions were assigned to proteins around 3277, 1533 and 1231 cm^{-1} and lipids around

2924, 2855, 1737 and 1452 cm^{-1}. Carbohydrates were also detected; the major absorption was found to be in the 1000–1200 cm^{-1} range. The samples from infected plants showed differences in their composition, comparedto those coming from healthy plants. This technique demonstrated its usefulness for investigating many aspects of the nematode-plant interaction.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE GENUS *LONGIDORUS* (NEMATODA: DORYLAIMIDA) IN CHINA WITH DESCRIPTION OF A NEW SPECIES. **Guo, K., P.T. Hoa, and J. Zheng.** Nematology Lab, Institute of Biotechnology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China.

The genus *Longidorus* was established by Micoletzky, 1922 with *Longidorus. elongatus* de Man 1876 as the type species. This genus contains migratory ectoparasitic nematodes that attack a wide variety of crops or trees. Some of the species in the genus are vectors of plant viruses. Given its possible vector role, the genus *Longidorus* has gradually received increased taxonomic attention in China. The geographical distribution and host associations of *Longidorus* in mainland China were determined from a comprehensive survey. Based on soil samples collected from 12 provinces of China. Eleven *Longidorus* species, including one new species, were identified based on morphological characters. These are *L. henanus*, *L. litchii*, *L. hangzhouensis*, *L. pisi*, *L. macromucronatus, L. fangi*, *L. camelliae*, *L. jonesi*, *L. fursti*, *L. nagnaensis*, and *Longidorus* n.sp. The new species is characterized by females with medium size (L = 4.9-6.6 mm), lip region 18-21 μm wide, guiding ring situated at 70-90 μm from anterior end, long odontostyle (142-168 μm), tail short, bluntly rounded. Ratio of males: females one to one, spicules 100-123 μm long, 10-13 ventromedian supplements, and three juvenile stages were observed. A phylogeny tree was constructed based on the D2D3 region of 28S rRNA of the *Longidorus* species found in the study.

POTENTIAL USE OF COMPOST FOR MANAGING NEMATODES, SOIL QUALITY AND CARROT YIELD. **Habteweld[1,2], A., D. Brainard[2], M. Ngouajio[2], S. Kravchenko[3], and H. Melakeberhan[1,2].** [1]Agricultural Nematology Laboratory Michigan, State University, East Lansing, MI 48824, USA; [2]Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA; [3]Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA.

In the absence of nematode resistant carrot cultivars, human- and environmental-health driven restrictions of broad-spectrum nematicides, developing multi-purpose soil amendments that suppress plant-parasitic and other soil-borne pests and diseases is one of the priorities identified in the US Midwest vegetable growers. A hypothesis that plant- (PC) and animal-based (AC) compost may suppress plant-parasitic nmeatodes, increase beneficial nematodes, soil conditions, and yield and quality of fresh market (Sugarsnax) and processing (Cupar) carrot cultivars was tested in a sandy clay loam field. PC and AC, at 1, 1.5 and 2 times the standard recommendation for nitrogen source, and urea and non-amended (as controls) were tested. Preliminary analyses indicate significantly higher Maturity index (MI) in compost-treated (2.1) plots than in the controls (1.6), and fertility index (FI) in compost-treated plots was 1.0 compared to 1.3 in the controls, suggesting improved soil conditions. Abundance of omnivore-predatory nematodes was positively correlated (r = 0.53) with root to shoot dry matter ratio in the processing carrot variety plots, implying improved soil condition and more carrot root than shoot growth. Average total abundance was about 50 nematodes/100 cc of soil, with herbivores and fungivores being most abundant and omnivore and predators least abundant. Multifactor analysis of the relationship among nematode guilds, trophic groups and soil physiochemical properties shows significant correlation (r = 0.68) between c-p 1 bacteriovores and NO_3-N. Soil food web structure appears to mature with time. Overall, the results support the hypothesis that compost treatment may increase beneficial nematodes and improving soil conditions.

NEW CHEMISTRIES, MODES OF ACTION, AND DIFFERENT FORMULATIONS FOR NEMATODE MANAGEMENT IN IDAHO. **Hafez, S.L. and M.P. Pudasaini.** University of Idaho, Parma Research and Extension Center, 29603 University of Idaho Lane, Parma, Idaho 83660, USA

Experiments were conducted to determine the efficacy of new chemistries such as Movento, Nimitz (MCW-2) and several numbered compounds for the management of major nematode species affecting potatoes, sugar beets and onions in Idaho. Potatoes, sugarbeets or onions were planted in a silt loam field infested with Columbia root-knot (*Meloidogyne chitwoodi*), sugarbeet cyst (*Heterodera schachtii*) and lesion nematodes (*Pratylenchus* spp.) at damaging levels. Treatments were replicated five or six times in a randomized complete block design. Movento was initiated at sufficient foliage and repeated every two weeks. Nimitz was applied pre-and post-plant. Within one hour of applications, all plots were disked twice to incorporate Nimitz to a depth of 10-15 cm. Numbered compounds were sprayed at 2.5-5 cm or 15-20 cm bands in furrow at planting, at 15 cm plant height, or chemigated at first irrigation and at monthly intervals. Total potato, sugar beet, onion, and infected potato yield was determined. The results demonstrated that the fall application of Vapam followed by Movento at 56 and 70 days after planting, or pre-plant application of Nimitz at low rate, or application of systemic number compound at 15 cm plant height and chemigated 60 days after planting are promising treatments for *M. chitwoodi* management on potatoes in Idaho. Movento applied 14 and 28 days after emergence and numbered compound applied at planting increased sugarbeet yield

12-36%. Two applications of Movento and numbered compound sprayed at pre-plant increased onion yield 12-14%. No standalone chemical or single application can control nematodes. Multiple applications in combination with these soft chemicals are promising nematode management alternatives.

INTERACTION OF LESION NEMATODES AND FUNGUS (*VERTICILLIUM DAHLIAE*) IN MINT. **Hafez, S.L. and M.P. Pudasaini.** University of Idaho, Parma Research and Extension Center, 29603 University of Idaho Lane, Parma, Idaho 83660, USA.

An experiment was conducted to study the interaction of root lesion nematodes (*Pratylenchus penetrans* and *Pratylenchus neglectus*) and fungus (*Verticillium dahliae*) on mint. Treatments included no nematode, no fungus, either species alone, or combination of nematode and fungus. Each treatment had five replications spread on the greenhouse bench in random complete blocks. One six-week old mint plant was transplanted into 1500 cm^3 pot filled with sand and soil mix (1:1 by v/v) with 10 % peat moss. Inoculations were done with 20 micro-sclerotia of *V. dahlia* or 4 nematodes per cm^3 of soil. The mint was allowed to grow until flowering stage (about 10 weeks), and cut at soil level and allowed to regrow again. Mint was cut four times and top dry weight was recorded each time. Data demonstrates that in all cuts fungus or nematodes significantly and progressively decreased the mint hay dry yield as compared to control check. Interactive effect of *V. dahliae* and lesion nematodes appears to be an additive on mint hay yield. *Verticilium dahliae* alone caused 44 % damage in mint hay yield. Root lesion nematode *P. neglectus* seems pathogenic to mint. A 23 % and 46 % reduction on mint hay were caused by *P. neglectus* alone or in combination with *V. dahliae*, respectively. *Pratylenchus penetrans* alone caused 44% yield reduction of mint hay while combination of *P. penetrans* and *V. dahliae* killed almost all plants. Population of *P. penetrans* increased by 41 fold, indicating that mint is an excellent host for *P. penetrans*.

TOLERANT VARIETIES AND REDUCED RATE OF TELONE II FOR SUGAR BEET CYST NEMATODE MANAGEMENT IN IDAHO. **Hafez, S.L. and M.P. Pudasaini.** University of Idaho, Parma Research and Extension Center, 29603 University of Idaho Lane, Parma, Idaho 83660, USA.

Three experiments; response of tolerant sugar beet varieties, in-row and broadcast fumigation of Telone II at reduced rate in-strip, and a combination of tolerant sugar beet varieties with low rate of Telone were conducted to determine the response of sugar beet yield and sugar beet cyst nematode at the Parma Research and Extension Center, Parma, Idaho. The experiments were carried out in a randomized complete block design with seven treatments including one control, each with five replications in silt loam field. Telone II was shanked to a depth of 30 cm. Temik 15G @ 20 at plant and 14.57 kg/ha at post-plant was applied. Nematode tolerant and susceptible (Hillshog 9036RR) sugar beet varieties were sown for these trials. Suager beets were harvested and weights were taken from all experiments. The results demonstrated that sugar beet yield was significantly increased in all tolerant varieties as compared to the susceptible variety. Final viable cysts were increased by four fold in the susceptible variety while there were no increases in tolerant varieties. The sugar beet yield was significantly increased in both in-row and broadcast application of Telone as compared to untreated control and Temik. All the rates of Telone in-row had significantly higher sugar beet yield as compared to broadcast applications. There were no significant differences in the sugar beet yield among the different rates in in-row or in broadcast applications of Telone. Sugar beet yield was increased in the susceptible variety when fumigated plots were compared with non-fumigated plots. Sugar beet yield of tolerant varieties were increased in fumigated plots compared to non-fumigated plots.

SCOPE OF NEW NEMATICIDES AND NUMBERED COMPOUNDS FOR COLUMBIA ROOT KNOT NEMATODE MANAGEMENT IN IDAHO. **Hafez, S.L. and M.P. Pudasaini.** University of Idaho, Parma Research and Extension Center, 29603 University of Idaho Lane, Parma, Idaho 83660, USA.

Experiments were conducted to determine the efficacy of Movento, Nimitz (MCW-2 15G), and numbered compounds against Columbia root-knot nematode (*Meloidogyne chitwoodi*). Ranger Russet potatoes were planted in silt loam fields with an average initial nematode population of 374 per 500 cm^3 soil. Treatments were replicated five times in a randomized complete block design. Movento was initiated at sufficient foliage and repeated every 2 weeks. Nimitz was applied pre-and post-plant. Within one hour of applications, all plots were disked twice to incorporate Nimitz to a depth of 10-16 cm and then hills were formed. Numbered compounds were sprayed at 2-5 cm or 15-20 cm bands in furrow at planting, or at first irrigation and at monthly intervals. After harvest, total and infected potato yield was determined. The findings demonstrate that Vapam in combination with Movento resulted in the lowest infected tuber yield (2.5 %) and provided superior performance as compared to either product alone, Movento (38-42 %) and Vapam (17 %). Nimitz lowered the infected potato yield in all treatments (3.4 to 7. 3%) as compared to untreated control (14.5%). The low rate of Nimitz applied pre-plant reduced infected tuber as compared to post-plant application. The infected yield was lower in all new numbered compounds tested (< 1.2 %) as compared to the untreated control (9.2 %). In conclusion, the fall application of Vapam followed by two foliar applications of Movento at 56 and 70 days after planting, or pre-plant application of Nimitz at low rate appears to be a promising treatment for Columbia root-knot nematode management on potatoes in Idaho.

THE MANAGEMENT OF PLANT-PARASITIC NEMATODES IN ORGANIC HORTICULTURE. **Hallmann, J.** Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Toppheideweg 88, 48161 Münster, Germany.

Plant-parasitic nematodes are a major threat to organically grown vegetables. High intensity of the production system allows the build-up of high nematode densities. The most commonly found plant-parasitic nematodes causing problems under temperate conditions in Europe are *Meloidogyne hapla*, *Pratylenchus penetrans* and *Paratylenchus bukowinensis*. Symptoms and economic losses are usually first seen on carrots and onions. In the past, organic farmers often used black fallow to reduce nematode numbers. However, as this will also diminish the organic matter content of the soil it is not a recommendable method. Over the past years, we developed rotation systems based on trap crops and non-host crops that reduce nematode numbers to a similar degree as black fallow but at the same time provide positive side effects like increasing organic matter content or nitrogen fixation. Good control of *M. hapla* can be achieved by growing fodder radish as trap crop during the season> If nitrogen fixation is desired, growing legumes over winter and incorporating them around mid-June just before the nematode cycle is completed. Best control of *P. penetrans* was achieved by growing *Tagetes* for three months. Growing fodder radish as green manure in late summer also helped to reduce *P. penetrans* significantly. *Paratylenchus bukowinensis* with its narrow host range comprising crops within the families Brassicaceae (e.g. fodder radish, rape) and Umbelliferae (e.g. carrot, celery, parsley) can be easily controlled by avoiding these host plants. However, a prerequisite for the success of all these measures is proper control of weeds, which are in general excellent hosts for *M. hapla* and *P. penetrans*.

PRELIMINARY RESULTS ALLUDE TO POTENTIAL ROOTKNOT NEMATODE TOLERANCE IN INDUCED POLYPLOIDS OF *PLECTRANTHUS ESCULENTUS*. **Hannweg[1, 2], K., W. Steyn[1], M. Daneel[1], A. Sippel[1] and I. Bertling[2].** [1]Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa; [2]Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Plectranthus esculentus, a member of the Lamiaceae family, is an important tuberous vegetable in Africa. It occurs in dry forest areas in central Africa, and southwards to Angola, the eastern parts of Limpopo and Mpumalanga and KwaZulu-Natal coast of South Africa, and Swaziland. *P. esculentus* is reported to be highly susceptible to rootknot nematode, *Meloidogyne* spp., which substantially reduces plant yield. In order to develop selections with potentially improved nematode tolerance, several polyploid lines were developed *in vitro* using colchicine treated shoot cultures and verified using flow cytometry. Hardened-off diploid (control) and tetraploid plants were inoculated with 1000 J2 eggs of *Meloidogyne* species and evaluated after 56 days for the number of egg masses and number of eggs and J2 per root system. The tetraploid plants had significantly fewer egg masses, eggs and J2 per root system compared with the diploid controls. Further, the tetraploids, had far lower Rf-values than the diploids indicating a higher tolerance for rootknot nematode infection. The results of a preliminary *in vitro* nematode challenge trial will also be presented. Further research concerning the mechanism of tolerance is planned.

SITE SPECIFIC APPLICATION OF FUMIGANTS FOR CONTROL OF NEMATODES IN TEXAS. **Haygood[1], R., J. Woodward[2], N. Foster[3] and C. O'Hara[1].** [1]Dow AgroSciences, Indianapolis, Indiana, USA; [2]Texas A&M AgriLife Extension, Lubbock, Texas, USA; [3]Texas Tech University, Lubbock, Texas, USA.

Control recommendations for plant pathogenic nematodes are based on species present, soil types, nematicide efficacy, economics, and other variables. Research results show that the use of soil maps and EC_a data can be used effectively to identify low and high risk, or ''responsive'' zones for root-knot nematodes (*Meloidogyne incognita*). As a result, input costs for the application of Telone® II fumigant can often be reduced by 30 – 40% by using this technological approach to assist in nematode management. Advances in Geographic Information Systems (GIS), Global Positioning Systems (GPS), apparent Electrical Conductivity (ECa) data collection, applicator equipment, and an increase in user expertise has enabled broader adaption and investigation of cotton yield responses. These studies were conducted to further define the value of site specific applications in fields with variable nematode populations and soil types in Texas.

BIOLOGICAL AND SYSTEMATIC IMPLICATIONS OF PHYLOGENETIC ANALYSES OF ~ 2,800 FULL LENGTH SMALL SUBUNIT RIBOSOMAL DNA SEQUENCES. **Helder[1], J., P. Mooijman[1], S. van den Elsen[1], H. van Megen[1], M. Vervoort[1], C. Quist[1], W. Bert[2], A. Karegar[3], G. Karssen[4] and W. Decreamer[2, 5].** [1]Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [2]Ghent University, Department of Biology, Nematology Unit, Ledeganckstraat 35, 9000 Ghent, Belgium; [3]Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran; [4]Plant Protection Service, Wageningen Nematode Collection, Geertjesweg 15, 6706 EA Wageningen,The Netherlands; [5]Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

As compared to other ancient and basal Ecdysozoan phyla such as the Nematomorpha (~ 350 known species), the Priapulida (~16 species) and the Kinorhyncha (~180 species), the phylum Nematoda stands out for being speciose

(~ 27,000 described species), highly abundant, and widespread in nearly all terrestrial, freshwater and marine habitats. We will present results of phylogenetic analyses of approximately 10% of the described nematode biodiversity (~ 2,800 taxa; with underrepresentation of marine and tropical terrestrial species). We have tried multiple genes for phylogenetic reconstruction but so far the small subunit of the ribosomal DNA (~ 1,700 bp) is the only gene that could easily be amplified and aligned for a wide range of nematode species. The resulting alignment including secondary structure information was analysed on the CIPRES Science Gateway (San Diego, USA), using RAxML, a maximum likelihood-based inference for large phylogenetic trees, and Bayesian inference, and the outcome of both analyses will be presented. Some relevant features of the resulting phylogenetic trees will be discussed, and attention will be paid as to whether or not such a single gene tree does provide us with useful information about organismal relationships. This talk will also be used to describe the positioning and the relationships between the nematode taxa that will be discussed in more detail in Theme 2: Morphology, taxonomy, phylogeny and classification.

EFFECTS OF SUNN HEMP AND PIGEON PEA ON BENEFICIAL AND PLANT-PARASITIC NEMATODES IN THE TROPICS. **Henmi, V.H. and S.P. Marahatta.** Science and Math Division, Kaua'i Community College, University of Hawai'i

Plant-parasitic nematodes such as burrowing nematode (*Radopholus similis*) and root-knot nematode (*Meloidogyne* spp.) are dominant in banana, *Musa* spp. ecosystems. Beneficial nematodes are also found in banana fields. A tropical cover crop, sunn hemp (*Crotalaria juncea*), can be used to suppress plant-parasitic and enhance beneficial nematodes. However, sunn hemp cultivation in Hawaii is under the threat of the flour beetle. Two experiments: Trial-I and Trial-II were conducted to compare the effects of another tropical cover crop, pigeon pea (*Cajanus cajan*)) with sunn hemp and no-cover crop control on *Radopholus similis* and *Meloidogyne* suppression and beneficial nematode enhancement. In both experiments soils infested with *R. similis* and *Meloidogyne* were sampled and amended with cover crop treatments or no-cover crop control and kept for two weeks. At the end of each experiment, nematodes were extracted through the Baermann funnel technique. The results of Trial-I and Trial- II showed that sunn hemp and pigeon pea did not reduce *R. similis* number ($P > 0.05$). However, *Meloidogyne* numbers were consistently reduced by sunn hemp and pigeon pea ($P < 0.05$). In both experiments sunn hemp increased beneficial nematodes number ($P < 0.05$). Pigeon pea increased beneficial nematode numbers in Trial -I ($P < 0.05$), but not in Trial –II ($P > 0.05$). However, the numbers of beneficial nematodes were consistently higher in pigeon pea compared to the no-cover crop control. Farmers could choose pigeon pea as an alternate to sunn hemp, as a cover crop for *Meloidogyne* suppression and beneficial nematode enhancement.

PRISTIONCHUS SCRATCHPAD – AN ONLINE PLATFORM FOR TAXONOMY, SYSTEMATICS AND PHYLOGENY. **Herrmann, M. and R.J. Sommer.** Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Spemannstraße 37, 72076 Tübingen, Germany.

The utility of the nematode *Pristionchus pacificus* as a model system in evolutionary biology and comparative developmental biology builds on the availability of a sophisticated genetic and molecular toolkit with a resolved phylogenetic context. Specifically, surveys have recovered several new species of *Pristionchus*, which are particularly diverse in East Asia. In total, 29 *Pristionchus* species have been characterized by molecular, morphological and in some cases genetic tools. All of these species, often including independently collected wild isolates, are available as live and frozen stocks in the Tübingen lab collection and can be distributed upon request. Similarly, a broad framework of 24 genera of the Diplogastridae family has been established, many of which are also available as live or frozen stocks. This includes among others, species of four newly described genera (e.g. *Parapristionchus*, *Sudhausia*), which have been characterized in recent years. To provide a transparent system for the taxonomy, biosystematics and phylogeny of *Pristionchus* and ultimately the Diplogastridae, we are establishing an online platform, *Pristionchus* Scratchpad (following the guidelines of scratchpads.eu), which can help researchers throughout the world to obtain an overview of published work and to request cultured isolates. Specifically, *Pristionchus* Scratchpad provides information on i) a species overview with published references and PDFs, ii) species distribution maps, iii) morphological characterization, iv) morphometrics and v) SSU rRNA and other molecular sequence tags. We hereby present *Pristionchus* Scratchpad as a model for an online platform of modern taxonomy, biosystematics and phylogenetic research in nematodes.

COMPARISONS OF NEMATODE BIODIVERSITY USING MORPHOLOGICAL, MOLECULAR, ECOLOGICAL AND BIOLOGICAL CRITERIA. **Hodda, M.** CSIRO Ecosystem Sciences, GPO Box 1700 Canberra ACT 2601 Australia.

There is an increasing trend towards assessments of nematode biodiversity based on molecular criteria only. This is understandable given the greater ease and automation possible assessing biodiversity using molecular techniques. Much greater expertise and resources are required to assess diversity using morphology in any sophisticated way corresponding to traditional taxonomy, or using ecological criteria such as host relationships, or using biological criteria such as interbreeding success. Even using ''morphospecies'', requires a considerable investment to assess nematode diversity in any meaningful way. Whatever their practicalities, when the results of these different measures of nematode biodiversity are compared, the

results are seldom exactly the same: diversity has been frequently underestimated by most methods, but that it may be overestimated by the new technique of ultrasequencing. However, these results depend on the species concept used, and the different species concepts may have different abilities to supply relevant measures of nematode biodiversity, depending on what diversity is being measured for. The different uses of nematode biodiversity are reviewed.

NEMATOLOGY COMMUNICATION & EDUCATION: FROM FARMERS TO POSTDOCTORAL RESEARCHERS & EVERYONE BETWEEN. **Hodda[1, 2], M., N. Tangchitsomkid[3], K.A. Davies[4], S.K. Singh[5] and N.C. Banks[1,6,7].** [1]CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, ACT 2601, Australia; [2]Australian National Insect Collection, GPO Box 1700, Canberra, ACT 2601, Australia; [3]Thailand Department of Agriculture, 50 Phaholyothin Road, Chatuchak, Bangkok, 10900, Thailand; [4]School of Agriculture Food & Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia; [5]Agricultural & Wine Sciences, Charles Sturt University, Locked Bag 588 Wagga Wagga, NSW 2678, Australia; [6]CSIRO Biosecurity Flagship, 41 Boggo Rd Dutton Park, QLD 4102 Australia; [7]School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch WA 6150, Australia.

Nematology is of necessity a very broad scienfic discipline, encompassing everything from the very practical (crop diseases) to the theoretical (species concepts); from the finely detailed (virulence genes) to the broad general (soil health); and from the local (species lists) to the global (biosecurity). This breadth should mean that interest is healthy, but too frequently the reaction is the opposite. Based on experience teaching everyone from farmers with no initial knowledge of nematodes to postdoctoral researchers, we suggest that there are several elements to successful education and communication about nematodes. First, a certain amount of basic knowledge is necessary as background. Second, information and communications need to be distilled into a few simple messages. This can be a considerable task. Third, that the details and exceptions and complicating factors are important, and recognizing how basic principles are modified in different situations is important in applying new knowledge about nematodes to particular situations. The skills to be able to recognize local variations are very important. Fourth, the practical uses of nematology knowledge need to be emphasized. Most people dealing with nematodes are non-specialists and require frequent updates, refreshers and a graded series of interactions, often best delivered over several presentations spaced some time apart to allow the information to sink in and avoid overload.

SUBERIN AND LIGNIN – BARRIERS TO NEMATODE ENTRY? **Holbein[1], J., R.B. Franke[2], F.M.W. Grundler[1] and S. Siddique[1].** [1]Rheinische Friedrich-Wilhelms University of Bonn, INRES, Molecular Phytomedicine, Karlrobert-Kreiten Str. 13, 53115 Bonn, Germany; [2]Rheinische Friedrich-Wilhelms University of Bonn, IZMB, Ecophysiology, Kirschallee 1, 53115 Bonn, Germany.

The essential function of a plant root is the uptake of water and minerals from the soil and their distribution to the aerial organs via the vascular bundle. Due to their vital function, these vessels are protected from external biotic and abiotic stresses by a tissue called endodermis. Special cell wall modifications in forms of biopolymer depositions enhance the barrier function of the endodermis. Primary walls of the endodermal cells are impregnated with suberin, while radial and transverse walls are additionally reinforced by belt like lignin structures, called casparian strip (CS). It has been proposed that suberin and casparian strip may act as insuperable barriers to nematodes invading the roots for feeding. In fact, the root knot nematode (RKN) *Meloidoygyne incognita* evade the endodermal barriers by first moving towards the root tip between cortical cells, and then once at the tip taking a U-turn to enter the vascular cylinder where they initiate feeding sites, whereas, the migratory nematode *Pratylenchus penetrans* feeds almost entirely from the cortex of roots suggesting an inability to cross endodermal barriers. To investigate the role of endodermal suberin and lignin during nematode infection, *Arabidopsis* lines and accessions were screened with altered levels of suberin and lignin against three different nematode species (*Heterodera schachtii, Meloidogyne incognita and Pratylenchus spp*.). These nematodes represent three different types of migration and feeding behavior. Our data showed that susceptibility of plants decreases significantly with increase in suberin content suggesting that root barriers play important role during plant-nematode interaction. Histochemical, analytical and molecular analyses have been performed to understand how enhanced suberin is mechanistically related to decrease nematode susceptibility.

LABEL-FREE COHERENT RAMAN SCATTERING (CRS) IMAGING FOR METABOLIC PROFILING OF NEMATODES. **Holden-Dye[1], L., N. Dalliere[1], P. Urwin[2], C. Lilly[2], V. O'Connor[1], J. Pertek[1] and S. Mahajan[1].** [1]University of Southampton, Institute for Life Sciences, Highfield Campus, University of Southampton, Southampton, SO17 1BJ, UK; [2]Centre for Plant Sciences, School of Biology, University of Leeds, Leeds LS2 9JT, UK.

Fundamental aspects of the biology of plant-parasitic nematodes are relatively underexplored however there is evidence to suggest that a major factor regulating their ability to infect the host crop is their lipid reserve. This is particularly important with respect to the phenomenon of field persistence whereby plant-parasitic nematodes that have thrived on a host crop deliver progeny with high lipid reserves, and thus high infectivity, back to the field. This positive feedback loop contributes to the persistence of crop infection and damage. Therefore there is an increasing appreciation that the key to identifying resistance breaking approaches and/or more efficacious ways of using existing practices will derive from improved understanding of the metabolic reserve of these agriculturally important parasites. We are deploying spectroscopic

methodologies that can detail metabolic analysis and enhance metabolomics investigation. Coherent Raman Scattering (CRS) spectroscopy and microscopy are label-free techniques which allow high-resolution, non-invasive and non-destructive chemically specific imaging. Thus CRS can monitor key biochemical signatures in vivo and is well suited to such an investigation. We have preliminary data to show precise quantification of lipid stores and their tissue redistribution, responding to environmental and chemical manipulation.

SPATIO-TEMPORAL DISTRIBUTION OF RENIFORM NEMATODE (*ROTYLENCHULUS RENIFORMIS*) AND RELATIONSHIPS WITH SOIL TEXTURE. **Holguín[1], C.M., P. Agudelo[1], P. Gerard[2], J.D. Mueller[3] and A. Khalilian[3].** [1]School of Agricultural, Forest, and Environmental Sciences. Clemson University, Clemson, South Carolina 29634, USA; [2]Mathematical Sciences. Clemson University, Clemson, South Carolina 29634, USA; [3]Edisto Research and Education Center, Clemson University. Blackville, South Carolina 29817, USA.

Densities of reniform nematode, *Rotylenchulus reniformis*, can change in time and space within a field affecting sampling and management strategies. A three-year study was conducted in two fields in South Carolina to assess the horizontal and vertical distribution of reniform nematode and determine potential correlations with soil texture. Cotton-peanut and cotton-corn-soybean rotation systems occurred in fields 1 and 2 respectively. Each year, soil samples were collected in both fields at planting and after harvest. In field 1, 40 samples were collected at random locations. Sample cores were separated into four depths: 0-15 cm, 15-30 cm, 30-60 cm, and below 60 cm. In field 2, samples were collected from 80 4 x 4 m plots distributed within four sections of the field representing different soil electrical conductivity readings and textures. Reniform nematode densities within each field/plot showed high levels of spatial variability during the length of the study. Horizontal distribution analysis using the variance/mean ratios showed a significant clustered distribution at planting and after harvest in both fields during the three years. However, a significant neighbourhood structure was only detected in the first two years in field 2, with patches between 8 and 18 square meters. Vertical analysis showed the highest numbers of reniform nematode between 15- and 30-cm deep. In some of the plots *R. reniformis* densities were significantly correlated with sand and clay content. In this study, the choice of sampling strategy, host as well as soil texture affected the spatial pattern of reniform nematode that was revealed.

STYLETCHIP: A MICROFLUIDIC DEVICE FOR RECORDING HOST INVASION BEHAVIOR AND FEEDING OF PLANT PARASITIC NEMATODES. **Hu[1], C., J. Kearn[2], P. Urwin[3], C. Lilley[3], V. O'Connor[2], L. Holden-Dye[3] and H. Morgan[1].** [1]Faculty of Physical Sciences and Engineering, and Institute for Life Sciences, Bassett Crescent East, University of Southampton, Southampton, SO17 1BJ; [2]University of Southampton, Centre for Biological Sciences, Bassett Crescent East, University of Southampton, Southampton, SO17 1BJ; [3]University of Leeds, Centre for Plant Sciences, School of Biology, University of Leeds, Leeds, LS2 9JT.

Plant parasitic nematodes (PPNs) infest the roots of crops and cause global losses with a severe economic impact on food production. Current chemical control agents are being removed from use due to environmental concerns and there is a need for new approaches in crop protection. A key feature of PPNs is a hollow stylet required for interaction with the host plant and feeding. This lance-like structure protrudes from the mouth of the worm and thrusts in a rhythmic manner to stab the host root. The underlying biology of stylet behaviour is poorly understood. We have addressed this by designing a microfluidic chip which traps the PPN *Globodera pallida* and permits the recording of an electrophysiological signal concomitant with stylet thrusting. The PDMS chip incorporates a precisely designed aperture to trap the nematode and valves for rapid application of test compounds and integral electrodes to facilitate acquisition of electrical signals. We show that stylet thrusting can be induced by controlled application of serotonin to the nematode. Each thrust and retraction produces an electrical waveform that characterises the physiological activity associated with the worm's behaviour. The ability to reproducibly record stylet activity provides a new platform for nematicide screening that focuses on a behaviour that is integral to the parasite host interaction. This is the first report of a microfluidic chip capable of electrophysiological recording from nematodes other than *Caenorhabditis elegans*. This approach may also be applied to other species of economic or medical importance.

MOLECULAR CLONING AND CHARACTERIZATION OF *MJ-1-1* FROM *MELOIDOGYNE JAVANICA*. **Hu[1,2], L., K. Zhuo[1,2], J. Liao[1,2].** [1]Laboratory of Plant Nematology, South China Agricultural University, Guangzhou 510642, China; [2]Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642, China.

Root-knot nematode, *Meloidogyne*, is one of the globally economically most damaging plant-parasitic nematodes causing great losses to many. Recently, some work has been done to explain the molecular mechanism involved in nematode infection. In the present study, a gene *Mj-1-1,* which encodes for a 149 aa protein with a predicted molecular mass of 17.982 KDa was isolated from *Meloidogyne javanica*. A real-time reverse-transcriptase polymerase chain reaction assay showed that expression of *Mj-1-1* was upregulated in later parasitic stages. The highest transcriptional level of *Mj-1-1* occurred in third-stage juveniles, and this high expression persisted in parasitic fourth-stage juveniles. *In planta* RNA interference targeting

Mj-1-1 suppressed the expression of *Mj-1-1* in nematodes and attenuated the parasitism ability of *M. javanica*. Meanwhile overexpression of *Mj-1-1* rendered tobacco susceptible to *M. javanica*. It can be concluded that *Mj-1-1* may play a role in parasitism of *M. javanica*, especially in later parasitic stages.

ENTOMOPATHOGENIC NEMATODES AND ENDOPHYTIC BACTERIA: A NOVEL APPROACH WITH POTENTIAL FOR PLANT HEALTH. **Hurley, M.J., D. Brazil and T. Kakouli-Duarte.** Molecular Ecology and Nematode Research Group, EnviroCORE, Department of Science and Health, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland.

Entomopathogenic nematodes (EPN) and bacterial endophytes have considerable potential in biological control and plant growth promotion, respectively. The use of EPN with various crop protection or improvement agents has been well documented. Similarly, there is evidence that bacterial endophytes can promote plant health and soil fertility. Although EPN and bacterial endophytes naturally function in a common habitat and have widespread agrobiological applications, the potential of synergy has yet to be explored. Mobilising the interaction of different nematode taxa with naturally occurring soil microbiota may result in increased plant growth and health, in addition to a reduction in the use of non-specific chemical fertilisers and pesticides. This project investigates the interaction between these two naturally occurring taxa. In preliminary experiments three EPN species, *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora,* were investigated for their interaction with a range of bacterial endophytes from our strain bank at EnviroCORE. In both laboratory and greenhouse trials the bacterial endophytes had no adverse effects on the ability of the EPN to kill *Galleria mellonella* hosts. In experiments where *G. mellonella* were exposed to increasing nematode doses, our results indicate that the presence of *Pseudomonas fluorescens* F113 and S118 caused *H. bacteriophora* to enter the insects at higher numbers. Experiments are currently ongoing in order to observe possible effects of the endophytes on *S. feltiae* and *H. bacteriophora* life cycle.

SUSCEPTIBILITY OF PASSION FRUIT SELECTIONS FROM THE ARC-ITSC BREEDING PROGRAMME TO ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA* AND *MELOIDOGYNE JAVANICA*). **Husselman, J.H., M.S. Daneel and W.P. Steyn.** Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa.

Passion fruit (*Passiflora edulis*) is a climber native to South America that is cultivated for its aromatic fruit in frost free areas around the world. In South Africa, production is primarily in Mpumalanga and Limpopo provinces with minor plantings in KwaZulu-Natal and the Eastern and Western Cape. The South African industry relies mainly on one cultivar namely 'Ester', a hybrid between the purple (*Passiflora edulis f. edulis*) and yellow granadilla (*Passiflora edulis f. flavicarpa*). Although viruses are the most important problem in the crop with no chemical cure available, leaf and soil diseases and nematodes also create considerable problems and selections from the breeding programme are screened for tolerance/resistance to these pests and diseases. Breeding parents are selected for good horticultural characteristics and tolerance to the pests and diseases. A trial to test nematode resistance was conducted with seven selections and twelve replicates in a complete randomised design. The purple commercial standard Ester (*Passiflora edulis f. edulis*) was included as a susceptible control and yellow granadilla (*Passiflora edulis f. flavicarpa*) as a tolerant control. The selections were multiplied by air-layers, transplanted in 2 litre plastic bags fill with sterilized sand eight weeks later after which the plants were placed in a glasshouse to establish for four weeks before being inoculated with 3200 *Meloidogyne* eggs/bag. Evaluation done at 56 days after inoculation showed low infection rate and evaluation was extended. Results will be discussed but differences were observed with *P. edulis* 'Ester' and *P. edulis f. edulis* having the highest nematode numbers.

ACTIVITY PROFILING REVEALS CHANGES IN THE DIVERSITY AND ACTIVITY OF PROTEINS IN *ARABIDOPSIS* ROOTS IN RESPONSE TO NEMATODE INFECTION. **Hütten[1], M., M. Geukes[1], J. C. Misas-Villamil[2], S. Habash[1], A. Elashry[1], R. A. L. van der Hoorn[2,3], F. M. W. Grundler[1] and S. Siddique[1].** [1]Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, 53115 Bonn, Germany; [2]Plant Chemetics lab, Max-Planck-Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany; [3]Plant Chemetics lab, Department of Plant Sciences, University of Oxford, South Parks Road, OX1 3UB Oxford, UK.

Cyst nematodes are obligate, sedentary endoparasites with a highly specialised biology and great economic impact in agriculture. The development of a syncytium is aided by a cocktail of nematode effectors that manipulate host plant activities in a complex network of interactions by post-translational modifications. Previous transcriptome and proteome studies of syncytia generated a wealth of data that is based on abundance rather than activity of transcripts or proteins. Activity-Based Protein Profiling (ABPP) was recently introduced in plant sciences and has been proven highly useful to display differential enzymatic activities of proteins by using activity based probes (ABPs). ABPs are small molecular probes (biotinylated or fluorescent) that react with a specific subset of enzymes in an activity-dependent manner ruling out all those proteins, which are inhibited, inactive or lack cofactors. To better understand functional proteomics that lead to formation of syncytia, ABPP was conducted on syncytia induced by the beet cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. This approach has identified genes and pathways that may play essential roles in feeding site development. Our data show that activity of several

papain-like cysteine proteases (PLCPs) and proteasomal subunits that are involved in activation of plant immune responses after pathogen attack is specifically suppressed in syncytia. Moreover, we identified effector proteins from *H. schachtii* that may target and inhibit PLCPs and proteasome in host plants. Our research contributes to a broader framework in understanding of cyst nematode parasitism and provides a platform to develop novel solutions against these pathogens.

GENE NETWORKS AND LIPID SIGNALS GOVERNING PLANT INTERACTIONS WITH THE ROOT-KNOT NEMATODE *MELOIDOGYNE JAVANICA*. **Iberkleid[1,2], I., N. Sela[1] and S. Brown Horowitz[1].** [1]Department of Entomology, Nematology and Chemistry units, Agricultural Research Organization, the Volcani Center, Bet Dagan, 50250, Israel; [2]Department of Plant Pathology and Microbiology, the Faculty of Agriculture Food & Environment, the Hebrew University of Jerusalem, Rehovot, 76100, Israel.

Plant parasitic nematodes produce a unique class of small helix-rich fatty acid and retinol binding proteins (FAR) with no counterpart in their plant hosts. *Meloidogyne javanica*'s Mj-FAR-1 is critical for the juvenile and sedentary development as well as the giant cell development presumably through compromising the plant defense by modulating plant lipid signals. For further insights into the role of Mj-FAR-1 protein in regulating disease development, we compared gene expression profiles of tomato hairy roots lines in which mj-far-1 is constitutively expressed and control roots at early and late stages after inoculation. Gene expression profiling revealed that 3970 transcripts were differentially expressed between the two lines; with 2069 up-regulated and 2205 down-regulated in the *mj-far-1* overexpressing line (OE) compared with the control root line over all inoculated and non-inoculated samples. A total of 61 up-regulated and down-regulated genes were overlapped between all non-inoculated and inoculated OE roots. This list might provide initial understanding of the mj-far-1 associated increased susceptibility that occurs in mj-far1-1 root line. These included genes involved in fatty acid metabolism, a group of hormone signals including Jasmonic acid and Auxin related genes, biotic stress related genes, and genes of the phenylpropanoid pathway that were also consistently differentially regulated. Validation of the data was confirmed through the use of quantitative real-time PCR of representative differentially expressed genes and through the use of β-glucuronidase reporter gene downstream of candidate gene promoters. Overall, the transcriptomic analysis indicates that, at the early time points samples clustered predominantly in relation to the differential genetics while at later time points samples clustered in relation to temporal dynamics related to nematode infection effect. Our results will shed light on the role of lipid signals and mj-far-1 effect in defining a common transcriptome that facilitate nematode infection.

STUDIES ON THE EFFECT OF *HETERODERA AVENAE* (WOLLENWEBER, 1924) ON YIELD LOSSES IN WHEAT VARIETIES IN THE EASTERN MEDITERRANEAN OF TURKEY. **Imren[1], M., H. Toktay[2], E.B. Kasapoğlu[3], A. Dababat[4] and I.H. Elekcioğlu[3]** [1]Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection Bolu, Turkey; [2]University of Nigde, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey; [3]Cukurova University, Faculty of Agriculture, Department of Plant Protection; [4]CIMMYT (International Maize and Wheat Improvement Centre), Wheat Improvement Program, Ankara, Turkey.

Globally *Heterodera avenae* (Wollenweber, 1924) is the most widely distributed and important species among cereal nematodes. The aim of the study was to determine yield losses caused by the *H*. Ha21 pathotype which was found in the Eastern Mediterranean region; one of the important spring wheat production centres in Turkey. Field trials were conducted during 2011 and 2012. The results of the study indicated that yield losses caused by *H.avenae* were between 4.36% - 25.7%, depending on the wheat variety. Bread wheat varieties, Adana 99 and Ceyhan 99, showed higher performance than the Silverstar wheat variety which is a known resistant variety against *H. avenae*. Although low nematode reproduction, high yield and less yield losses rates were found in Adana 99, high nematode reproduction rate, high yield rate and less yield losses were determined in Ceyhan 99. These findings suggest that Adana 99 is a moderately tolerant variety and Ceyhan 99 is a tolerant variety.

FUNGAL PARASITES OF EGGS AND CYSTS OF *GLOBODERA ROSTOCHIENSIS* AS POTENTIAL BIOLOGICAL CONTROL AGENTS OF POTATO CYST NEMATODE IN INDONESIA. **Indarti, S., Mulyadi, D. Widiayanto and J. Widada.** Agriculture Faculty of Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia.

Globodera rostochiensis produces considerable damage in potato production areas in Indonesia, and the infested area continues to increase each year. Potato cyst nematodes (PCN) is difficult to control because the cyst protects the eggs from unfavourable environmental conditions allowing the eggs to survive for years, and hatch only in the presence of host root exudates. The aim of this research was to develop biocontrol agents using fungal parasites of eggs and cysts, isolated from soil found to be suppressive to PCN. The research was carried out in three stages: 1) identification of the soil that had the ability to suppress the development of PCN and analysis of antagonistic activity of fungi potentially involved in the suppression of nematode populations, 2) isolation of fungi and testing their ability to parasitize PCN eggs and cysts and 3) testing the effectiveness of the fungi to control nematode populations in laboratory and pot trials, thus at mesocosm as well as macrocosm levels. PCN infested soils with ability to suppress PCN multiplication were found in Central Java, Indonesia. Twelve fungal isolates with the ability to parasitise more than 50 % of the PCN cysts and with extracellular proteolytic

activity were isolated from these locations. These fungi gave effective control to PCN populations in laboratory and pot trials compared to untreated treatments. The best performing isolate of this group suppressed the PCN cyst multiplication by 2.4 times (30.07 cysts/ 100 g soil compared with 73.53 cysts/ 100 g soil on control or without fungal treatment). Tuber yield from treated plants were also 1.6 times higher than the control.

PARTIAL SURVEY OF *MELOIDOGYNE* SPECIES ON COFFEE IN PARANA STATE, BRAZIL. **Ito, D.S., A.C.Z. Machado, S.A. Silva and O.F. Dorigo.** Insituto Agronômico do Paraná, Rodovia Celso Garcia Cid, km 375, 86047-902, Londrina, PR, Brazil

Paraná is an important State of Brazil in coffee production. However, root-knot nematodes have been causing great losses for the coffee crop. The aim of this study was to carry out a survey on the distribution of root-knot nematodes in coffee crops in Paraná State. For this, 50 samples of soil and roots were collected from coffee crops showing symptoms of nematode infestation, located in municipalities of Alto Paraná, Altônia, Bela Vista do Paraíso, Cambira, Carlópolis, Cianorte, Corumbataí do Sul, Esperança Nova, Kaloré, Lupionópolis, Moreira Sales, Munhoz de Melo, Paranavaí, Perobal, Pérola, Pinhalão, Pitangueiras, Rolândia, Santa Luzia, São Jorge do Patrocínio, São Tomé, and Xambré. From the total of samples analyzed, 31 (62%) contained *Meloidogyne paranaensis*, nine (18%), *M. incognita* and two (4%), *M. javanica*. Four samples (8%) showed a mixture of *M. incognita* and *M. paranaensis* and four (8%) presented *M. incognita* and *M. javanica*. The main nematode species present in Paraná State is *M. paranaensis*. These results will help in the mapping the distribution of nematodes in the coffee growing regions of Paraná, allowing to follow the dispersion of nematodes in the State and to manage more efficiently the populations present at each region.

ON THE DIVERSITY OF NEMATODES PARASITIC IN INVERTEBRATES OF MINDANAO ISLAND, PHILIPPINES. **Ivanova[1], E.S., N.H.N. Sumaya[2], A.B. Mohagan[3] and S.E. Spiridonov[1].** [1]A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr., 33, Moscow, 119071, Russia; [2]Biology Department, Mindanao State University-Iligan Institute of technology (MSU-IIT), Andres Bonifacio Ave., Tibanga, Iligan City, Philippines; [3]Biology Department, University of Central Mindanao, University Town, Musuan, Maramag, Bukidnon, Philippines.

Several representatives of interesting taxa of the nematodes of invertebrates were collected in the Island of Mindanao during a short field trip in November 2013. Large tropical millipedes of the order Spirobolida were found infected with a rich array of rhigonematids and ransomnematids inhabiting the hind gut of their host. For the first time since its description, predacious nematodes of the genus *Zalophora* Hunt 1994 were reported. Analysis of the D1D3 expansion segment sequences of Hethidae from Mindanao indicated close relationships with Indonesian species of *Heth*. Large tropical earthworms of the family Megascolecidae were collected in two habitats near Iligan City. The earthworms collected near Tinago Falls were found to be infected by the *Synoecnema* nematodes (Ungellidae). Phylogenetic analysis of LSU rDNA sequences has confirmed their independent position among other studied ungellids thus proving the validity of the genus *Synoecnema*. The earthworms collected in Initao-Libertad Protected Seascape and Landscape Forest were parasitized by nematodes of the genus *Filiponema* (nominal representative of the family Drilonematidae). These nematodes primarily found in the Philippines were also never molecularly characterised. Analysis of partial LSU rDNA sequence of *Filiponema* from Mindanao revealed their phylogenetic relationships with other tropical families of nematodes parasitic in earthworms (Ungellidae and Homungellidae), but not with Drilonematidae from temperate lumbricids (genus *Dicelis*). A mermithid from a spider caught on the Central Mindanao University campus, Musuan, Bukidnonwas characterised with LSU and SSU rDNA sequencing.

NEMATODE ASSEMBLAGES AND MICROBIAL COMMUNITIES IN SOYBEAN CROPPING SYSTEMS. **Jansen, C., S. Claassens and H. Fourie.** Unit of Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa.

Soil is an important ecosystem that supports a wide variety of organisms such as arthropods, bacteria, fungi, and nematodes. This sensitive ecosystem is influenced by various factors of which intensive agricultural management practices are the most destructive. With the introduction of genetically modified (GM) glyphosate-resistant (Roundup Ready: RR) crops, herbicides such as glyphosate (Roundup) has been increasingly used. With the proximity that microorganisms and nematodes have with the roots of these plants, these organisms can be used as bio-indicators. The aim of this study was to compare microbial community structure and nematode diversity in soil samples from conventional- and Roundup Ready- soybean fields in soybean production areas of South Africa to that in adjacent, uncultivated grassland areas. Microbial community structure was determined by phospholipid fatty acid analyses. Nematode diversity was determined by extracting the nematodes from soil samples with standard methods and conducting faunal analyses. Results indicated a definite difference in microbial community structure between the various localities; however, no differences in community structure between Roundup Ready- and conventional soybean samples were evident. Soils of both Roundup Ready - and conventional soybean crops were primarily dominated by bacteria. Nematode analyses also indicated no differences between the soils of these two treatments. A complete faunal analysis will be discussed including correlations that might exist between microbes and nematodes associated with Roundup Ready and conventional soybean plantings as well as natural veld areas.

NEMATODES FROM THE SEEKOEIVLEI NATURE RESERVE, MEMEL, SOUTH AFRICA. **Jansen van Rensburg, C. and A. Mobara.** Department of Zoology & Entomology, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa.

In South Africa, the amount of taxonomic information for freshwater nematodes from wetlands is lacking, when comparing it to Europe. The Seekoeivlei Nature Reserve (45 km^2), situated in the north-eastern corner of the Free State Province near the town of Memel, is the largest protected area of wetlands on the Highveld and in South Africa. This marshland is a very important sponge area for the Klip River which is a tributary to the Vaal River, providing water to the highly industrialised and densely populated Gauteng Province. Nematode samples were collected from three localities within the reserve with the aims of determining the nematode genera present, taxonomically describing some of the species belonging to these genera, and to determine whether the nematodes present could give an indication of what the ecological status of the wetland is. Soil samples were collected using the core-syringe method and transported back to the laboratory at the University of the Free State, Bloemfontein campus where nematodes were extracted and prepared for light and scanning electron microscopy using standard techniques. A total of 45 genera belonging to 24 different nematode families were identified. Nematode genera data was used to determine the: Maturity Index (MI), Shannon-Wiener diversity index (H'), as well as Hill's infinity (N∞). Results showed that nematode diversity was higher during the summer months compared to the winter months, with the Maturity index indicating that there may be some kind of ecological disturbance where the Klip River enters the Seekoeivlei Nature Reserve. Descriptions are currently underway for species belonging to the genera *Brevitrobrilus, Chronogaster, Eutobrilus* and *Tobrilus*.

PROVIDING A LINK BETWEEN MORPHOLOGICAL DESCRIPTIONS AND DNA BARCODING TO STUDY SPECIATION OF THE GENUS *PRATYLENCHUS* IN A ROBUST PHYLOGENETIC FRAMEWORK. **Janssen[1], T., G. Karssen[2], L. Waeyenberge[3], M. Couvreur[1] and W. Bert[1].** [1]Nematology Research Unit, Ghent University, Department of Biology, Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Plant Protection Service, Nematology section, P.O Box 9102, 6700 HC Wageningen, The Netherlands; [3]Institute for Agricultural and Fisheries Research, Crop Protection, Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium.

Root-lesion nematodes of the genus *Pratylenchus* are an important pest in economic crops. They have a worldwide distribution (except for the Polar Regions) and are poplyphagous. At the moment the genus consists of more than 70 species. The vast majority of these descriptions is based only on morphological and morphometric characters. However, it is well known as many authors have already stated that reliable identification of many species is impossible due to the lack of diagnostic characters, morphological interspecific plasticity and incomplete taxonomic descriptions. This often results in the impossibility to link original descriptions, based on morphological characterization, and contemporary molecular taxonomy. This task is further impeded as we demonstrated cryptic speciation in *Pratylenchus goodeyi,* providing additional evidence that cryptic speciation is common within the genus. To assess this species complex we investigated a new set of promising mitochondrial DNA markers for molecular species delimitation and barcoding. These new mitochondrial markers will be used to develop primers for mitochondrial genome sequencing. Finally, these mitochondrial genomes will be used to construct a robust phylogenetic framework to study speciation and allow an improved characterization and proper identification of *Pratylenchus* species.

MOLECULAR INSIGHTS INTO THE INTERACTION BETWEEN RICE AND ENDOPARASITIC NEMATODES. **Ji, H., K. Nahar, L. Bauters, G. Gheysen and T. Kyndt.** Department of Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium.

Rice (*Oryza sativa L.*) is one of the major staple foods in the world, and an interesting model monocot plant. Two nematodes, causing extensive crop yield losses, are *Meloidogyne graminicola* and *Hirschmanniella oryzae*. The response of rice upon infection with these nematodes was studied using transcriptome analyses on local and systemic tissues, at different time points and comparing the two different nematodes. The role of hormone pathways in the plant defence system was investigated using infection experiments after exogenous chemical treatments and in rice mutants/transgenics altered in different hormone pathways. Our transcriptome data show a strong and fast reduction of the plant defence system upon rice infection with the root-knot nematode *M. graminicola*, not only in local but also in systemic tissues. The SA and JA pathways were both strongly suppressed inside the nematode feeding sites, but activation of these pathways could counteract nematode infection substantially. The brassinosteroid pathway promotes plant susceptibility for root knot nematodes by its antagonistic interaction with the classical defence pathways. The strong systemic defence suppression upon root knot nematode infection leads to a significantly enhanced susceptibility for rice blast (*Magnaporthe oryzae*) in the above-ground tissues. In case of *H. oryzae* infection, a systemic suppression of the SA pathway was detected, potentially caused by the nematodes secretion of two effectors: chorismate mutase and isochorismatase. Over-expression of these effectors in transgenic rice lines leads to a significant interference with genes involved in SA-production.

TOP 10 PLANT PARASITIC NEMATODES IN MOLECULAR PLANT PATHOLOGY. **Jones[1], J.T., A. Haegeman[2], E. G.J. Danchin[3], H. S. Gaur[4], J. Helder[5], M. G.K Jones[6], T. Kikuchi[7], R. Manzanilla-López[8], J. E. Palomares-Rius[7], W. M.L. Wesemael[9,10] and R. N. Perry[8,10].** [1]James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK; [2]Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Caritasstraat 21, B-9090 Melle, Belgium; [3]INRA UMR 1355, CNRS UMR 6243, UNSA, 400 route des Chappes, F-06903 Sophia-Antipolis, France; [4]Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250 110, India; [5]Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [6]Plant Biotechnology Research Group, School of Veterinary and Life Sciences, WA State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia, WA 6150, Australia; [7]Division of Parasitology, Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Miyazaki 889-1692, Japan; [8]Department of AgroEcology, Rothamsted Research, Harpenden Herts, AL5 2JQ, UK; [9]Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit - Crop protection, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium; [10]Ghent University, Department of Biology, Ledeganckstraat 35, B-9000 Ghent, Belgium.

A survey of researchers working with plant-parasitic nematodes was undertaken in order to determine a ''top 10'' list of these pathogens based on scientific and economic importance. Any such list will not be definitive as economic importance will vary depending on the region of the world in which a researcher is based. However, care was taken to include researchers from as many parts of the world as possible when carrying out the survey. The top 10 list emerging from the survey was composed of (1) root-knot nematodes (*Meloidogyne* spp.), (2) cyst nematodes (*Heterodera* and *Globodera* spp.), (3) root lesion nematodes *Pratylenchus* spp., (4) the burrowing nematode *Radopholus similis*, (5) *Ditylenchus dipsaci*, (6) the pine wilt nematode *Bursaphelenchus xylophilus*, (7) the reniform nematode *Rotylenchulus reniformis*, (8) *Xiphinema index* (the only virus vector nematode to make the list), (9) *Nacobbus aberrans*, and (10) *Aphelenchoides besseyi*. The biology of each nematode (or nematode group) is reviewed briefly. A review article based on this survey has been published in *Molecular Plant Pathology* as part of the ''Top 10 pathogens'' series.

CANDIDATE SECRETED PROTEINS AND EFFECTORS OF *PRATYLENCHUS* SPP. **Jones[1], M.G.K., J.C.H. Tan[1] and J. Fosu-Nyarko[2].** [1]Plant Biotechnology Research Group, School of Veterinary and Life Sciences, Western Australia, Australia; [2]State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia 6150, Australia.

Root lesion nematodes (*Pratylenchus* species) are a group of economically important migratory endoparasitic plant pathogens that attack host roots of major crops. We have used Roche 454 deep sequencing of RNA extracted from mixed stages of *Pratylenchus thornei* and *Pratylenchus zeae* to generate reads, which following assembly and annotation, have provided transcriptomic data on sequences of genes expressed by these nematodes. Using this data to provide target sequences we have shown that both *P. thornei* and *P. zeae* are amenable to gene silencing (RNAi). By applying bioinformatics tools we have interrogated the transcriptome data for the presence of sequences that encode candidate secreted proteins, and from these about 30 candidate genes for such proteins have been identified. Some of these are common to sedentary endoparasitic nematodes, in particular genes encoding wall degrading enzymes, some found in the sedentary endoparasites are absent, and some candidate sequences have no known function. *In silico* comparative analysis of proteins and putative effectors present in the migratory and sedentary endoparasites will be provided as well as functional characterisation of some identified effectors using RNAi. Since *Pratylenchus* spp. do not reprogram cell identity and metabolism to form giant cells or syncytia, it would be expected that classes of effectors involved in those activities by root knot and cyst nematodes would be lacking, whereas the types of proteins secreted to aid entry and migration of nematodes through plant tissues, and to suppress host defences would be present in all endoparasitic nematodes.

DETERMINATION OF RACES OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN TURKEY. **Kacar, G. and I. H. Elekcioğlu.** University of Çukurova, Faculty of Agriculture, Department of Plant Protection, 01360, Sarıçam, Adana Turkey.

Root-knot nematodes are one of the most economically important pest species with an extremely wide host range and high damage potential worldwide. Nearly a 100 *Meloidogyne* species have been described. *Meloidogyne arenaria*, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, *Meloidogyne incognita* and *Meloidogyne javanica* are the most common and the most important *Meloidogyne* spp. in Turkey. In this study 50 *Meloidogyne* populations were collected from different region of Turkey. Races of *Meloidogyne* species were investigated by using differential host tests. The results indicated that *M. incognita* race 1, race 2, race 5, race 6; *M. arenaria* race 2, race 3; *M. javanica* race 1, race 5 and *M. chitwoodi* race 1 and race 2 are present in Turkey. *Meloidogyne incognita* race 5, *M. javanica* race 5 and *M. chitwoodi* race 1 and race 2 constitutes the first record of these races in Turkey.

ECOTOXICITY OF SILVER NANOPARTICLES SYNTHESIZED FROM *AZADIRACHTAINDICA* AND *CURCUMA LONGA* ON ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. **Kalaiselvi[1], D., P. Sundararaj and S.L. Hafez[2].** [1]Department of Zoology, Bharathiar University, Coimbatore, India; [2]University of Idaho, Idaho, USA.

Experiments were carried out to establish the efficacy of green synthesized silver nanoparticles by using two different tropical plants *Azadirachtaindica* and *Curcuma longa* on the mortality of second stage juveniles of the root-knot nematode

Meloidogyne incognita. Characterization of nanoparticles was carried out using scanning electron microscopy (SEM), X-ray diffraction studies, FT-IR techniques, DLS techniques, zeta potential studies and the nano size was confirmed. Initially, the formation of silver nanoparticle was confirmed by UV spectroscopy after adding the respective plant extract as a reducing agent to the 0.01 M silver nitrate solution. Second stage larvae of *M.incognita* hatched out from the eggs of adult female nematodes cultured on tomato roots were exposed to respective plant extract concentrations of 10 mg L^{-1}, 25 mg L^{-1}, 50 mg $L^{-}1$ and 100 mg L^{-1}. There was a significant difference in the mortality of second stage juveniles as a result of their exposure to the hextracts of *A. indica* or *C. longa*. Differences were also observed in mortality induced by different concentrations of both plant extracts and there is a linear positive relationship between the larval mortality and the concentration of plant extract.

UNRAVELING MI-1 IMMUNE RECEPTOR PERCEPTION AND SIGNALING IN TOMATO. **Kaloshian, I.** Department of Nematology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521.

The tomato (*Solanum lycopersicum; Sl*) gene *Mi-1* confers broad-spectrum resistance to three species of root-knot nematodes (*Meloidogyne* spp.) and three phloem feeding insects including potato aphid (*Macrosiphum euphorbiae*). It is not clear how *Mi-1* is able to recognize avirulence effectors from these diverse groups of pests and whether the detection of nematode and insect pests involve similar recognition complexes. *Mi-1* encodes a nucleotide-binding leucine-rich repeat immune receptor with no subcellular localization signal. Surprisingly, using confocal microscopy and biochemical fractionation, we found that *Mi-1* is localized to three subcellular pools including the plasma membrane, cytoplasm and the nucleus. Using forward genetics, we identified *Somatic Embryogenesis Receptor Kinase 1* (*SERK1*) to be required for *Mi-1*-mediated aphid resistance but not for root-knot nematode resistance. SERK1 is a transmembrane protein localized to the plasma membrane. Co-immunoprecipitation experiments in both *Nicotiana benthamiana,* transiently expressing *Mi-1* and *Sl*-SERK1, and in 35S-*Sl-SERK1*-HA resistant tomato cultivar Motelle showed that *Mi-1* and *Sl*-SERK1 are present in a complex in the microsomal fractions. Using reverse genetics, we have identified among others, members of WRKY transcription factors, known regulators of plant immunity inducible transcriptional network. *Sl-WRKY72a* and *Sl-WRKY72b* are upregulated by both root-knot nematode and aphid feeding and required for resistance to both pests. Interestingly, *Arabidopsis thaliana* WRKY72 seem to regulate a network of genes independent of the known defense hormone salicylic acid. Our work suggests similar *Mi-1*-mediated defence responses to *Meloidogyne* spp. and aphids but distinct *Mi-1* recognition complexes to these organisms.

DIVERSITY AND TRANSMISSION OF SOIL TRANSMITTED STRONGYLID NEMATODES BETWEEN HUMANS AND OTHER PRIMATES IN THE WILD. **Kalousova[1], B., H. Hasegawa[2], D. Modry[1,3,4], M. Kitagawa[2], K. A. Shutt[5], A. Todd[6], I. Profousova[1] and K. J. Petrzelkova[1,4,7 8].** [1]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic; [2]Department of Biology, Oita University, 1-1 Idaigaoka, Hasama, Yufu, 879-5593 Oita, Japan; [3]Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, Czech Republic; [4]Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic; [5]Department of Anthropology, Durham University, South Road, DH1 3LE Durham, United Kingdom; [6]WWF, Dzanga Sangha Protected Areas, Avenue des Martyrs, BP 1053 Bangui, Central African Republic; [7]Liberec Zoo, Masarykova 1347/31, 460 01 Liberec, Czech Republic; [8]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Kvetna 8, 603 65 Brno, Czech Republic.

Humans have historically shared habitat with nonhuman primates. However, the dynamics of human-primate interactions have recently changed dramatically and increased as a result of forest fragmentation, habitat encroachment and tourism. Little is known about the diversity and transmission of helminthes between humans and other primates and the presence of potential zoonotic strongylids should be viewed as a major concern for both public health and primate conservation. Generic diagnosis of strongylids is traditionally facilitated by morphological examination of L3 larvae; however, coprocultures for the development of larvae are not commonly performed in routine diagnostics of strongylids in primates. The difficulties in diagnosing hookworms combined with their clinical relevance have driven the need for molecular research/diagnostic approaches. We therefore studied the diversity and transmission of soil transmitted strongylid nematodes between humans and other primates inhabiting Dzanga Sangha Protected Areas, Central African Republic and Campo Ma'an National Park, Cameroon. We developed the filariform larvae of hookworms from faeces by using Harada-Mori filter-paper culture and identified them based on morphology. We sequenced and compared the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA and partial cytochrome *c* oxidase subunit 1 (*cox1*) gene of mtDNA obtained from larvae to identify the molecular type. Our data revealed an as yet undocumented diversity of hookworms (Ancylostomatoidea: Ancylostomatidae) in western lowland gorillas, chimpanzees, agile mangabeys and humans and provided evidence for the transmission of some parasite taxa among primates and humans as well as the discovery of a novel nematode species affecting humans.

THE ROLE OF JASMONIC ACID AND ETHYLENE DURING ATTRACTION AND EARLY DEFENCE REACTIONS TO *HETERODERA SCHACHTII* IN *ARABIDOPSIS THALIANA*. **Kammerhofer[1], N., P. Dobrev[2], R. Vankova[2], M.A.J. Regis[1], J. Hofmann[1] and K. Wieczorek[1].** [1]Division of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, UFT Tulln, Konrad Lorenz Strasse. 24, 3430 Tulln, Austria; [2]Academy of Sciences of the Czech Republic, Institute of Experimental Botany, Rozvojová 263, 165 02 Prague 6 – Lysolaje, Czech Republic.

The root penetration, intracellular migration and syncytium initiation of *Heterodera schachtii* were suggested to induce plant defence responses in their hosts that were impaired at later stages of syncytium expansion and nematode development. The potential induction of plant defence during nematode invasion and migration is however poorly studied so far even though it may determine host plant susceptibility at that stage. Accordingly, phytohormones were shown to play significant roles in feeding site induction and nematode development, but their effect on nematode attraction and invasion during the first 24 hours was only studied to a minor extent. Thus the aim of the present work was to elucidate stress hormone based defence pathways which occur at the onset of infection. First, the potential role of salicylic acid, jasmonic acid and ethylene during nematode migration and syncytium induction was tested by analysing hormone levels and also the expression of frequently applied marker genes in infected compared to non-infected plants. Next, hormones or their inhibitors were applied to the shoots of *Arabidopsis* to be transported towards the roots. The successful translocation and induction of plant responses was controlled by analysing hormone and transcript levels as before. After this system was proved successful the potential effects of hormone application on the attraction, infection and development of *H. schachtii* was studied. Results suggest a pivotal role of jasmonic acid and ethylene in attraction as well as early infection process of *H. schachtii*. Findings of this work can contribute in understanding plant – nematode interactions and could be integrated in future resistance breeding programmes.

BURSAPHELENCHUS SP. (TYLENCHOMORPHA: APHELENCHOIDIDAE) A PHORETIC ASSOCIATE OF *XYLEBORINUS GRACILIS* (COLEOPTERA: SCOLYTINAE) FROM AVOCADO IN FLORIDA. **Kanzaki[1, 2], N., R.M. Giblin-Davis[1], D. Carrillo[3], L. Duncan[3] and R. Gonzalez[1].** [1]Fort Lauderdale Research and Education Center, University of Florida/IFAS, 3205 College Avenue, Davie, FL 33314, USA; [2]Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan; [3]Tropical Research and Education Center, University of Florida/IFAS, 18905 SW 280 Street, Homestead, FL 33301, USA.

During an experimental host-plant survey for the invasive red bay ambrosia beetle, *Xyleborus glabratus*, in Homestead, Florida, a native species of ambrosia beetle, *Xyloborinus gracilis* was found to be associated with a *Bursaphelenchus* species. This nematode species was isolated from *X. gracilis* and cultured on *Monilinia fructicola* or *Botrytis cinerea* for further study and was determined to be new to science and a putative sister species to *B. kiyoharai* because of two derived characters in males; possession of a tail spike versus the typical bursal flap, and the apparent absence of the P1 ventral single papilla, both typically plesiomorphic characters for the genus. Additionally, *B. kiyoharai* is associated with *X. serriatus* suggesting that the host and microbiome associations that are shared between these two species that are carried by ambrosia beetles may have ecological and biological significance in their evolution and lineage radiation. Molecular phylogenetic analyses of the near-full-length small subunit (SSU: 18S) and the D2/D3 expansion segments of the large subunit (LSU: 28S) confirmed that *B. peñai* n. sp. is very closely related to *B. kiyoharai* which is a member of the *B. fungivorus* clade which includes *B. thailandae* and *B. willibaldi*. The newly-found *Bursaphelenchus* sp. and *B. kiyoharai* both share very similar overall spicule morphology with the *B. fungivorus* clade. The new species is described and can be typologically differentiated from *B. kiyoharai* by the position of the hemizonid and its different geographical and host associations.

DIVERSIFICATION OF TERMITE-ASSOCIATED NEMATODES IN RELATION TO EVOLUTIONARY PATTERNS OF DIVERSITY. **Kanzaki[1, 2], N., R.M. Giblin-Davis[2] and K.A. Davies[3].** [1]Forestry & Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [2]Fort Lauderdale Research & Education Center, University of Florida/IFAS 3205 College Avenue, Davie, Florida 33314 USA; [3]Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia.

Entomophilic nematodes are important subjects as indicators of biodiversity, potential genetic resources, and potential biological control agents. The nematode fauna associated with termites and their relatives were examined as predictors of nematode diversity. The termites were collected in the forests in the American and East Asian tropics and subtropics, and dissected to determine the nematode species associated with each termite species. In addition, as relatives of termites, some roach and woodroach species were also collected and examined for their associated nematodes. Information from the literature was also examined to complement deficiencies from our survey work. The termites and related insects were separated into four groups according to the pattern of associated nematodes. Roaches, phylogenetically the most basal group, were associated with external and internal parasites (*e.g.*, Thelastomatids); woodroaches, intermediate between roaches and termites, were not found to be associated with any nematodes; lower termites (Kalotermitidae) were associated with some phoretic *Poikilolaimus* spp. and *Halicephalobus* spp. which were isolated only from kalotermitids; and the other more derived termite groups were associated with various phoretic rhabditids, diplogastrids and aphelenchoidids and a few

parasitic species. Comparing the associated nematode assemblages and phylogenetic relationships of their insect hosts, the nematode association is hypothesized to have been lost by the common ancestor of woodroach/termites, *i.e*., the insects might have escaped from nematode associations, and then re-obtained and expanded the associated nematode fauna as the termite lineages radiated.

DIVERSITY OF FIG-ASSOCIATED NEMATODES AS A MODEL SYSTEM FOR STUDYING BIOGEOGRAPHY, HOST SWITCHING AND COLONIZATION OF NEW HABITATS. **Kanzaki[1, 2], N., R. Giblin-Davis[2] and K.A. Davies[3].** [1]Forestry & Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [2]Fort Lauderdale Research & Education Center, University of Florida/IFAS 3205 College Avenue, Davie, Florida 33314 USA; [3]Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia.

Trees belonging to the genus *Ficus*, comprise a very interesting plant group. Fig trees are associated with their mutualistic pollinator fig wasps, and the patterns of their host associations with pollinator wasps, inquilines and nematodes are serving as an excellent model system for studying co-speciation and host switching. Several different lineages of nematodes are involved in this interaction and promise high species diversity. However with over 700 species of *Ficus* worldwide, the information about the diversity and biological interactions of this group and other fig-associated nematodes has not been sufficiently studied. Therefore, the authors have conducted several field surveys of fig-associated nematodes in Central America, Australia and the East Asian tropics and subtropics, as well as some African specimens deposited by previous investigators. During these surveys, many new species/genera have been observed, and new species (*e.g*., *Parasitodiplogaster* spp. and *Schistonchus* spp.), and a new genus (*Teratodiplogaster*) have been described. Furthermore, by comparing the nematode fauna from figs from the surveyed areas, region-specific nematode lineages were discovered. Also, several different confamilial genera (lineages) were confirmed with evidence of morphological and life history trait convergence, *e.g*., at least four clades of aphelenchoidids have converged around the general morphology and trait of plant-parasitism of ''*Schistonchus*'', and three lineages of diplogastrids have converged around the traits of parasitism/necromeny of the ''*Parasitodiplogaster*''. The authors will summarize current knowledge of the diversity of fig-associated nematodes from field surveys.

PARASITODIPLOGASTER SPECIES ASSOCIATED WITH PHARMACOSYCEA FIGS IN PANAMA. **Kanzaki[1, 2], N., R.M. Giblin-Davis[1], W. Ye[1,3], E.A. Herre[5] and B.J. Center[1].** [1]Fort Lauderdale Research and Education Center, University of Florida, 3205 College Ave, Davie, Florida 33314–7799, USA; [2]Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [3]Present address: Nematode Assay Section, Agronomic Division, North Carolina Department of Agriculture & Consumer Services, 4300 Reedy Creek Road, Raleigh, NorthCarolina 27607, USA; [4]Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama.

Parasitodiplogaster species that are associated with figs in the *Ficus* subsection *Pharmacosycea*, *i.e*., *Ficus maxima*, *Ficus yoponensis*, *Ficus insipida* and *Ficus glabrata* were examined based on morphological characters and molecular sequences. Some nematodes isolated from the fig sycones were observed and recorded for their morphotype, and then digested for their DNA, while others were fixed in formalin-glycerin for additional morphological study. High resolution microscopic observation of the morphological materials yielded five morphotypes including two nominal species, *Parasitodiplogaster maxinema* and *Parasitodiplogaster pharmaconema*, and these morphotypes were distinguished from each other by male tail characters. In contrast, five and six genotypes were recognized by D2/D3 expansion segments of the large subunit (LSU) and near-full-length sequences of small subunit (SSU) ribosomal RNA genes, respectively. Except for the two nominal species, the genotypes and morphotypes were not clearly correlated because of limitations in the microscopic resolution in the initial morphotyping. Although the morphotypes and genotypes were not clearly paired, *Pharmacosycea*-associated *Parasitodiplogaster* species which are tentatively referred to as the ''*P. maxinema* group'' formed a monophyletic clade in both D2/D3 LSU and SSU analyses, and are morphologically characterized by their stomatal morphology, *i.e*., a tube-shaped stoma with two stick-like teeth and male tail morphology, presence of nine paired papillae and relatively slender spicule.

A *BURSAPHELENCHUS* SPECIES HAS BECOME A PLANT-PARASITE IN FIGS AND CONVERGED IN APPEARANCE WITH *SCHISTONCHUS*. **Kanzaki[1], N., R. Tanaka[2], R.M. Giblin-Davis[3] and K.A. Davies[4].** [1]Forestry & Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687 Japan; [2]Division of Parasitology, Faculty of Medicine, University of Miyazaki, Miyazaki 889-1692, Japan; [3]Fort Lauderdale Research & Education Center, University of Florida/IFAS 3205 College Avenue, Davie, Florida 33314, USA; [4]Centre for Evolutionary Biology and Biodiversity, School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia.

Some morphological and biological characters of an undescribed *Bursaphelenchus* species are reported. The species was found from syconia of a Japanese native fig, *Ficus variegata* in Ishigaki Island, Okinawa, Japan. Although the *Bursaphelenchus* sp. shares an important typological character, male spicule possessing a strongly recurved condylus, with the

Bursaphelenchus eremus group and the *Bursaphelenchus leoni* groupof the genus, it was inferred to be monophyletic with the *Bursaphelenchus fungivorus* group based on the molecular sequences of ribosomal RNA genes. Further, the stylet morphology of the species is very unique in the genus, i.e., it is very long, thick and possessing long (ca 50% of total length) conus and large basal swellings and well-developed pharyngeal glands. The morphology is rather similar to that of the *Schistonchus* paraphyletic assemblage of the fig-floret parasitic group. Thus, the very unique stylet morphology found in the species appears to be an extreme example of convergent evolution in the nematode family, Aphelenchoididae inside figs. Whereas the other characters shared by the new species and its close relatives, *i.e*., lack of ventral P1 male genital papilla, female vulval flap, and papilla-shaped P4 genital papillae in males, corroborate the molecular phylogenetic inference. The unique biological character of obligate plant parasitism and highly derived appearance of the ingestive organs of *Bursaphelenchus* sp. expands our knowledge of the potential morphological, physiological and developmental plasticity of the genus *Bursaphelenchus*.

STRUCTURE FUNCTION OF SOIL NEMATODE COMMUNITIES AFTER APPLE ORCHARD FLOOR MANIPULATIONS. **Kapp[1], C., S.G. Storey[2] and A.P. Malan[1].** [1]Department of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa; [2]Nemlab Diagnostic Laboratories, c/o R44, Anyswortelrug Road, Klapmuts, South Africa.

As an essential non-renewable resource in all land-based ecosystems, it is imperative to develop methods in which to maintain the health, biological productivity and sustainability of our agricultural soil. The importance of this is indeterminate when considering the increase in the global human population and the consequent proliferation on the demand for food production. This has prompted research to develop uses of different biological indicators of soil health. Nematodes are one of the most abundant groups of Metazoa and form an integral part of the soil food web at several trophic levels, responding rapidly to any environmental changes. Characterization according to functional guild can impart valuable insight into the condition of the soil food web. A study was undertaken to determine the biodiversity and functionality of soil nematodes associated with the effects of various orchard floor manipulations in an apple orchard in the Western Cape province of South Africa. Samples were collected from the ridge of the tree row of each treatment plot. The nematodes present within each treatment were identified to family level, in addition to being quantified. Indices for the determination of ecosystem function were calculated for each treatment and a faunal analysis was done. Results will be discussed in terms of soil enrichment and structure as well as the increase or decrease in the levels of maturity over time.

THE EFFECT OF DIFFERENT TOMATO GENOTYPE ON ROOT-KNOT NEMATODE-FUSARIUM WILT COMPLEX IN COASTAL KENYA. **Kariuki[1], P.M., F. Kariuki[1], G. M. Kariuki[2] and D. L. Coyne[3].** [1]Department of Plant and Microbial Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya; [2]Department of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya; [3]International Institute of Tropical Agriculture (IITA), c/o *icipe*, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya.

Tomato is one of the most popular vegetable crops grown worldwide, owing to its nutritive value and diversified use. The disease complex involving root-knot nematodes (*Meloidogyne* spp.) and *Fusarium oxysporum* is a common occurrence on tomato, and especially along the coast in Kenya. The effect of the disease complex was studied under screen house conditions in pots using five cultivars with varying levels of resistance to the two pathogens. The effects of both pathogens individually and sequentially were assessed on the five cultivars. Isolates of root-knot nematodes and *F. oxysporum* singly and concomitant caused significant reduction in both shoot and root growth on tomatoes over the un-inoculated control. The reduction was however, greater when both pathogens were inoculated together, as opposed to when root-knot nematodes were inoculated singly. Fusarium wilt inoculation alone had the least reduction in the growth parameters. Galling index, egg mass index and the number of juveniles in the soil reduced significantly while the Fusarium wilt index increased on sequential inoculation of the pathogens. Among the five cultivars tested tomato cv. Kilele showed the least galling index, egg mass index, juveniles' population in the soil as well as the least Fusarium wilt index. It was followed by cv. Bt Okitu 101 and cv. T- R-3034-3-10-N-UG respectively. The most susceptible cultivars were Cal J and Hawai 7996. The study concludes that both pathogens interacts and are virulent against the tomato cultivars.

SAFE AND EFFECTIVE NEMATODE AND OTHER PESTS MANAGEMENT STRATEGIES TO STRENGTHEN THE TOMATO VALUE CHAIN IN COASTAL KENYA. **Kariuki[1], G.M., L. Muriuki[1], A.K. Thuo, J.W. Kibunja[2], P.M. Kariuki,[2] and D.L. Coyne[3].** [1]Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya; [2]Department of Plant and Microbial Sciences, Kenyatta University, Nairobi, Kenya; [3]International Institute of Tropical Agriculture (IITA), c/o ICIPE, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya.

With rising population and urbanization in the developing world, peri-urban agricultural systems become more intensely cultivated and plagued by pests and diseases. Since individual pest control measures each have limitations, the integration of several tactics is often necessary. When available, host resistance should be incorporated as a first line defence mechanism to reduce pesticide reliance. Unfortunately, vegetable crops, particularly tomato are usually susceptible to a range of pests,

including root-knot nematodes (*Meloidogyne* spp.), bacterial wilt, fusarium wilt and viruses. Resistance needs to be locally appropriate to species or strain variability and durable under multiple pest attack. To address this problem the current study is being conducted along the East African coast to: 1) determine the distribution and relative occurrence of virulent species, races and/or strains of begomoviruses, *Ralstonia solanacearum* and *Meloidogyne* spp. and fusarium wilt on tomato, 2) select tomato germplasm with multiple resistance against begomoviruses, *R. solanacearum* and *Meloidogyne* spp. and 3) explore and develop novel bio-pesticides for ttomato cultivation for action against begomoviruses (or vectors), *R. solanacearum* and *Meloidogyne* spp. Molecular techniques are being employed to attain accurate pest diagnostics and marker assisted identification of resistance and host interactions. The use of endophytic fungi *Trichoderma* spp. alongside moderately resistant varieties have been found to be effective against *Meloidogyne* spp. on tomato and also against disease complexes involving root-knot nematodes, Bacterial wilt and fusarium wilt. The beneficiaries of this study will be the small-scale farmers in coastal East Africa.

NIMITZ™: A PARADIGM CHANGE IN NEMATODE CONTROL. **Karmon, D.** ADAMA Agricultural Solutions, Golan Street, Airport City 7019900, Israel.

Worldwide damage to crops by nematodes is currently estimated to be greater than US$ 120 billion per annum. Existing measures taken by farmers to cope with nematode damage vary and include crop rotation, resistant varieties, sanitation, ''suppressive crops'', organic substances and chemical PPP's (?). The availability of PPP's has declined over recent years. Methyl bromide has been phased out while other soil fumigants like metham sodium & 1,3-dichloropropen are under increasing regulatory pressure. Specific nematicides belong mostly to organophosphate (OP's) and carbamate groups are also under increasingly heavy regulatory pressure in many countries. ADAMA is currently in the process of registering NIMITZ™, which contains the active ingredient fluensulfone. Fluensulfone belongs to novel chemical class - Fluoroalkenyl (-thioether). NIMITZ™ is a true nematicide, with irreversible activity, causing the death of nematodes, as compared to OP's and carbamates that only have reversible nematostatic activity. NIMITZ™'s novel mode of action affects the different developmental stages of nematodes in a variety of ways. NIMITZ™ has toxicity and ecotoxicity profiles which are much better than the existing nematicides. NIMITZ™ registration is expected shortly in several leading countries with the main target crops being the families of Solanaceae and Cucurbitaceae. Additional crops will be added to the registration at a later stage.Global field trials program proved the excellent efficacy of NIMITZ™ in controlling root knot nematodes (*Meloidogyne spp.*) as well as a variety of other economically important species. Trial results are presented here.

INVESTIGATING THE NEUROPHARMACOLOGICAL REGULATION OF STYLET BEHAVIOUR IN *GLOBODERA PALLIDA*. **Kearn[1], J., A. Dorey[1], P. Urwin[2], C. Lilley[2], V. O'Connor[1], L. Holden-Dye[1].** [1]University of Southampton, Centre for Biological Sciences, Bassett Crescent East, University of Southampton, Southampton, SO17 1BJ, UK; [2]University of Leeds, Centre for Plant Sciences, School of Biology, University of Leeds, Leeds, LS2 9JT, UK.

All plant parasitic nematodes possess a stylet; a, protrusible mouth spear. The stylet is essential in the life cycle as it is required for the emergence of second stage juvenile (J2) from the egg, the penetration of host roots,or migration through the host and feeding. The thrusting of the stylet when the nematode is in contact with the host root mechanically pierces or separates the host cells and enables the first step in the life cycle. Due to the importance of the stylet in plant parasitic nematodes, its behaviour presents an attractive target for agrochemical control of plant parasitic nematodes. The neurobiology of this behaviour is however relatively uncharacterised. Here, we have used the potato cyst nematode *Globodera pallida* to study the effects of neurotransmitters, neuromodulators and pharmacological inhibitors on stylet thrusting and movement to gain a greater insight into how the behaviour of this crucial structure is regulated and controlled. Our data suggest that there are similarities between the neuropharmacological regulation of stylet behaviour in plant parasitic nematodes and the regulation of feeding in other nematode species. Using our data we have begun to develop a putative model of the neurotransmitters and neuromodulators involved in this behaviour and we look to develop this model further and to study the pharmacology of the stylet in other plant parasitic nematodes.

MODE OF ACTION STUDIES ON FLUENSULFONE, A NEW NEMATICIDE. **Kearn, J., E. Ludlow, V. O'Connor, L. Holden-Dye.** University of Southampton, Centre for Biological Sciences, Bassett Crescent East, University of Southampton, Southampton, SO17 1BJ.

Plant parasitic nematodes present an unacceptable burden to global food security, with nematode infection directly responsible for $125 billion of yield losses every year. The nematode problem is exacerbated by a lack of nematicides, as many chemicals have had their use restricted due to concerns over their non-selective toxicity. Fluensulfone is a new nematicide that is selectively toxic towards nematodes and thus has reduced potential for environmental damage. The mode of action of fluensulfone is however not known. Here, we have performed mode of action studies, utilising the model organism *Caenorhabditis elegans*, and the cyst nematode *Globodera pallida*. *Caenorhabditis elegans* has been previously used as a model organism in mechanism of action studies on anthelmintic compounds, due to the wealth of knowledge concerning its neuropharmacology and its experimental tractability. *Globodera pallida* was used to determine the relevance

of these observations to the activity of fluensulfone against plant parasitic nematodes. We have found that fluensulfone has complex and pleiotropic actions on *C. elegans*, including effects on locomotion, development, egg laying, egg hatching and feeding behaviour. These observations and analysis of *C. elegans* mutants suggest a mode of action that is distinct from other nematicides and anthelmintics. The effects of fluensulfone on the feeding behaviour of *C. elegans* and *G. pallida* are complex, and suggest an interaction with neural signalling pathways, a hypothesis that is supported by further pharmacological studies. Further studies on *C. elegans* and *G. pallida* are in progress to delineate the molecular mechanism(s) underpinning these responses to fluensulfone.

INVESTIGATING *STEINERNEMA FELTIAE* AS A SUITABLE INDICATOR ORGANISM FOR HEAVY METAL TOXICITY ASSAYS. **Kelly, E. and T. Kakouli-Duarte.** Molecular Ecology and Nematode Research Group, EnviroCORE, Department of Science and Health, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland.

Heavy metals occur in the environment naturally, but through mining and industrialization they can accumulate to toxic levels. This work explores the suitability of *Steinernema feltiae* as a sentinel species for heavy metal pollution. Nematodes are widely used as environmental indicators. *Steinernema feltiae* is a widespread soil-dwelling nematode, normally parasitizing insects to complete its life cycle, and possessing an age-synchronized developmental stage particularly suitable for toxicity testing. Here we report on laboratory based toxicity assays where third stage juveniles of the nematode were used as biomarkers for the effects of cadmium, lead and zinc. Nematodes were exposed to specific levels of heavy metals for different time periods. Cadmium and lead had a most profound negative effect on nematode life span in a time dependent manner. Experiments were also conducted in sand columns and on agar plates, in order to observe the effects of heavy metals on nematode motility and attraction to insect hosts. The host seeking ability of the nematodes was not negatively affected by heavy metals in sand, but their movement was compromised by heavy metals on agar so they did not respond directionally to the hosts. When nematode development in larvae of the moth *Galleria mellonella* was investigated, lead appeared to delay J1 and J2 formation and cadmium was detrimental to fecundity and J3 emergence. These results constitute a first report of metal toxicity end points that may be developed to use this nematode as a sentinel for heavy metal pollution.

VARIABILITY IN RESPONSE OF WHEAT AND *AEGILOPS* GENOTYPES TO MEDITERRANEAN CEREAL CYSTS NEMATODE (*HETERODERA LATIPONS*). **Kherfan[1], W.I., F. C. Ogbonnaya[2,3], L. Al-Banna[1].** [1]Department of Plant Protection, University of Jordan: University of Jordan Amman-11942-Jordan; [2]International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria; [3]The Grains Research & Development Corporation, PO Box 5367, Kingston, ACT 2604, Australia.

The Mediterranean cereal cyst nematode, *Heterodera latipons*, is one of the major pests that affect wheat production in Jordan and other countries in the Mediterranean region. To successfully deploy genetic resistance as a control measure, the reaction of bread wheat cultivars, synthetic hexaploids of wheat and *Aegilops* genotypes to a Jordanian isolate of the Mediterranean cereal cysts nematode, *H. latipons* were investigated. Furthermore, the impact of different sources of genes for resistance to this cyst nematode was compared. The examination of stained roots harvested after two, three and four weeks of inoculation from all genotypes, showed that the penetration of J2s occurred in all the genotypes. However, the total number of the penetrated J2s varied among lines and were less in *Aegilops* genotypes. The presence of different developmental stages of the nematode were higher in number and developed faster in the susceptible and moderately susceptible wheat genotypes than in the resistant ones. Interestingly, some *Aegilops* genotypes did not support the development of J2s to further stages. The development of J2s to J3s and J4s were less in wheat genotypes carrying the *Cre3* resistant gene than those genotypes carrying *Cre1* and *Cre8* resistant genes. These findings suggest that different pre- and post-penetration resistance mechanisms may be associated with the differential response of the genotypes. Results from this study confirmed that the previously identified cereal cyst nematode resistance genes (*Cre1*, *Cre3* and *Cre8*) confer resistance to the Jordanian pathotype of *H. latipons* used in this study.

IN VITRO EVALUATION OF EXTRACT AND FRACTIONS OF *MAERUA ANGOLENSIS* AND *TABERNAEMONTANA ELEGANS* ON MOTILITY AND HATCH INHIBITION OF *MELOIDOGYNE INCOGNITA* RACE 2. **Khosa[1], C., A. Mc Donald[2], M. Daneel[1], D. De Waele[3], G. Fouche[4], M. Tselanyane[4] and F. Calitz[5].** [1]ARC-Institute of Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa; [2]North-West University, School of Environmental Sciences and Development, Plant Protection, Private Bag X6001, Potchefstroom, 2520, South Africa; [3]Laboratory for Tropical Crop Improvement; Catholic University of Leuven, Kasteelpark Arenberg 13, 3001, Heverlee, Belgium; [4]Biosciences, Council of Scientific and Industrial Research, P.O. Box 395, Pretoria, 0001, South Africa; [5]ARC-Central Office, P.O. Box 8783, Pretoria, 0001, South Africa.

Plant-derived metabolites have attracted the most attention of all prospective alternatives to pesticides, either as plant extracts, formulated phytochemicals or as organic amendments to soil. Several plant species have been reported to contain metabolites with nematoxic activity and there is continuous effort in discovering new plant sources with such characteristics. Plant extract and fractions of *Maerua angolensis* and *Tabernaemontana elegans* were tested for nematicidal activity in terms

of nematode second-stage juvenile (J2) motility and egg hatch inhibition at 10 different concentrations (0.1-1.0 mg ml^{-1}) each in 96-well test plates. Four independent trials for each plant extract concentrate were arranged in a randomized-complete block design, with the eight treatments in each test replicated four times each. *In vitro* bioassay studies confirmed that extract and fractions of varying polarity of both plant products *M. angolensis* and *T. elegans* might be toxic to J2 stages of the root-knot nematode *Meloidogyne incognita*. All extract/fractions of *M. angolensis* tested caused immobility of J2, whereas only three extract/fractions of *T. elegans* affected mobility of J2 adversely. Methanol/dichloromethane (B) and evaporated dichloromethane (D) from both plants were the only extract/fractions with potent egg hatch inhibition activity at concentration levels 0.9 to 1.0 mg ml^{-1} after 21 days of exposure. This information provided further insight into the usefulness of these materials in root-knot nematode control in small-scale farming communities, domestic gardens and commercial farming.

THE USE OF *TRICHODERMA* SPP. ALONGSIDE RESISTANT TOMATO VARIETIES FOR ROOT-KNOT NEMATODE MANAGEMENT UNDER FIELD CONDITIONS IN COASTAL KENYA. **Kibunja[1], J.W., J.K. Birgen[1], G.M. Kariuki[2] and D.L. Coyne[3].** [1]Department of Plant and Microbial Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya; [2]Department of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya; [3]International Institute of Tropical Agriculture (IITA), c/o icipe, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya.

The endophytic activity of *Trichoderma spp.* strains were assessed for their potential biological control against root-knot nematode (*Meloidogyne* spp.) on both resistant and susceptible tomato varieties in the humid tropical conditions of coastal Kenya. Screenhouse and field experiments were carried out at Pwani University. *Trichoderma* spp. was evaluated alongside tomato cv. Hawaii, Okitu, Nug (AVRDC) and Kilele and Cal J (local varieties). One week after sowing seedlings were drenched with a suspension of *Trichoderma* spp. which had been obtained in the course of this research and not yet characterized and *T. harzianum* T-22 (sold under trade name Trianum-P in Kenya) in the nursery bed. Seedlings were transplanted three weeks after planting and inoculated with 500 J2s. The trial was laid out in randomized complete block design (RCBD) replicated four times. Experiments were terminated at 60 days (greenhouse) and 90 days (field). There was no significant difference between treated plants and the control on growth parameters. However there were significant differences for galling and egg mass index, J2 density, and reproductive index between the treatments and the control. The study revealed a considerable significant variation in response against *Meloidogyne* spp. infection among the germplasm tested when used alongside the *Trichoderma* spp. Interaction was observed between the varieties and the treatments. The cultivar Cal J was highly susceptible to RKN followed by Hawaii. The cv. Kilele which is commonly used in Kenya had less galling and egg mass index with a low reproduction rate indicating its ability to suppress the adult female reproduction. The *Trichoderma spp.* used significantly suppressed the disease severity caused by root-knot nematodes. The results of this study points to a combined use of locally available *Trichoderma spp.* alongside resistant varieties in the humid tropics where temperatures are relatively high as part of integrated pest management against *Meloidogyne* spp.

CHARACTERIZATION OF NEMATODE PESTS OF ENSET (*ENSETE VETRICOSUM* WELW. CHEESMAN) AND THEIR MANAGEMENT. **Kidane[1], S., D. Coyne[1] and S. Haukeland[2].** [1]International Institute of Tropical Agriculture, P.O. Box 30772-00100, Nairobi, Kenya; [2]Bioforsk, Plant Health and Plant Protection, Norway.

Enset (*Ensete vetricosum* Welw. Cheesman) is an important starch staple crop, cultivated primarily in southern and south west Ethiopia. Related to the banana family, enset is similarly infected by plant-parasitic nematodes. From previous survey studies *Pratylenchus goodeyi* appears to be the dominant nematode pest, which is believed to contribute to reduced productivity of enset. However, while surveys have demonstrated high *P. goodeyi* infection levels, there is relatively scant information on how damaging the nematode is to enset production. There is also little information on the variability of the nematode pest in terms of levels of pathogenicity on enset and if so, how this may relate to variability in climate and temperature zones under which enset is grown. Our study is being undertaken to assess the possible damage of nematode pests, with emphasis on *P. goodeyi*, and in relation to the presence of other diseases and how climate and agro ecology may affect this. Specifics of the study are presented.

EFFICACY OF BIOACT WG (*PAECILOMYCES LILACINUS* STRAIN 251) TO CONTROL ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) IN HIGH VALUE CROPS. **Kiewnick, S.** Agroscope Competence Center for Nematology, Institute for Plant Production Sciences, IPS Schloss 1, CH-8820 Waedenswil, Switzerland.

Root-knot nematodes (*Meloidogyne* spp. cause severe losses in high value crop production systems. As plant resistance or chemical control options are either not available or being phased out, a management strategy based on the biological bionematicide BioAct WG (active ingredient *Paecilomyces lilacinus* strain 251) was tested in on-farm and semi-commercial trials for efficacy in reducing root-knot nematode damage and nematode population densities in soil. It could be demonstrated that a pre-planting soil treatment followed by repeated post-plant applications significantly reduced the root damage in commercial greenhouse production systems. In contrast to chemical soil disinfestation, root-knot nematode soil population densities remained at low levels after application of BioAct WG. In greenhouses where *Meloidogyne enterolobii* had caused up to 50% yield loss, application of BioAct WG reduced the damage to a minimum and ensured high yield levels. Studies on

the interaction between *P. lilacinus* strain 251 and *M. enterolobii* revealed particularly high levels of egg parasitism when compared to other RKN species which explains the high efficacy in controlling this extremely aggressive species. In addition, semi commercial trials revealed a significant reduction in root damage and increase in tomato fruit yield following a BioAct WG application. The combination of a BioAct WG application with measures to reduce the nematode population densities in soil further enhanced efficacy of this root-knot nematode management strategy.

DEVELOPMENT OF A STRAIN SPECIFIC SYBR GREEN I BASED REAL-TIME PCR ASSAY FOR ENVIRONMENTAL MONITORING OF *PAECILOMYCES LILACINUS* STRAIN 251. **Kiewnick, S., S. Wolf, A. Lehmann and J. Frey.** Agroscope, Institute for Plant Production Sciences, IPS, Schloss 1, CH-8820 Waedenswil, Switzerland.

Monitoring of nematode antagonists after application allows for better understanding of the tri-trophic interactions between target nematode species, the host plant and the fungal biocontrol agent. Previous molecular assays were based on the internal transcribed spacer (ITS) region of the fungal genome, but only specific to species level. Therefore, other gene regions were tested to develop molecular assays for the detection and identification of *Paecilomyces lilacinus* strain 251 (PL251). Ribosomal RNA genes were evaluated for containing strain specific single nucleotide polymorphisms (SNPs), but, comparison of all sequence data obtained for individual *P. lilacinus* strains revealed no strain specific SNPs. Furthermore, a RAPD-analysis was conducted, but no specific fragments were detected. Therefore, a new approach using GS Junior 454 sequencing technology was used. 22 RAPD primers were used to generate amplicons for different *P. lilacinus* strains. All amplicons were sequenced and a total of 34 MB sequence data generated. Contiguous sequence of strain 251 was concatenated with addition of 100bp N linkers, to facilitate read mapping. Raw reads of the other libraries were then mapped against reference PL251. The consensus sequence of the other strains was aligned (MUSCLE, Geneious Pro v. 6.1.3). For the multiple alignment 8 contiguous sequences were found to be singleton. For these sequences, primer pairs were generated with Geneious Pro v. 6.1.3 and 2 combinations were suitable for use in SYBR Green I assays. Initial testing demonstrated a very high specificity for PL251 and no cross reaction with other closely related strains or biocontrol fungi.

NEW CYST NEMATODES FROM SOUTH AFRICA: A VALUABLE RESOURCE FOR THE STUDY OF THE EVOLUTION AND BIOGEOGRAPHY OF THE GROUP. **Knoetze[1,2], R., A. Swart[3] and L.R. Tiedt[4].** [1]Department of Conservation Ecology and Entomology, Department of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa; [2]Directorate Inspection Services, Department of Agriculture, Forestry and Fisheries, Private Bag X5015, Stellenbosch 7599, South Africa; [3]National Collection of Nematodes, Biosystematics Division, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa; [4]Laboratory for Electron Microscopy, North-West University, Potchefstroom Campus, Potchefstroom 2520, South Africa.

The theory that South America or Africa is a centre of origin for *Globodera* prompted the initiation of a survey for the presence of indigenous cyst nematodes in The Cape Floristic Region of South Africa. The ITS-rDNA regions of four indigenous isolates of cyst nematodes, discovered in the survey, were amplified by PCR, sequenced and aligned with selected sequences from the Heteroderidae. Phylogenetic analyses of the aligned sequences were conducted using the Maximum Parsimony (MP) method. The morphology of the cysts was also studied using both light microscopy (LM) and scanning electron microscopy (SEM). Phylogenetic analysis of the sequences from three of the isolates, places them in a clade of *Globodera* species that parasitise non-solanaceous plants, confirming their membership of the genus. LM and SEM studies confirmed that these specimens can be classified as *Globodera* on the basis of their morphological characteristics. Morphometric data and phylogenetic analyses indicate that they are unique species, previously undescribed. Another isolate forms a monophyletic group with *Heterodera* species in the MP tree, but the morphology of these specimens is more consistent with that of *Betulodera*. However, unique labial patterns, as well as the presence of a vulva and functional gonads in some of the juvenile specimens, set them apart from both *Betulodera* and *Heterodera*. The discovery of new *Globodera* species in Southern Africa supports the theory of a Gondwanaland origin of the genus and the theory that divergence of the two main *Globodera* lineages might have occurred subsequent to the break-up of Africa and South America.

NEMATODE MANAGEMENT IN FLORIDA VEGETABLE AND ORNAMENTAL PRODUCTION. **Kokalis-Burelle[1], N., F.B. Iriarte[2], D.M. Butler[3], J.C. Hong[1] and E.N. Rosskopf[1].** [1]USDA-ARS, US Horticultural Research Lab, Ft. Pierce, Florida, USA; [2]Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA; [3]Department of Plant Sciences, University of Tennessee, Knoxville, Tennessee, USA.

Restrictions on fumigants necessitate development of new options for plant-parasitic nematode management in vegetable and ornamental crop production. USDA-ARS researchers are developing new nematode control tactics, including low-risk chemicals (SPK), steam, and anaerobic soil disinfestation (ASD). These approaches are integrated into site-specific management plans. SPK is a unique formulation of organic acids with efficacy against root-knot nematodes (*Meloidogyne* spp.), as well as soilborne plant pathogenic fungi and bacteria. Steam is effective for broad-spectrum soilborne pest control, however, logistics and expense of application may limit adoption. ASD uses a combination of solarization, organic amendments, and soil saturation to raise soil temperatures, stimulate microbial activity, and create anaerobic conditions in

soil covered with polyethylene mulch. In a double-crop pepper-eggplant trial, *Meloidogyne incognita* numbers were low through the first season following ASD. By the second eggplant crop, solarization (no amendments or water) averaged > 200 nematodes/100 cm^3 soil compared to 10/100 cm^3 in ASD treatments with amendments and irrigated with 5-10 cm of water. While integrated nematode control tactics are preferred, stand-alone approaches are also being investigated including vegetable grafting, and biocontrol with *Pasteuria penetrans*. Grafting for root-knot nematode control in tomato has been successful; however melon grafting did not improve root-knot nematode control, although yield of some rootstock scion combinations improved compared to non-grafted plants. Greenhouse and microplot trials on *P. penetrans* are ongoing but preliminary results indicate good potential for field application.

IDENTIFICATION AND DIVERSITY OF THE ROOT-KNOT NEMATODES AFFECTING YAM (*DIOSCOREA* SPP.), IN NIGERIA. **Kolombia[1,2], Y.A., G. Karssen[2,3], N. Viaene[2,4], P Lava Kumar[1], D. Coyne[5] and W. Bert[2].** [1]International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, Ibadan, Nigeria; [2]Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent Belgium; [3]National Plant Protection Organization, 6706 EA Wageningen, The Netherlands; [4]Institute for Agricultural and Fisheries Research (ILVO), 9820 Merelbeke, Belgium; [5]IITA-Tanzania, Plot 25, Mikocheni, P. O. Box 34441, Dar es Salaam, Tanzania.

Root-knot nematodes, *Meloidogyne* spp. represent an important threat to yam (*Dioscorea* spp.) production in West Africa. With the aim to characterize the species affecting yam plants, for control and resistance screening purposes, surveys were conducted in the main yam producing areas. Infected, galled tubers were collected from farmers and markets in 12 states in Nigeria. To trap *Meloidogyne* species, infected yam peels were inoculated in a ring surrounding the root system of susceptible tomato cv ''Ibadan local'' and celosia plants. Young egg-laying females were extracted from roots of infected tomato and or celosia plants and used for enzymatic (Esterase: EST and Malate dehydrogenase: MDH) analysis with PhastSystem. Out of 46 populations examined, results revealed that yam tubers were mostly infested by *Meloidogyneincognita* (68%), followed by *Meloidogyne javanica* (16%), *Meloidogyne enterolobii* (2%) and *Meloidogyne arenaria* (5%). In most of the samples, tubers were infected by a single species. However, in 9% of the analysed samples, mixed populations of *M. enterolobii* and *M. incognita* (7 %) and *M. enterolobii*, *M. incognita* and *M. javanica* (2 %) were recorded. The distribution of the *Meloidogyne* spp. in the main yam producing areas is discussed. This constitutes the first report of *M. arenaria* and *M. enterolobii* on yam in West Africa.

IDENTIFICATION AND MOLECULAR ANALYSIS OF *MYLONCHULUS PAITENSIS* YEATES, 1992 BASED ON 18S RDNA. **Koohkan, M. and E. Shokoohi.** Plant Protection Department, Agricultural Faculty, Shahid Bahonar University of Kerman, Kerman, Iran.

The genus *Mylonchulus* is one of the most widely distributed genera among the order Mononchida. *Mylonchulus paitensis* was originally described from New Caledonia and then reported from Pakistan and Iran. During a survey of grasses in the Kerman province, Iran, a species of *Mylonchulus* was extracted using the adapted Baermann tray method and identified according to morphological characters as *M. paitensis*. Molecular study of 18S rDNA region of *M. paitensis* and comparison with available sequences deposited in GenBank, demonstrated that the studied population resembles *Mylonchulus sigmaturus* (AB361446, AB361447 from Japan and AY284755 from the Netherlands) and *Mylonchulus arenicolus* (AF036596 from UK) with 98% similarity. Phylogenetic analysis using the neighbor-joining method revealed that this population forms a monophyletic group with *M. sigmaturus*, *Mylonchulus brachyuris*, *Mylonchulus oceanicus, Mylonchulus hawaiiensis, Mylonchulus rotundicaudatus, Mylonchulus arenicolus* and *Mylonchulus mulveyi*. The maximum genetic distance (using Maximum Composite Likelihood method) was 0.084 between Iranian *M. paitensis* and *M. mulveyi* (AB361448; AB361449). This species has 17 and 20 nucleotide difference with Japanese and the Netherland population of the same species, respectively. In addition it differs from *M. arenicolus* by 18 nucleotides. The content of G+C in the 18s rDNA sequence of this isolate is 42.3%. This is the first study of 18S rDNA of *M. paitensis* in Iran. In conclusion, morphological and molecular study indicates that the Iranian population is *M. paitensis*. Furthermore, exploring more genes (e.g. mtDNA and 28S rDNA) are necessary for better understanding the relationship of the species of this group of nematodes.

LONG-TERM EFFECTS OF EIGHT TREATMENTS TO CONTROL PLANT-PARASITIC NEMATODES AND *VERTICILLIUM DAHLIA*. **Korthals, G.W., T.C. Thoden, W. van den Berg, L.P.G. Molendijk and J.H.M. Visser.** Applied Plant Research, Wageningen University and Research Centre, Edelhertweg 1, 8219 PH Lelystad, The Netherlands.

There is an urgent need to develop sustainable methods for management of soil pathogens, such as *Pratylenchus penetrans* and *Verticillium dahliae*. Ultimately this should be investigated with long-term measurements of biological and chemical parameters and their final impact on crop yield. The present study focusses on eight soil treatments (compost, chitin, marigold, grass-clover, biofumigation, anearobic soil disinfestation, a physical control method and a combination of marigold, compost and chitin) and two reference treatments (a chemical control with 300 L/ha Metam sodium and an untreated control). The effects of the10 treatments were studied for 6 years. The present study did demonstrate that in comparison to chemical control, additions of chitin, anaerobic soil disinfestation and marigold are already excellent alternatives for the

control of plant-parasitic nematodes and *V. dahliae*. Grass-clover, biofumigation, Cultivit and compost are not effective alternatives for chemical control yet and further development is needed. All treatments caused a yield increase in comparison with the control. The biggest increases of more than 60 % were found for the treatments with chitin. Furthermore it was demonstrated that these yield increases were probably less influenced by changes in chemical soil properties, but the consequence of changes in the soil biota, in this case especially the effective control of *P. penetrans* and *V. dahliae*. Furthermore it has been demonstrated that most of these soil treatments could be implemented in an arable crop rotation and probably adapted for many areas of the world.

INVESTIGATIONS OF AN EMERGING PATHOGEN, *BELONOLAIMUS* SP., INFECTING PEANUT IN FLORIDA, USA. **Kutsuwa[1], K., D.W. Dickson[1], J.A. Brito[2], A. Jeyaprakash[2] and A. Drew[3].** [1]Entomology and Nematology Department, University of Florida, Bldg. 970, Natural Area Dr., P.O. Box 110620, Gainesville, Florida 32611, USA; [2]Division of Plant Industry, Florida Department of Agriculture and Consumer Services, P.O. Box 147100, Gainesville, Florida 32614, USA; [3]MultiCounty Extension Specialist, University of Florida, Bronson, Florida 32621, USA.

Sting nematodes (*Belonolaimus* spp.) are important ectoparasitic nematodes, highly pathogenic on a wide range of plants in the sandy soils of the southeastern United States. Although this nematode is commonly found in Florida as a soilborne pathogen on turfgrasses and numerous agronomic and horticultural crops, it has not been reported infecting peanut. In the summers of 2012 and 2013, a sting nematode was found infecting three different peanut cultivars being grown on two separate peanut farms in Levy County, Florida. *Belonolaimus* spp. nematodes extracted from soil averaged 44/100 cm^3 on peanut 'TifGuard', 39/100 cm^3 on 'Bailey' in 2012 and 2013; and 28/100 cm^3 on 'Georgia 06' on another farm in 2013. The damage was seen as large irregular patches at both farms. The foliage of infected plants were severely stunted and showed symptoms of nutrient deficiency. The root systems were severely abbreviated and there were numerous small, round brown lesions observed on pegs and pods of infected plants. Harvest taken from infected areas showed a yield reduction of 64% compared with non-sting nematode infested sites. Phylogenetic analysis of the D2-D3 expansion fragments of 28S rRNA and ITS1RNA genes from the nematodes infecting peanut were found to be 99% identical to *Belonolaimus longicaudatus* as reported by previous investigators. The sequences were deposited to GenBank (Accession No. KF963097-KF963100). Additionally, preliminary studies using the isolates from peanut showed similar morphology to that reported for *B. longicaudatus*. To our knowledge this is the first report of sting nematode infecting peanut grown under field conditions in Florida.

STANDARDIZING DIAGNOSTIC QPCR ASSAYS FOR IDENTIFICATION AND DETECTION OF NEMATODES. **Landeweert, R. and W. Mulder.** Clear Detections, Binnenhaven 5, 6709 PD Wageningen, The Netherlands.

A molecular detection test enables accurate fast screening of nematodes and is well suited for high throughput analyses. The accuracy of Molecular methods can be influenced by many factors. Various factors need to be taken into consideration upon chosing a molecular test as an identification tool, including most optimal gene, primer sequence and (q) PCR chemicals Therefore, the transformation of a promising primer pair into a robust diagnostic (q)PCR assay needs a lot of care and should be well documented. To promote consistency between nematode diagnostic laboratories, standardization of molecular methods is a prerequisite, just like proper method validation. Full understanding of the variables influencing the final results – ranging from proper nematode DNA extraction to proper functioning of the (q)PCR test and data interpretation – is therefore very critical. A number of examples will be presented during this congress on factors that influence (q)PCR test performance, which will be of help when deciding upon the development of one's own test ('homebrew'), either published or not, or the purchase of a ready-made diagnostic assay. For standardization purposes is will be of great value if laboratories either provide transparency on all experimental details, or choose to work with pre-defined and well-validated (q)PCR tests.

A NEW IN-FURROW NEMATICIDE FOR *ROTYLENCHULUS RENIFORMIS* AND *MELOIDOGYNE INCOGNITA* NEMATODE MANAGEMENT IN COTTON. **Lawrence, K.S., C. Land and R. Sikkens.** Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama 36832 USA.

Experimental nematicide in-furrow combinations were evaluated for *Rotylenchulus reniformis* and *Meloidogyne incognita* nematode management for cotton production in Alabama. Field tests were placed in RCBD with five replications and were irrigated. The experimental nematicide was applied at planting in furrow spray application and some treatments added a foliar spray application of the experimental or Vydate C-LV applied at the 6 to 8 leaf stage. Initial population densities were 3553 vermiform life stages per 150 cm^3 of soil for *R. reniformis* and 160 J2 per 150 cm^3 of soil for *M. incognita*. Plant stand or plant survival was similar between all nematicide treatments and the untreated control in both nematode fields and no phytotoxicity was observed with the experimental nematicides. All the experimental nematicide treatments applied at 0.53 l/ha or 1.06 l/ha reduced ($P < 0.05$) *R. reniformis* and *M. incognita* numbers compared to the untreated control in both tests. The average reduction for the 1.06 l/ha application was 85% and 74% for *R. reniformis* and *M. incognita,* respectively. Seed cotton yield were increased ($P < 0.10$) by the .06 l/ha application. The 1.06 l/ha rate increase seed cotton by 737 and 655 kg/ha for *R. reniformis* and *M. incognita,* respectively. *Rotylenchulus reniformis* and *M. incognita* 40DAP population's densities

($P < 0.05$) negatively affected seed cotton yield. Regression equations of yield = $-0.0503x + 3147.9$; $R^2 = 0.4515$ for *R. reniformis* and yield = $-0.0743x + 1787.2$; $R^2 = 0.6236$ for *M. incognita*.

MACROECOLOGY OF FREE-LIVING INTERTIDAL NEMATODES ALONG THE COAST OF CHILE AND THE ANTARCTIC PENINSULA. **Lee, M.R.** Centro i~mar, Universidad de Los Lagos, Camino a Chinquihue km6, Puerto Montt, Chile.

Standard macroecological hypotheses were explored using the free-living intertidal nematode fauna from sandy beaches along the coast of Chile and the Antarctic peninsula. Species richness, abundance, range-size (Rapoport's rule) and body size (Bergman size clines) were all analysed over the latitudinal gradient. Replicate quantitative samples were collected in the intertidal of 111 beaches between Arica (18.412 °S, 70.326 °W) on the border with Peru down to Margarite Bay (67.818 °S, 67.201 °W) in Antarctica. Meiofauna were extracted and the nematode species present identified and enumerated. The trends were assessed for their association with the principal environmental variables, surface sea water temperature, coastal primary productivity and coastline complexity. Species richness in exposed beaches declined with increasing latitude, as did abundance. Range size increased with increasing latitude. Body size neither declined nor increased with increasing latitude at the phylum level. At the level of individual species changes in body size were inconsistent, with some species increasing, and others decreasing or displaying no trend with increasing latitude. The latitudinal trend in species richness was most strongly associated with temperature, with one possible hypothesis being that the fauna has equatorial origins and that southward dispersal is limited by temperature. Antarctic nematode fauna was less diverse and abundant than the nematode fauna in southern Chile, and contrary to predictions nematodes in Antarctica were not larger than those from more northerly latitudes. These results are relevant as they are based on samples and not data extracted from the literature.

THE CHOLECYSTOKININ SIGNALING PATHWAY AS A TARGET FOR THE DEVELOPMENT OF NOVEL NEMATICIDES. **Lemoine[1] L., T. Janssen[1], N. Suetens[1], W. Grant[2], B. Landuyt[1], L. Schoofs[1] and L. Peeters[1].** [1]KU Leuven (University of Leuven), Oude Markt 13, 3000 Leuven, Belgium; [2]La Trobe University, Melbourne VIC 3086, Australia.

Cholecystokinin (CCK) neuropeptides and their corresponding receptors are known to play a role in the management of energy supplies and feeding behavior. Both the receptor and its ligands are structural and functional very well conserved among different species, including nematodes. We conducted a differential feeding micro-array experiment and found that this signaling system affects a lot of genes involved in sugar- and fat metabolism and responses to starvation. The expression of the receptor and its ligands are upregulated under starvation and during the dauer stage, when food consumption is stopped. We also noticed that worms with a defect CCK system display abnormalities in food-consumption, locomotion and egg laying. Localization constructs revealed expression of the receptor in the intestine. We also identified an NLP-12-like neuropeptide precursor (the nematode orthologue of CCK) in *Strongyloides ratti* and in *Parastrongyloides trichosuri*, gastrointestinal parasites of rats and Australian possums, respectively. *P. trichosuri* NLP-12 peptides are able to activate the *C. elegans* CKR-2 receptor in the nanomolar range, as does *C. elegans* NLP-12. In *C. elegans* NLP-12's inhibitory effects on locomotion and egg laying, two important targets for nematicides, were rescued with *P. trichosuri* genomic *nlp-12*. We could conclude from these experiments that the CKR-2 receptor is an interesting target for intervention in the development of nematicides. Therefore, we determined the core receptor-activating peptide in *C. elegans*, which is conserved among free-living and parasitic nematodes. As a result we use this as a starting point in the design of non-peptide mimetics and have begun testing analogues.

PLURONIC GEL, A USEFUL MEDIUM TO STUDY ENTOMOPATHOGENIC NEMATODE HOST HABITAT FINDING BEHAVIOR IN RESPONSE TO ENVIRONMENTAL CUES FROM THE PLANT. **Li[1], C., Y. Wang[2], F. Pan[1] and C. Wang[1].** [1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China; [2]Center for Vector Biology, Rutgers University, New Brunswick, New Jersey, 08903, USA.

Pluronic F-127 is a non-toxic and thermoreversible transparent copolymer, which allows free nematode movement inside the gel. Pluronic gel has been proved to be a useful medium to study plant parasitic nematode host finding behaviour. For the first time, we used pluronic gel to investigate how plant roots affect localisation and host habitat seeking of entomopathogenic nematodes (EPN). One isolate of EPN, *Heterorhabditis bacteriophora* HBN (Hb-HBN) from north-eastern China was found to be an effective bio-control agent against Chinese chive maggot (*Bradysiaodoriphaga* sp.). Among the nematodes tested, Hb-HBN had the strongest attraction to chive roots, stronger attraction to garlic and green onion roots, and weak attraction to tomato and soybean roots 4hr post-exposure ($P < 0.05$). The result may explain why HB-HBN was the most effective nematode against the maggot. Further tests have shown that the nematodes were more attracted to wounded roots than non-wounded roots, younger roots than older roots, which indicated that the nematodes prefer the habitat where their hosts are more likely present. Moreover, we found that nematode age, species, and strains significantly affected their attraction to chive root. Further studies about whether chive roots emit compounds to recruit EPN to protect their root system from herbivores

are being conducted in pluronic gel. These studies will provide further understanding of EPN infection mechanisms and an important parameter to predict the success of field application.

TOWARDS INTEGRATED MANAGEMENT OF CEREAL CYST NEMATODE: AN EXAMPLE FROM RESEARCH IN JIANGSU PROVINCE, CHINA. **Li, H. and X. Wang.** Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China.

The cereal cyst nematode (CCN) was first reported on wheat in Jiangsu Province, China in May, 2009. Jiangsu Province is the fifth largest producer of wheat in China (approx. 2.3 Mha). Knowledge of the biology of CCN and the development of control methods are essential for integrated management of this nematode. During a survey in the province, CCN was detected from 152 towns in 47 counties. The wide distribution and high population numbers had notable adverse impact on wheat production. All CCN populations were identified as *Heterodera avenae* and finished only a single life cycle on winter wheat during a growing season. The hatch peak and therefore the largest infection to roots were annually at the end of February. Five granular nematicides (GR) were screened in field trials for effectiveness in CCN control and 0.5% avermectin GR was successful in both inhibiting nematode reproduction and as well as improving wheat growth. A dosage of 30 kg/ hm^2 was recommended to be applied in heavily infested fields during the regreening period. Seed-coating chemicals were also screened and Gannong seed-coating III exhibited high control efficacy. The pathotypes of *H. avenae* Jiangsu populations were determined as belonging to the *Ha1* group. Planting resistant cultivars is usually the most economical and practical measure for CCN control and three wheat cultivars were evaluated as highly resistant to *H. avenae*. The Huamai No.1 cultivar can successfully be planted in heavily infested areas.

INTERACTIONS BETWEEN COTTON AND RENIFORM NEMATODE. **Li[1], W., P. Agudelo[1], and C. Wells[2].** [1]School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, South Carolina, USA, 29634; [2]Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA, 29634.

Reniform nematode (*Rotylenchulus reniformis*) is an important problem for cotton production in the United States, but knowledge of cotton resistance to reniform nematode is very limited. The objectives of this project were: to generate a histological and gene expression time course for the cotton root-reniform nematode interaction; to identify and validate key candidate genes involved in cotton response to reniform nematode infection; and to identify metabolic and regulatory pathways associated with syncytium formation. Resin sections of root tissue from susceptible and resistant cotton were made after inoculating with reniform nematode, using a split-root system, and observations were made at 3, 6, 9, and 12 days after inoculation with the nematode. Trinity *de novo* transcriptome assembly was performed using root RNA from multiple infection stages, and transcripts were functionally annotated with Blast2GO. Candidate genes that were strongly up- and down-regulated during syncytium development were identified. Genes for detailed characterization were selected based on differential expression and our understanding of nematode-host root biology. Target proteins were localized in the root cells with fluorescent microscopy. The information gathered in this project is critical to the breeding and engineering of nematode-resistant cotton plants.

SOYBEAN CYST NEMATODE RESISTANCE IN PI 567516C SOYBEAN: A PROMISING NEW SOURCE OF RESISTANCE. **Lian[1], L., F. Wang[2], R.L. Denny[1], J.H. Orf[3], N.D. Young[1] and S. Chen[1,4].** [1]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108, USA; [2]Life, Agriculture and Forestry College, Qiqihar University, Qiqihar, Heilongjiang 161006, China; [3]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota 55108, USA; [4]Southern Research and Outreach Center, University of Minnesota, Waseca, Minnesota 56093, USA.

Soybean cyst nematode (*Heterodera glycines*) is the most serious yield-limiting pathogen on soybean (*Glycine max*), which is most effectively managed by host resistance. However, most commercial soybean cyst-resistant cultivars in the USA are developed from PI88788 and Peking, and frequent use of the limited number of resistance sources has shifted virulence phenotypes of *H. glycines* populations (HG Types) to new types that can overcome originally resistant cultivars. A recently identified soybean line PI 567516C is a promising alternative source of soybean cyst nematode-resistance. PI 567516C is highly resistant to HG Type 2.5.7 (race 1) which is virulent to PI 88788. Interestingly, PI 567516C is highly resistant to some populations of HG Type 1.3.6 (race 14) which is virulent to Peking, but not other populations of race 14. Genetic studies revealed that the major QTLs conferring H. Glycines-resistance in PI567516C are distinct from the quantative trait loci (QTL) in PI 88788, Peking, and many other -resistance sources. Two QTLs located on chromosomes 10 (LG O) and 8 (LG A2) in PI 567516C were identified to be resistant to *H. glycines*. The QTL on chromosome 10 was the major one with 32.0% additive heritability and 68.7% of the genetic variance of resistance to HG Type 2.5.7, and the closest single nucleotide popymorphism (SNP) ()marker was BARC-008021-00209. The QTL on chromosome 8 displayed 6.8% additive heritability and 14.8% of the genetic variance of resistance to HG Type 2.5.7 and the closest SNP marker was BARC-028207-05794. The two SNP markers can be used to select soybean cyst nematode-resistance from PI 567516C in soybean breeding through marker-assisted selection.

PLANT NEMATOLOGY, EDUCATION AND TRAINING IN CHINA. **Liao[1], J. and H. Li[2].** [1]Laboratory of Plant Nematology/Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642, People's Republic of China; [2]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China.

Due to the great losses caused by some parasitic nematodes on many crops in China, more and more researchers are involved in this area. Presently, there are about two hundred researchers located in different provinces in China. They focus on nematodes of agricultural importance, especially on species identification, basic biology, molecular aspects and disease management. In recent years, the Chinese government funded research on root-knot nematode, cyst nematode and pine wood nematode because of their serious damages on crops and forest. The interaction between nematodes and host plants and novel management techniques will be the ''hot'' research topics which attract Chinese nematologists. A good system for Nematology education and training has been developed through the efforts of Chinese nematologists. These include Bachelor, Master and PhD degree programmes. Incorporation of Nematology curriculums from USA and Europa at local universities is also a possibility.

THE GENOME SEQUENCE AND LIFE-STAGE SPECIFIC TRANSCRIPTOMES OF POTATO CYST NEMATODE. **Lilley[1], C.J., J.A. Cotton[2], V. Blok[3], S. Eves-van den Akker[1,3], L.M. Jones[1], A.J. Reid[2], P. Thorpe[1,3], M. Berriman[2], J.T. Jones[3] and P.E. Urwin[1].** [1]Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, UK; [2]Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; [3]James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK.

Potato cyst nematodes are major pathogens of potato crops in temperate regions, making them some of the most economically important plant parasitic nematodes. Research to develop novel approaches for control of these and other cyst nematodes will be significantly enhanced by a greater understanding of the molecular basis of the parasitic interaction and the key nematode genes required for this. A complete draft genome sequence of the white potato cyst nematode *Globodera pallida* has been assembled, together with transcriptomic data from most of the nematode life cycle, particularly focusing on the life cycle stages involved in root invasion and establishment of the biotrophic feeding site. Despite the relatively close phylogenetic relationship with root-knot nematodes, there is a very different gene family content between the two groups and in particular extensive differences in the repertoire of effectors, including an enormous expansion of the SPRY domain protein family in *G. pallida*, which includes the SPRYSEC family of effectors. This highlights the distinct biology of cyst nematodes compared to the root-knot nematodes that were, until now, the only sedentary plant parasitic nematodes for which genome information was available. The repertoires of genes likely to be important in understanding the unique biology of cyst nematodes and those that represent potential chemical targets and other targets for control have been analysed. A recently assembled draft genome for the closely related species *Globodera rostochiensis* will allow valuable comparative studies.

MOLECULAR CHARACTERIZATION OF RESISTANCE RESPONSES OF *COFFEA CANEPHORA* 'CLONE 14' UPON INFECTION WITH *MELOIDOGYNE PARANAENSIS*. **Lima[1,2], E.A., F.A. Carneiro[2], T.S. Costa[2], E.C.S. Rêgo[2], A. Jorge Júnior[2], C. Furlanetto[1], P. Marraccini[2,3], R.M.D.G. Carneiro[2] and A.C. Andrade[2].** [1]Dep. Fitopatologia, Universidade de Brasília, 70910-900 Brasília, DF, Brazil; [2]Embrapa Recursos Genéticos e Biotecnologia, 70770-917 Brasília, DF, Brazil; [3]CIRAD UMR AGAP, 34398, Montpellier, France.

Coffee is one of the major commodities in the world and an important source of income for producing countries. However, biotic and abiotic stresses are great limiting factors to coffee yield. In Brazil, root-knot nematodes cause considerable yield reduction and the use of resistant plants is the most promising method to control *Meloidogyne* spp. The aim of this work was to characterize the molecular mechanism underlining the previously identified resistance to *M. paranaensis* in *C. canephora* 'Clone 14' by means of RNAseq experiments. Differential expression using RNA extracted from roots of plants from clones 14 and clone 22 of *C. canephora,* previously identified as resistant and susceptible to *M. paranaensis,* respectively, were grown in sand and inoculated. Root samples were collected at different time points post inoculation as well as roots from an uninfected plant. The RNA was treated with DNAse and subsequently, a portion of the sample was lyophilized for RNAseq experiments and another portion kept for validation by qPCR experiments. Results of the identified candidate genes with differential expression among resistant (Clone 14) and susceptible (Clone 22) genotypes will be presented and discussed.

AGGRESSIVENESS OF *MELOIDOGYNE JAVANICA* POPULATIONS ON COMMERCIAL POTATO CULTIVARS. **Lima-Medina[1], I., J.T. Schafer[2], C.B. Gomes[1], M. Vizzoto[1], A.C. Krolow[1], R.M.D.G. Carneiro[3] and V. Correa[3].** [1]Embrapa Clima Temperado, Cx Postal 403, Pelotas/RS, Brazil; [2]Graduated student in Plant Pathology, PPGFS/Universidade Federal de Pelotas, Campus Universitario s/n C. P. 354, Pelotas/RS, Brazil; [3]Embrapa Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-979, Brasília DF, Brazil.

The genus *Meloidogyne* is widely found in the different potato production regions of Brazil. However *Meloidogyne javanica* is the most frequent species that causes damage in potato crop. In order to study the aggressiveness of *M. javanica*, four populations (P1, P3 and P4: Est J3; P2: Est J2a) from southern Brazil was evaluated in two commercial potato cultivars (BRS Clara and Agata) in greenhouse conditions. Individual plants of the two cultivars, kept in pots with sterilized soil, were

inoculated with 5,000 eggs and second stage juveniles of *M. javanica* using six replicates. Fifty-five days after inoculation, each plant was evaluated for number of galls/root, galls number/1.76 cm^2/tuber, nematode reproduction factor (RF). Subsequently, the sensorial quality and the respective total phenolic compound levels in the tubers were also determined. P2 and P4 were the most aggressive *M. javanica* populations exhibiting the higher values of all studied nematode variables interfering in the expression of symptoms on both potatoes cultivars. Additionally, all the populations affected the flavour of cooked potatoes in both of the tested cultivars and higher levels of phenolic compounds were observed in potatoes infected with *M. javanica* P2 population.

REACTION OF POTATO CULTIVARS TO *MELOIDOGYNE HAPLA* AND *M. MOROCCIENSIS*. **Lima-Medina[1], I., J.T. Schafer[2] and C.B. Gomes[1].** [1]Embrapa Clima Temperado, Cx Postal 403, Pelotas/RS, Brazil; [2]PPGFS/Universidade Federal de Pelotas, Campus Universitário s/n C. P. 354, Pelotas/RS, Brazil.

In Brazil, different species of root-knot nematodes, affect potatoes. However, there are few studies of genetic resistance to *Meloidogyn*e species. The objective of this study was to evaluate the reaction of nine commercial potato cultivars to *Meloidogyne hapla* and *Meloidogyne morocciensis* in greenhouse conditions. Potato plants kept in pots with sterilized soil were inoculated with 5,000 eggs and second stage juveniles of *M. hapla* or *M. morocciensis*/plant using six replicates/genotype. 'Santa Cruz' tomato plants received the same inoculum level and were used as control. Fifty-five days after inoculation, each plant was evaluated for the number of galls, eggs and juveniles./root system. Subsequently, the reproduction factors (RF) of the two *Meloidogyne* species were determined in the different genotypes. Among the tested cultivars, BRS Ana, Asterix, BRSIPR Bel, Cota, Cristina, BRS Clara, Catucha, and Eliza were susceptible to both *Meloidogyne* species. Besides being susceptible to *M. morocciensis*, 'Agata' was also moderately susceptible to *M. hapla*.

FREE LIVING NEMATODES AS INDICATORS OF THE BIOLOGICAL STATUS OF AUSTRALIAN CEREAL SOILS. **Linsell[1], K., A. Stirling[2], D. Hartley[3], Herdina[1], A Cheshire[4], J. Nobbs[1], A. McKay[1], G. Stirling[2] and K. Ophel Keller[1].** [1]South Australian Research & Development Institute (SARDI), GPO Box 397, Adelaide, 5001, South Australia, Australia; [2]Biological Crop Protection Pty. Ltd., 3601 Moggill Road, Moggill, 4070, Queensland, Australia; [3]CSIRO Ecosystems Science, GPO Box 1700, Canberra, 2601, ACT, Australia; [4]Science to Manage Uncertainty, 24 Winding Way, Belair, South Australia, 5052, Australia.

The impact of management practices on the biological status of cereal-growing soils was investigated across a range of Australian soil types and climates through nematode community analysis, across multiple years. A multivariate statistical analysis approach identified the two key drivers influencing changes within free-living nematode communities to be soil type which is linked to regional rainfalls, particularly 1-3 months prior to crop sowing and the application of certain nutrients, particularly N, P, S and Cu. Significant shifts in nematode population structures were also characterised by tillage regimes when analysed by soil type. Stubble management and prior plantings did not influence community changes except where canola and legumes were included in the cereal rotations. A Bray–Curtis measure of similarity characterised the contribution of each species/genera driving the changes between each management/environmental treatment and seventeen free-living species were identified as good indicators. Since manual nematode community analysis is laborious and requires specialised taxonomic skills, molecular technologies were developed to allow routine indicator identification and quantification in soil, which can be delivered as part of diagnostic service for soil-borne pathogens. Nine DNA tests were developed incorporating eleven of the free-living nematode indicators and are predicted to detect more than 80% of the species present in Australian cereal cropped soils. There was a very strong correlation between free-living nematode community structures obtained from the manual count and DNA tests. Therefore, we concluded that DNA tests are a sensitive, quick and robust tool for assessing free-living nematode communities, and provide a useful indication of a soil's biological status.

DAMAGE FUNCTIONS OF *MELOIDOGYNE JAVANICA* ON ZUCCHINI SQUASH AND RELATIVE LEAF CHLOROPHYLL CONTENT. **López-Gómez[1], M., F.J. Sorribas[2], M. Talavera[3] and S. Verdejo-Lucas[1,4].** [1]IRTA. Crta de Cabirls Km 2. 08348 Cabrils, Barcelona, Spain; [2]Universitat Politècnica de Catalunya. 08860 Castelldefels, Barcelona, Spain; [3]IFAPA. Camino de Purchil s/n. Granada. Spain; [4]IFAPA. Camino de San Nicolás, 1. 04745 La Mojonera, Almería. Spain.

Yield losses in cucurbits have been reported in many horticultural regions. Zucchini-squash is an important crop in southern Spain accounting for one third of the production in the country. For sustainable management of root-knot nematodes in susceptible crops, it is essential to develop accurate information on population densities that cause yield losses and quantify them into plant damage functions. This study was conducted to determine maximum multiplication rate and equilibrium density of *Meloidogyne javanica* on zucchini squash cv Amalthee in response to increasing initial population densities and to develop damage function models. Seedlings were inoculated with 0, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 eggs of *Meloidogyne* per cm^3 of soil in repeated experiments in a greenhouse. The maximum multiplication rate was 511, and the equilibrium density 8135 eggs + J2 cm^{-3} soil. The relationship between Pi and relative plant top dry weight fitted the Seinhorst damage function model (R^2=0.53; P=0.0002). Values for minimum relative yield, tolerance limit, and constant z

were 0.77, 3.4 J2 cm^{-3} soil, and 0.95, respectively. Relative leaf chlorophyll content measured 40, 50, 60 and 70 days post-inoculation (dpi) showed a significant reduction ($P<0.05$) 50 dpi onward. Regression analyses indicated an inverse relationship between increasing Pi and relative leaf chlorophyll content but there was no correlation with dry top weight.

MODIFICATIONS IN THE PHENYLPROPANOID METABOLISM IN CHILLI PEPPER INFECTED BY *NACOBBUS ABERRANS*. **López-Martínez[1], N., M. Biesaga[2] and E. Zavaleta-Mejía[3].** [1]Departamento de Fitotecnia, Universidad Autónoma Chapingo Km. 38.5 carr. México-Texcoco, Chapingo, Estado de México CP 56230, México; [2]Departament of Chemistry, University of Warsaw, Pasteura 1, 02-093. Warsaw, Poland; [3]Instituto de Fitosanidad, Colegio de Postgraduados, Km. 36.5, Carr. México-Texcoco. Montecillo, Edo. De México, C.P. 56230, México.

Phenylpropanoid pathway plays an important role in plant-pathogen interactions. Infection of chilli pepper (*Capsicum annuum*) CM334 by the false root-knot nematode *Nacobbus aberrans* (*Na*) induces changes in this metabolic pathway. These changes affect the resistance of CM334 to the oomycete *Phytophthora capsici*. The L-phenylalanine ammonia-lyase (PAL) activity was evaluated in CM334 plants inoculated with *N. aberrans, P. capsici* or both pathogens (*N. aberrans-P. capsici*). Besides, the profiles of phenolic acids and flavonoids were monitored in chilli pepper plants at 7, 14, 21 and 28 days after nematode inoculation (dai) and the toxic effect of rutin was tested on second stage juveniles (J_2) of *N. aberrans* and *Meloidogyne incognita* (*Mi*). At 21 dai, the highest PAL activity (28.2 nM *trans*-cinnamic acid μg^{-1} protein min^{-1}) was registered in plants inoculated only with *P. capsici,* while those plants inoculated with *N. aberrans* showed the lowest activity ($P < 0.05$). In some sampling points, inoculated plants had lower content of both phenolic acids and flavonoids than non-inoculated plants (control), particularly at 7 dai ($P < 0.05$). Chlorogenic acid was the phenolic acid in the highest quantity in shoot (from 277 to 458 $\mu g.g^{-1}$ of dry matter). In all sampling points, plants inoculated with *N. aberrans* showed lower content of *p*-hidroxy benzoic and chlorogenic acid than control ($P < 0.05$). Rutin was the only flavonoid found in the root whereas apigenin and luteolin were also found in shoot. Usually, the flavonoids content in shoot of inoculated plants was significantly ($P < 0.05$) lower than in control plants. Rutin had a nematocidal effect on J_2 of *N. aberrans*, whereas it only had a nemastatic effect on *M. incognita*.

MARKER-ASSESSED SELECTION IN TANDEM WITH BIOASSAY PREDICT SOYBEAN RESPONSE TO INFECTION WITH *HETERODERA GLYCINES*. **Lopez-Nicora[1], H.D., B.W. Diers[2], A.E. Dorrance[3] and T.L. Niblack[1].** [1]Department of Plant Pathology, Ohio State University, Columbus, Ohio 43210, USA; [2]Department of Crop Sciences, University of Illinois, Urbana, Illinois 61810, USA; [3]Department of Plant Pathology, Ohio State University, Wooster, Ohio 44691, USA.

Heterodera glycines, the soybean cyst nematode (SCN), is an economically important pathogen of soybean worldwide. The use of soybean cultivars resistant to *H. glycines* remains the best management practice to reduce the impact of this pathogen. Resistance to the nematode is a multigenic, quantitative trait and *Rhg1*, a quantitative trait locus (QTL) on chromosome 18 is the major quantitative trait locus for resistance to *H. glycines*. Marker-assisted selection for this locus is widely used in plant breeding; however, the selection of lines for resistance based on markers linked to *Rhg1* should be accompanied with a bioassay to evaluate the soybean cyst nematode resistance level of cultivars. The purpose of this study was to evaluate the *H. glycines* resistance levels of 40 soybean back-cross lines (BC_4F_2 derived), half with the resistance allele for the marker Satt309, which is closely linked to *Rhg1*, and the other half susceptible. A standardized protocol for resistance screening was used to measure the response of these lines to *H. glycines in* the greenhouse. Lines were also planted in a soybean cyst nematode-infested field in ten randomized complete blocks. Soybean yield and initial (Pi) and final (Pf) population densities of *H. glycines* eggs were determined for each plot. The resistance phenotypes of the lines in the greenhouse bioassay were predicted by the presence or absence of the marker for *Rhg1*. The field performance of soybean lines was reliably predicted by the marker alleles of lines for *Rhg1* and a greenhouse bioassay. In the field tests, the susceptible lines yielded significantly less than resistant lines.

SECRETED VENOM ALLERGEN-LIKE PROTEINS OF PLANT-PARASITIC NEMATODES MODULATE DEFENCE RESPONSES IN HOST PLANTS. **Lozano-Torres, J.L., R.H.P. Wilbers, S. van Warmerdam, A. Finkers-Tomczak, C.C. van Schaik, H. Overmars, J. Bakker, A. Goverse, A. Schots and G. Smant.** Laboratory of Nematology, Wageningen University, Droevendaalsesteeg 1, Wageningen, The Netherlands.

The venom allergen-like proteins form a family of effectors that seems to be conserved among all parasitic nematodes of plants and animals studied to date. Recently, we have shown that the venom allergen-like protein of the potato cyst nematode *Globodera rostochiensis* Gr-VAP1 interacts with the apoplastic cysteine papain-like proteases Rcr3pim of *Solanum pimpinellifolium*. Gr-VAP1 and Rcr3pim are both required to activate defence-related programmed cell death and resistance to nematodes mediated by the extracellular plant immune receptor Cf-2 in tomato. Thus, Gr-VAP1 is able to trigger defence responses in a host plant of *G. rostochiensis*, but the virulence function of Gr-VAP1 or of any other venom allergen-like protein of an animal- and plant-parasitic nematode is not known. A specific knock-down of *Gr-VAP1* expression in *G. rostochiensis* showed that the effector is indeed important for virulence of infective juveniles in host plants. Similarly, the ectopic expression of venom allergen-like proteins in transgenic plants alters their response to nematodes and other plant

pathogens. Transcriptopme analysis of these transgenic plants has shed light on the molecular mechanisms underlying the virulence function of venom allergen-like protein of plant-parasitic nematodes in plants.

POTENTIAL OF BIOLOGICAL CONTROL AGENTS, ORGANIC AMENDMENTS AND CROP ROTATION ON ROOT-KNOT NEMATODES. **Luambano-Nyoni[1], N., J.W. Kimenju[2], R.D. Narla[2], W.J. Wanjohi[3].** [1]Sugarcane Research Institute, P.O. Box 30031, Kibaha, Coast, Tanzania; [2]University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya; [3]Kenyatta University, P.O. Box, 43844 Nairobi, Kenya.

Integrated approaches are fast gaining popularity in the management of root knot nematodes (*Meloidogyne* spp.). This study was undertaken to assess the potential of integrating a biological control agent (*Pochonia chlamydosporia* isolate 10), crop rotation (maize) and organic amendment (maize stover) in the management of root knot nematodes in tomato production. One greenhouse and two field (dry and rainy seasons) experiments were conducted with tomato as the preferred crop. *Pochonia chlamydosporia* isolate 10, maize stover and maize were used as treatments either singly or in combinations before tomato was grown in the second season. Results from the field experiments showed that application of *P. chlamydosporia* and maize stover into the plots where maize was planted, significantly ($P<0.005$) reduced numbers of root knot nematodes. Moreover the combination reduced in season two galling of tomato roots planted in the glasshouse and in the field. Application of *P. chlamydosporia* in plots where maize was planted increased the yield of tomato by 63% in the first season compared to plots where no nematode management measure was done. Therefore, in this study rotating maize crop with tomato has been effective on its own and also in combination with a biological control agent and organic amendments.

HOST STATUS OF GREEN MANURES TO FOUR SPECIES OF ROOT-KNOT NEMATODES IN BRAZIL. **Machado, A.C.Z., D. Mattei, S.A. Silva, O.F. Dorigo, D.S. Ito.** Insituto Agronômico do Paraná, Rodovia Celso Garcia Cid, km 375, 86047-902, Londrina, PR, Brazil.

Green manures are responsible for the improvement of soil health, increased nitrogen fixation and for the reduction of the nematode population in agricultural soils. However, information about the host reaction of green manures to phytonematodes is scarce. Therefore, in this present work we aimed to characterize the host response of some green manures, under greenhouse conditions, to four species of root-knot nematodes, *Meloidogyne incognita* (Mi), *M. javanica* (Mj), *M. paranaensis* (Mp) and *M. enterolobii* (Me), based on nematode reproductive factor (RF) and number of nematodes per gram of roots (nem/g). Results showed a great variation in the susceptibility of the plants to the nematodes tested. Among 30 different species tested, at least four had showed resistance to the root-knot nematodes, i.e., *Clitoria ternata*, resistant to all four root-knot species, *Crotalaria juncea, C. spectabilis,* and *C. breviflora*, resistant to Mi, Mj, and Mp. *Lupinus albus, Mucuna pruriens, M. deeringeana, M. aterrima,* and *M. pruriens* var. *utili* were resistant to Mp, a pathogen of concern in coffee plantations, at Paraná State, in Brazil. *Mucuna pruriens* and *M. deeringeana*, *Cajanus cajan* ''Fava Larga'', ''Anão'' and ''BRS Mandarim'' were also resistant to Me, a minor root-knot species with rising importance in Brazil. Therefore, there are many plant species with resistance to root-knot nematodes that could be used on infested areas, in order to reduce the population on soil.

THE UNKNOWN-UNKNOWN OF NAGASAKI 1905. **Magnusson, C.** Norwegian Institute for Agricultural and Environmental Research, Høgskoleveien 7, 1430 Ås, Norway.

The outbreak of pine wilt disease in Nagasaki, Japan, in 1905 was impossible to foresee. It was something scientists didn't know they didn't know. In 1934, a harmless wood nematode *Aphelenchoides xylophilus* was described from pine logs in Louisiana U.S.A. The disease in Nagasaki was suspected caused by fungi, or insects. In 1934 pine wilt disease was spreading on Kyushu Island and in southern Honshu. Inoculation of a *Bursaphelenchus* species detected in the wood of dead pine trees, revealed the nematode as the causal agent of pine wilt disease. It was described as pine wood nematode *Bursaphelenchus lignicolus,* and it proved to have a phoretic association with the beetle, *Monochamus alternatus*. Pine wood nematode may have spread from North America to Japan in infested wood and established a successful phoretic relationship with the beetle. In the susceptible pine species *Pinus densiflora* and *Pinus thunbergiii*, it caused a disease unknown of in North America. Taxonomic studies demonstrated that *A. xylophilus* belonged to the genus *Bursaphelenchus* and that *B. lignicolus* was a junior synonym of *Bursaphelenchus xylophilus*. Sixty years delay in understanding causality, and a failure in eradicating latent infections resulted in a dramatic epidemic, which spread to neighbouring countries, and to Portugal. Today we are aware of the importance of man-assisted movement of wood for the dissemination of pine wood nematode. We need to pay more attention to changes in trade of wood, and control risks associated with wooden pallets. We need to find strategies to detect latent infections and to prepare better for emerging risks.

INTEGRATING ENTOMOPATHOGENIC NEMATODES IN MANAGEMENT STRATEGIES AGAINST KEY LEPIDOPTERAN PESTS OF FRUIT TREES IN SOUTH AFRICA. **Malan[1], A.P., S.D. Moore[2] and M.F. Addison[1].** [1]Department of Conservation Ecology and Entomology, Private Bag X1 Matieland 7602, South Africa; [2]Citrus Research International, PO Box 20285, Humewood 6013, South Africa.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae have been successfully utilised as biological control agents in classical, conservation, and augmentative insect pest control management

programmes. Research into the biological control of insects has shown that no single biocontrol method, including the use of EPNs, can, by itself, effectively replace pesticide usage. Research into EPNs in South Africa has mostly been directed toward the control of insect pests on a commercial scale. To integrate nematodes into an integrated pest management system, it is important to conduct research under local climatic conditions for a specific crop. Especially for commercial application, the unique environmental conditions in the various production areas need to be assessed to allow for the effective use of various nematode species. Research into endemic EPNs, mainly targeting the two key South African lepidopteran pests, codling moth (*Cydia pomonella*) on apples and pears, and false codling moth (*Thaumatotibia leucotreta*) on citrus, forms the current bulk of our knowledge. Application of EPNs against codling moth will target the diapausing larval overwintering population above ground, whereas, in the case of false codling moth, the application will be aimed at the soil stages of the insect, which include cocooned larvae, pupae and the emerging moths. Orchard application, either onto trees or the soil surface poses its own unique challenges with regard to the inundative application of EPNs. The potential for integrating EPNs in the current chemical, biological, and orchard management practices will be discussed.

ATTRACTION/REPULSION OF *MELOIDOGYNE HISPANICA* TO *PINUS PINASTER* BARK EXTRACTS. **Maleita[1], C., M. Braga[1], H. Sousa[1] and I. Abrantes[2].** [1]CIEPQPF, Department of Chemical Engineering, FCTUC, University of Coimbra, 3030-790 Coimbra, Portugal; [2]IMAR-CMA, Department of Life Sciences, FCTUC, University of Coimbra, 3004-517 Coimbra, Portugal.

Natural nematicides obtained from residues derived from agricultural activities have shown potential to the management of plant-parasitic nematodes. In this way, these can be considered as high-value residues and thus valorised by the extraction of their bioactive/nematicide compounds reducing environmental problems and waste recycling costs associated with the management of those residues. The aim of this study was to assess the effect of six extracts obtained from *Pinus pinaster* bark, an agro-industrial residue, on *Meloidogyne hispanica* second-stage juveniles (J2) attraction/repulsion. Chemotaxis assay was carried in Petri dishes with 1% water-agar and at the opposite sides on the plates two wells were made and filled with 50 μl of each of the extracts, 3 h before the inoculation of 20 J2, at the plate centre. After 2 h, the position and number of J2 in the attractive and repellent zones were recorded. Each treatment was replicated three times and the experiment twice. All bark extracts with no effect in *M. hispanica* J2 mortality at 5000 ppm, except for extract obtained with HCOOH (1%) and Na_2SO_3 (1%) as solvent, where 26% J2 mortality was achieved after 48 h at 5000 ppm, were classified as repellents at 10000 ppm. Avoidance responses suggest that these extracts if added to the rhizosphere may have an important role in the orientation of J2 to the roots which could be an advantage in the control of plant-parasitic nematodes.

DECIPHERING THE INVASION ROUTES OF *BURSAPHELENCHUS XYLOPHILUS*: A POPULATION GENETICS APPROACH. **Mallez, S., C. Castagnone, T. Guillemaud and P. Castagnone-Sereno.** INRA UMR1355, Université de Nice Sophia Antipolis, CNRS UMR7254, Institut Sophia Agrobiotech, 06903 Sophia Antipolis, France.

The pinewood nematode, *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease and is currently considered as one of the most important pests in the forest ecosystems. Native to North America, it has been introduced and spread to pine forests in Asia and more recently to Europe. In all invaded areas, *B. xylophilus* has considerable economic and environmental impacts, and has been designated as a quarantine organism. Therefore, identifying its invasion routes and determining the origin of new outbreaks are of crucial importance to establish and improve regulatory measures and to potentially limit the caused damages. For that purpose, we performed population genetics analyses on a worldwide sampling of natural pinewood nematode populations. Their genetic characterization was achieved using a set of 16 validated microsatellite loci previously identified *in silico* in the *B. xylophilus* genome. The genetic diversity was low to moderate in the native area, and low to extremely low in the invaded areas. Subsequent analyses indicated a strong spatial genetic structuration in *B. xylophilus* populations from the native area, thus indicating a dramatic genetic drift effect. The almost complete absence of polymorphism in Madeira and continental Portugal hence suggested a single event of introduction in these areas and a small effective size of the introduced populations. However, the classical analytical methods used identified two alternative options, i.e., the native area and Japan as possible sources for Europe. More powerful inference methods will be used to quantitatively evaluate and statistically compare the various scenarios in competition.

THE DEVELOPMENT OF NEMATODE MANAGEMENT PRACTICES IN MENTORING UNDERGRADUATE STUDENTS. **Marahatta, S.P., B. Yamamoto, V.H. Henmi and P.V. Fewkes.** Science and Math Division, Kauai Community College, University of Hawaii, Hawaii, USA.

The need and benefits of undergraduate student's research experience have been advocated in higher education. Thus, undergraduate students at Kauai Community College, Hawaii were mentored on nematode management internships in 2012 -13. The objective of the internships was to develop students' research skill. Internships were focused on management of multiple plant-parasitic nematodes, reniform nematode (*Rotylenchulus reniformis*), root-knot nematode (*Meloidogyne* spp.) and burrowing nematode (*Radopholus similis*), and beneficial nematodes through cover crops. Effects of tropical cover crops, sunn hemp (*Crotolaria juncea*) and pigeon pea (*Cajanus cajan*), on nematodes were evaluated through two separate

shadehouse experiments. A third shadehouse experiment was conducted to determine the effects of sunn hemp at 0.5% and 1.0% (w/w) on *R. reniformis*. All experiments were conducted in pots by mixing nematode infested soil and cover crops for two weeks. In each experiment, separate no cover crop control pots were maintained. Sunn hemp and pigeon pea did not suppress *R. similis* ($P > 0.05$), but did suppress *Meloidogyne* ($P < 0.05$). Soil incorporation of 0.5% and 1.0% sunn hemp was insignificant for *R. reniformis* ($P > 0.05$). However, sunn hemp consistently enhanced beneficial nematodes number in all experiments ($P < 0.05$). Effects of pigeon pea on beneficial nematodes were inconsistent. Interns had recommended that farmers should add at least 0.5% sunn hemp in their fields to enhance beneficial nematodes, and could choose pigeon pea as an alternate cover crop. Results were presented by interns at Regional or National conferences. It is suggested to include research focused internship projects in undergraduate student'scourse curriculums.

THE GENUS *HELICOTYLENCHUS* IN THE GRASSLAND BIOME OF SOUTH AFRICA. **Marais[1], M., A. Swart[1], A.S. Schoeman[2] and L.R. Tiedt[3].** [1]National Collection of Nematodes, Biosystematics Programme, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa; [2]Sports Turf Solutions, Pretoria, South Africa; [3]Laboratory of Electron Microscopy, North West University, Potchefstroom, 2520, South Africa.

The grassland biome is one of the most threatened biomes because it is here that the bulk of South Africa's maize, beef, and milk production are anchored. Moreover, urbanisation and unbridled mining are also placing immense pressure on this biome. For example 77 % of the Gauteng Province is classified as grassland, but only 5 % of this area is protected. Datasets of the distribution of *Helicotylenchus* species contained in the South African Plant-Parasitic Nematode Survey (SAPPNS) database were used to test the theory that grassland disturbance leads to decline in biodiversity of nematode fauna. In this paper the diversity of *Helicotylenchus* species found in pristine grassland, agricultural ecosystems, and urban areas were taken as indicators of ecological disturbance. Sports fields were chosen as representatives of urban ecosystems. The genus *Helicotylenchus* is widespread in South Africa, with 25 of the 33 South African *Helicotylenchus* species reported from the grassland biome. The two most common species were *Helicotylenchus digonicus* and *Helicotylenchus dihystera,* and both were found in 13 of the 15 different vegetation types that comprise the grassland biome. According to the SAPPNS datasets, there was a decline in biodiversity as the habitat changed from pristine grassland to crop plants to urban areas. Twenty three species were reported from uncultivated areas, 14 species from crop plants, and 10 species from the grasses grown on sports fields. *Helicotylenchus* is ideally suited for a study on the impact of cultivation on nematode diversity.

EVIDENCE OF INCREASING NEMATODE NUMBERS AND THE POTENTIAL OF BIOFRIENDLY PRODUCTS IN THEIR MANAGEMENT. **Martin, T.J.G. and C.C. Fleming.** Agri-Food and Biosciences Institute, Newforge Lane, Belfast, Northern Ireland.

Nematodes are one of the most important pests worldwide causing crop losses of between 9% and 15%. Considering that the projected increase in food consumption by 2050 is assessed at 75%, it becomes imperative that monitoring takes place to have a current up to date knowledge of the pest status for any region. Current trends throughout Northern Europe would indicate that there are nematode populations establishing in regions where previously they had not been detected. These facts combined with the decision to decrease the amount of inorganic pesticides leads to the need for new and novel nematode population management techniques which are more acceptable and environmentally friendly. It will be demonstrated that within the last eight years genera and species of nematodes, not previously detected in Irish soils, have been found and have become established. These are causing major damage in amenity grassland and on some occasions in agricultural crops. The potential of some bio-friendly products are investigated for their ability to manage nematode populations or for their capacity to protect the plant against attack.

TIME INTERVAL BETWEEN SUNN HEMP COVER CROPPING AND CASH CROPPING FOR NEMATODE AND CROP MANAGEMENT. **Martiney, C.L. and S.P. Marahatta.** Science and Math Division, Kaua'i Community College, University of Hawai'I, Hawaii, USA.

Sunn hemp (*Crotalaria juncea*) is a tropical legume that when used as a cover crop, has allelopathic properties that suppresses plant-parasitic nematodes, enhances beneficial nematodes, and improves soil health. However, the allelopathic effects of sunn hemp could also reduce cash crops' germination, if sunn hemp is planted immediately after cover cropping. To find out an optimum interval of days between sunn hemp cover cropping and cash cropping, a field experiment and a shade house experiment was conducted in 2013. In the field experiment, sunn hemp and pigeon pea (*Cajanus cajan*) cover crops were separately grown for one month, tilled and incorporated into the soil. During the cover cropping period, a separate plot with no cover crop fallow was maintained as a control.Corn, *Zea mays*, was seeded immediately after incorporating cover crops into the soil. In the shade house experiment, fresh sunn hemp was incorporated with soil samples at 1.0% (w/w) (sunn hemp+) or non-incorporated (sunn hemp-), kept in a 10-cm diam plastic pot, and left for zero week (W-0), one week (W-1) and two weeks (W-2). At W-0, W-1 and W-2, corn seeds were seeded. In the field experiment, compared to pigeon pea and control, sunn hemp reduced corn germination ($P < 0.05$). In the shade house experiment, compared to sunn hemp(-), sunn hemp(+) reduced corn germination on W-0 ($P < 0.05$), but not on W-1 and W-2 ($P < 0.05$). When farmers use sunn hemp as a cover crop, it is best to wait one week before seeding a cash crop in order to optimize seed germination.

POCHONIA CHLAMYDOSPORIA AND PLANT AQUEOUS EXTRACTS, A COMBINED APPROACH FOR MANAGEMENT OF *MELOIDOGYNE INCOGNITA* IN BEANS. **Martínez[1], K.V., R.M. Belmont[1] and R.H. Manzanilla López[2].** [1]Centro de Desarrollo de Productos Bióticos Instituto Politécnico Nacional. Apartado postal 24. Yautepec Morelos México 62731; [2]Department of AgroEcology, Rothamsted Research. Harpenden Herts, AL5 2JK, UK.

Pochonia chlamydosporia is a facultative parasite of *Meloidogyne* spp. eggs, and is increasingly being used in combination with other crop management strategies, such as the nematicidal properties of extracts from several plant families. In the present work, the combination of both management strategies was tested in beans against *M. incognita*. Bean plants grown under greenhouse conditions in a soil infested with *M. incognita* were added with powder from three milled plants (6 g/ 600 g of soil) belonging to three plant species with nematicidal properties (*Chenopodium album*, *Raphanus raphanistrum*, *Thymus vulgaris*) alone or in combination with *P. chlamydosporia*. One treatment also included the fungus and the three plant species together. All treatments were compared with the nematicide carbofuran. There was a significant reduction in root galling with carbofuran, the combination of the fungus with the three plant species applied together, and the combination of the fungus with *Thymus vulgaris*. There was no difference in green bean production between treatments. It was concluded that it is possible to combine both management strategies against *M. incognita* but further studies are required.

BIOCIDAL EFFICACY OF ENTOMOPATHOGENIC NEMATODES AGAINST CATTLE TICKS. **Maru[1], A.K., S. Kachhawaha[2], A.U. Siddiqui[3] and S.K. Sharma[3].** [1]Department of Entomology, B.A.C., Bihar Agricultural University, Sabour, Bhagalpur-813210, India; [2]Krishi Vigyan Kendra, CAZRI, Pali-Marwar -306401, India; [3]Department of Nematology, R.C.A., MPUAT, Udaipur-313001, India.

Entomopathogenic nematodes have been successfully used as biological control agents of insects of economically important crops. In the present study, the biocidal efficacy of two different strains of entomopathogenic nematodes, *Steinernema carpocapsae* STSLU and *S. carpocapsae* STUDR against two different cattle ticks, *Rhipicephalus microplus* and *Hyalomma savignyi* was evaluated on the basis of percentage mortality under laboratory conditions. The adult female cattle ticks were inoculated with infective juveniles (IJs) of both the strains of *S. carpocapsae* at different inoculum levels. All the treatments were replicated four times at 20° C in a B.O.D. incubator. The percentage mortality of the cattle ticks was determined every 24 hours up to 120 hours from the time of inoculation. Rhipicephalus *microplus* was more susceptible to both strains than *H. savignyi*.*Steinernema carpocapsae* STSLU was more efficient than *S. carpocapsae* STUDR and cause 100 and 97.5 % mortality of *R. microplus* and *H. savignyi*, respectively at a concentration of 250 IJs/Petri dish after 120 hours. Both the tested strains showed promise for the control of *R. microplus* and *H. savignyi,* and will be evaluated further in field conditions.

THE LEVAMISOLE-SENSITIVE NICOTINIC ACETYLCHOLINE RECEPTOR OF *GLOBODERA PALLIDA*. **Marvin[1], J., A. Crisford[2], L. Jones[1], C. Lilley[1], V. O'Connor[2], L. Holden-Dye[2] and P. Urwin[1].** [1]Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK; [2]Centre for Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK.

The potato cyst nematode *Globodera pallida* costs the UK potato industry over £50 million per annum and novel effective control of the pathogen is essential. The ability to locate and migrate to host-roots in the soil is a common requirement among many plant-parasitic nematodes and may be a key target for control. A target for control may be the mechanism of muscle contraction by the neurotransmitter acetylcholine. The paralytic drug levamisole is known to act via acetycholine receptors. *Globodera pallida* is more resistant to levamisole than *Caenorhabditis elegans*. In *C. elegans* the levamisole-sensitive acetylcholine receptors of body wall muscle are largely comprised of five subunits; UNC-38, UNC-63, UNC-29, LEV-1 and LEV-8. Orthologues of *unc-38; unc-63* and *unc-29* have been identified and cloned from *G. pallida*. Orthologues for *lev-1* and *lev-8* have not been identified in *G. pallida*. The predicted amino acid sequence of *Gp-unc-38* lacks important determinants for binding of acetylcholine and other agonists like levamisole. *Caenorhabditis elegans* mutants that are functionally null for *unc-38* display an uncoordinated phenotype and increased resistance to levamisole. Transgenic expression of *Gp-unc-38* in this mutant background rescues normal movement suggesting a functional reconstitution of the levamisole sensitive receptor, but does not restore full sensitivity to levamisole. This raises important questions about the arrangement and pharmacology of this receptor in *G. pallida* that are currently being investigated. Other members of the order Tylenchida share the same complement of receptor subunits and features of UNC-38, which may provide a unique target for control of plant-parasitic nematodes.

TYLENCHULUS SEMIPENETRANS BIOTYPE IN SOUTH AFRICA: PONCIRUS BIOTYPE. **Mashela[1], P.W., Z.P. Dube[1] and K.M. Pofu[2].** [1]School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa; [2]Agricultural Research Council- Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria 0001, South Africa.

The reproductive potential (Pf/Pi) previously used to established the citrus nematode (*Tylenchulus semipenetrans*) biotype among differential hosts did not allow for inter- and intra-continental comparability. Therefore, the reproductive potential was used to re-assessed the citrus nematode biotype from *T. semipenetrans* isolates collected from 18 different citrus-producing district municipalities in South Africa on rough lemon (*Citrus jambhiri*), trifoliate orange (*Poncirus trifoliate*) and

olive (*Olea europaea*) under greenhouse conditions. Inoculum from each of the 18 districts constituted a single experiment, with three differential hosts arranged in a randomised complete block design, with 15 replications. Three months after inoculation in all 18 experiments, the reproductive potential (eggs + juveniles) suggested that the *T. semipenetrans* isolates did not reproduce on olive, but reproduced on the other two hosts. In conclusion, the use of reproductive potential provided conclusive evidence that the *T. semipenetrans* biotype in South Africa was poncirus and the information was comparable with those from other citrus-producing countries.

SOIL ALLELOCHEMICAL RESIDUE EFFECTS FROM NEMARIOC-AL PHYTONEMATICIDE ON *BRADYRHIZOBIUM JAPONICUM* NODULATION, *VIGNA UNGUICULATA* AND *MELOIDOGYNE* SPECIES. **Mashela[1], P.W., K.M. Pofu[2] and Z.P. Dube[1].** [1]University of Limpopo, School of Agricultural and Environmental Sciences, Private Bag X1106, Sovenga 0727, South Africa; [2]Agricultural Research Council – Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria 0001, South Africa.

Nemarioc-AL phytonematicide consistently stimulated growth of tomato (*Solanum lycopersicum*) and suppressed population densities of *Meloidogyne* species under diverse environments. However, information on its soil allelochemical residual (SAR) effects on growth of successor crops and related population nematode densities is scant. A field trial was therefore conducted to investigate the SAR effects of nemarioc-AL on growth of cowpea (*Vigna unguiculata*) as a successor crop and the related population nematode densities. The SAR conditions were created in a 5 × 5 factorial tomato experiment, with the first and second factors being concentrations and application intervals, respectively. The concentration × application interval contributed 21% to total treatment variation (TTV) in number of galls, with permutations consistently reducing number of galls. The concentration factor contributed 12% and 8% to TTV in dry seed mass and number of nematodes, respectively, increasing (39-83%) dry seed mass and decreasing (88-94%) nematode densities. The application interval contributed 7% to TTV in dry shoot mass and number of nematodes each, increasing (39-69%) dry shoot mass and reducing (45-92%) nematode densities. In conclusion, appropriate permutations of concentration and application interval are necessary to allow for cowpea production as a successor crop to a tomato crop after using Nemarioc-AL phytonematicide.

MANAGING PHYTOTOXICITIES IN PHYTONEMATICIDES: THE DOSAGE MODEL. **Mashela[1], P.W., K.M. Pofu[2] and Z. Dube[1].** [1]School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727 South Africa; [2]Agricultural Research Council Roodeplaat-VOPI, Private Bag X293, Pretoria, 0001, South Africa.

Phytonematicides are inherently allelopathic to plants protected against plant-parasitic nematode damage. The dosage model was developed to manage phytotoxicity in phytonematicides. The objective of this study was to use empirically-derived information to provide the three-step process of developing the dosage model. First, mean concentration stimulation range (MCSR) was established by subjecting test plants inoculated with nematodes to various concentrations of the phytonematicides for 56 days. Significantly affected plant variables were subjected to the Curve-fitting Allelochemical Response Dosage computer-based model to generate seven biological indices, where D_m and R_h indices were used to compute the non-phytotoxic concentration using the MCSR = $D_m + (R_h/2)$ relation. Second, the application interval (T) for the MCSR value was established using the concept of a ''30-day-week-period'', where T comprised 0, 1, 2, 3 and 4 application intervals. Significantly affected plant variables were further subjected to lines of the best fit, where quadratic curves ($bx^2 + bx + c$) were optimised using $-b/2b_2$ to generate the appropriate T for the empirically-based MCSR value. Using the proportion of T to the crop cycle (T_{cc}), the application frequency (T_{ca}) was derived ($T_{ca} = T/T_{cc}$) to allow for computation of the dosage ($D = MCSR \times T_{ca}$, which is currently being successfully used under diverse environments to manage *Meloidogyne* species without causing phytotoxicity to tomato plants.

NAPDH OXIDASES PRODUCE ROS THAT LIMIT CELL DEATH AND FACILITATE NEMATODE INFECTION IN *ARABIDOPSIS THALIANA*. **Matera[1], C., M.S. Hasan[1], Z.S. Radakovic[1], P. Gutbrod[1], E. Rozanska[2], M. Sobczak[2], M. Angel Torres[3], F.M.W. Grundler[1] and S. Siddique[1].** [1]INRES, Department of Molecular Phytomedicine, University Bonn, Bonn, Germany; [2]Department of Botany, Warsaw University of Life Sciences (SGGW), Warsaw, Poland; [3]Centro de Biotecnología y Genomica de Plantas (UPM-INIA), ETSI Agrónomos, Universidad Politecnica de Madrid, Pozuelo de Alarcón, Madrid, Spain.

Plants produce reactive oxygen species (ROS) in response to infection. These ROS activate defence responses and are mainly generated by plasma membrane-localized NADPH (Nicotinamide adenine dinucleotide phosphate) oxidases. Mutation of NADPH oxidase genes eliminates pathogen-induced ROS production and compromises immune responses. However, the functions of ROS in compatible plant-pathogen interactions remain largely unknown. Here, we demonstrated that the roots of *Arabidopsis thaliana* with mutation in NADPH oxidases failed to produce ROS when infected by the plant parasitic nematode *Heterodera schachtii*. Inhibition of ROS production by NAPDH oxidase with a small molecule Diphenlyl iodonium limited cell death spread and thus facilitated nematode-infection. Moreover, we discovered that the role of the ROS in antagonizing cell death during nematode infection is independent of salicylic acid. Our study provides understanding of how a pathogenic organism with destructive invasion behaviour is able to switch to a biotrophic life-style that requires viable

host cells for feeding. The molecular mechanisms and signalling pathways involved in ROS-mediated restriction of cell death will be discussed.

STUDY OF BRAZILIAN SPECIES OF *MELOIDOGYNE*: ENZYMATIC AND MOLECULAR CHARACTERIZATIONS. **Mattos[1], V.S., J.M.S. Monteiro[1], J.E. Cares[1], V.R. Correa[1, 2], M.R.A. Almeida[2], J.P. Borges[3], R.M.D.G. Carneiro[2].** [1]Dep. Fitopatologia, Universidade de Brasília, 70910-900 Brasília, Distrito Federal, Brazil; [2]Embrapa Recursos Genéticos e Biotecnologia, 70849-979 Brasília, Distrito Federal, Brazil; [3]Embrapa Hortaliças, 70359-970 Brasília, Distrito Federal, Brazil.

Root-knot nematodes (*Meloidogyne* spp.) are amongst the world's major crop pathogens, due to their wide distribution, extensive host ranges, and ability to cause considerable economic losses. Efficient control of these pathogens depends upon correct species identification. Recently, five Brazilian species of *Meloidogyne* were described (*Meloidogyne petuniae*, *Meloidogyne pisi*, *Meloidogyne phaseolus*, *Meloidogyne brasiliensis* and *Meloidogyne polycephannulata*) without detailed molecular characterization. Therefore, the aim of this study was to characterise the type isolates using enzymatic profiles and SCAR markers. Esterase profiling and SCAR analyses were done according to described protocols. *Meloidogyne petuniae* Est Pe2 (Rm: 0.95, 1.08) and *M. pisi* Est Pi5 (Rm: 0.91,0.95,1.12,1.25,1.33) showed new species-specific esterase phenotypes. *Meloidogyne phaseolus*, *M. brasiliensis* and *M. polycephannulata* had the same species-specific esterase phenotypes as *Meloidogyne morocciensis (*Est A3, Rm: 1.1,1.2,1.3), *Meloidogyne ethiopica* (Est E3, Rm: 0.9,1.05,1.20) and *Meloidogyne incognita* (Est I2, Rm:1.0, 1.1), respectively. PCR with species-specific SCAR primers confirmed the results of esterase phenotypes: *M. phaseolus* and *M. morocciensis*, both showed a single specific fragment of 420 base pairs (bp), as well as in *M. brasiliensis* and *M. ethiopica* (350 bp) and *M. polycephannulata* and *M. incognita* (399 bp). Preliminary, morphological studies revealed similarities in characters of females, males and second stage juveniles among these species. Additional studies, including morphology, sequencing of 18S rRNA, internal transcribed spacer rRNA (ITS), D2-D3 fragment of 28S rRNA and phylogenic analyses have been carried out in order to clarify the taxonomic status of these Brazilian root-knot nematode species.

EFFECTIVENESS OF ECOSYSTEM CONDITION AND FUNCTION INDICES USED IN SOIL NEMATOLOGY. **Matveeva, E., A. Sushchuk and D. Kalinkina.** Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, 11 Pushkinskaya St., 185910 Petrozavodsk, Russia.

Monitoring of soil nematodes in the Republic of Karelia has provided extensive data on the taxonomic diversity, abundance and community structure of nematodes in coenoses differing in geographical location, type of vegetation and scope of disturbance. The large data pool allowed us to check the effectiveness of the ecological indices derived from nematode fauna analysis (structure *SI*, enrichment *EI*, and channel *CI* indices) for assessing soil ecosystem conditions. It is shown that the indices reflect both changes in the soil or plant cover of natural coenoses, and the consequences of environmental disturbance caused by human activities. E.g., differences depending on the geographical location were found in *CI* and *SI* indices. In meadows *CI* significantly decreased from southwards (from 56 to 23). Northern coenoses exposed to extreme climatic conditions or anthropogenic transformation had a high *SI* index owing to a large proportion of omnivores in the nematode community structure. In natural coenoses *EI* and *CI* indices were associated with the type of vegetation. A combination of the indices enables clear differentiation between nematode communities of meadow and forest habitats. The nematode communities of meadows had higher *EI* and lower *CI* values. Low *SI* and high *EI* values were detected in disturbed habitats (urban areas, industrial zones). Agrocoenoses yielded similar values, indicating habitat instability and simplification of the soil food web. Thus, ecological indices are effective tools for assessment of the state and functioning of soil ecosystems.

PRIMING OF POTATO PLANTS BY TEMPERATURE FOR ENHANCEMENT OF RESISTANCE TO COLD STRESS AND NEMATODE INVASION IN THE NORTH. **Matveeva[1], E., V. Lavrova[1], E. Sherudilo[1], M. Seppänen[2] and P. Palonen[2].** [1]Institute of Biology, Karelian Research Centre of Russian Academy of Sciences, Pushkinskaya St. 11, 185910 Petrozavodsk, Karelia, Russia; [2]University of Helsinki, P.O. Box 27, FIN-00014, Helsinki, Finland.

Priming of plants to abiotic and biotic stresses is an important component in plant survival and normal functioning in the North, where low temperatures with sharp fluctuations during day-cycle are experienced and consequences of environment disturbances, including pest invasion, are strongly expressed. Research on priming of plants by low temperature was carried out on a parasite system ''potato - potato cyst nematode *Globodera rostochiensis* (Wollenweber)Behrens, Ro1''. Plants of potato genotypes derived from wild species *Solanum commersonii,* possessing different freezing tolerances and cultivated potato *Solanum tuberosum* (resistant and susceptible to nematode cultivars) were subjected to a temperature drop from 23 to 5°C for 2 h at the end of the night. Afterwards half of plants were infested by potato cyst nematode, (10-20 cysts per plant). It was established that nematode infestation promoted plant growth and development and led to a slight decrease in content of photosynthetic pigments and Fv/Fm ratio in potato genotypes in comparison with non-infested plants. Plant growth and development of susceptible cultivars were inhibited and photosynthetic pigment content was slightly increased. Morphological and physiological traits of resistant cultivars were not affected under *G. rostochiensis* infestation. Temperature

priming diminished differences between infested and non-infested plants and enhanced plant resistance to potato cyst nematode (final nematode population was decreased on 30%). In addition temperature drop enhanced cold resistance in both potato genotypes and cultivars compared with control.

NEMATODE COMMUNITIES IN MODERN SOYBEAN CROPPING SYSTEMS IN SOUTH AFRICA. **Mbatyoti[1], A., D. Fourie[1], A. Swart[2] and A.H. Mc Donald[1].** [1]North-West University, Unit of Environmental Sciences and Management, Private Bag X6001, Potchefstroom, 2520, South Africa, 24088978@nwu.ac.za; [2]National Collection of Nematodes, Biosystematics Division, Agricultural Research Council - Plant Protection Research Institute, Private Bag X134, Queenswood, 0121, South Africa.

Local soybean (*Glycine max* (L.) Merr.) crops host a variety of plant-parasitic nematode species, with *Meloidogyne incognita* and *Meloidogyne javanica* being the predominant nematode pests. Information on nematode-soybean associations exists for conventional soybean crops but not for Roundup Ready soybean cultivars, which constitute more than 65% of modern plantings. Nematode surveys were thus conducted during 2012 and 2013 by sampling soil and roots at 11 localities where conventional and Roundup Ready soybean crops were grown in close proximity. Grass in natural areas adjacent to soybean fields was also sampled concurrently. *Meloidogyne* spp. was generally the predominant nematode pest associated with conventional and Roundup Ready soybean roots. Their population levels ranged from ca. 23000/50g roots of Roundup Ready to ca.175000/50g roots of conventional soybean cultivars. Natural grass hosted up to ca. 1800 *Meloidogyne* eggs and J2/50g roots. Other plant-parasitic nematodes that were recorded from root and soil samples from soybean and natural vegetation were *Pratylenchus* spp *Helicotylenchus* spp., *Rotylenchus* spp., *Scutellonema* spp., *Criconemoides* spp., *Criconema* spp. and *Tylenchorhynchus* spp. In terms of non-parasitic nematodes a variety of fungivores, bacterivores, omnivores and predators were recorded in the soil samples from conventional and Roundup Ready soybean, as well as natural fields.

OCCURRENCE OF ROOT KNOT NEMATODES IN AFRICAN LEAFY VEGETABLES PRODUCTION SYSTEMS OF WESTERN KENYA. **Mbogoh[1], J.M., E. Omami[1], L. Ngode[1], J. Ochuodho[1], P. Njira[2] and W. Sunda[1].** [1]University of Eldoret, P.O. Box 1125-30100 Eldoret, Kenya; [2]Moi University, P.O. Box 1035-30100, Eldoret, Kenya.

The current market supply of African leafy vegetables does not meet domestic demand due to production constrains attributed to pests especially root-knot nematodes. A survey was conducted in Busia, Kakamega and Bungoma counties to assess the occurrence of root-knot nematodes in leafy vegetable production systems. Structured questionnaires were administered to 120 selected respondents. Soil and roots were sampled for the presence of *Meloidogyne* spp. and the data analysed using SPSS. The number of galls/root system differed significantly at $P<0.05$ with a mean of 4.8 for *Solanum* spp., 3.7 for *Cleome* spp., 1.0 for *Crotalaria* spp., and 3.0 for *Amaranthus* spp. The incidence of root-knot nemataodes was highest in Bungoma at 83%. Mixed cropping system was practiced by over 60% of the farmers with 80% of them growing all their vegetables in home gardens. There was no significant difference in nematode levels in African leafy vegetables grown in the home garden or commercial production area in the three counties. No discernable differences were also observed in leafy vegetables grown in mixed or mono cropping systems in Busia and Kakamega counties. *Solanum* spp. was found to be very susceptible to root-knot nematodes while *Crotalaria* spp. was resistant.

USE OF *CROTALARIA INTERMEDIA* IN CROP ROTATION FOR MANAGEMENT OF ROOT KNOT NEMATODES (*MELOIDOGYNE SPECIES*). **Mbogoh[1], J.M., E. Omami[1], J. Ochuodho[1], L. Ngode[1], P. Njira [2] and W. Sunda[1].** [1]University of Eldoret, P.O. Box 1125 -30100; [2]Moi University, P.O. Box 1035 -30100, Eldoret, Kenya.

The use of rotational crops that are poor or non-hosts, or antagonistic to nematodes is an effective cultural practices for nematode management, as it is less environmentally harmful and an alternative to chemical nematicides. An experiment was carried out in a greenhouse in pots to evaluate the capacity of *Crotalaria intermedia* to suppress population buildup of root knot nematodes in soils when used in rotation with nematode susceptible crops. Seven different crop combinations of *Crotalaria intermedia*, *Solanum nigrum* and *Cleome gynandra* were evaluated. The experiment was a complete randomized design with 21 treatments, three replicas, two rotation cycles (two seasons) and seven treatments that comprised four rotational programmes and three mono crops. *Crotolaria intermedia* mono crop was the control. The soils were inoculated with 3000 root-knot nematode juveniles. Data was analysed using Genstat statistical package and means separated using Tukeys. The result showed that in season one *C. intermedia* had no galls and had a reproduction factor (RF) of less than 1 while *C. gynandra* and *S. nigrum* had galling index ranging from 3-5 respectively and RF of more than 2. In season two crops that were rotated with *C. intermedia* showed a reduced galling index and RF while mono crops of *C. gynandra* and *S. nigrum* had the galling index and RF increase up to 5 and above 2 respectively. Mono crops of *C. intermedia* had no galls and recorded a reduced final inoculum and RF. Use of *Crotalaria intermedia* in rotation with nematode susceptible crops in nematode infested areas is an effective way of managing nematodes.

INTRODUCTION TO NEMATODES: A MULTIMEDIA PRESENTATION FOR TEACHING NEMATOLOGY. **McGawley[1], E.C., C. Overstreet[1], M.J. Pontif[1] and A.M. Skantar[2].** [1]LSU AgCenter, Baton Rouge, Louisiana 70803, USA; [2]USDA-ARS Nematology Laboratory, Beltsville, Maryland 20705, USA.

Teaching in general, and in the agricultural protection sciences in particular, is a challenging endeavour in the 21st century. A large monitor or projection screen and a range of multi-media resources have replaced the traditional blackboard and multi-coloured chalk. Hardbound textbooks are increasingly being supplemented with or replaced by software-based instructional modules. In order to successfully engage and educate students, instructors must enter the classroom equipped with ''seasoned'' experience in and up to date knowledge of subject matter, a keen awareness of the scientific acuity of the student body and a genuine enjoyment for the teaching activity. *Introduction to Nematodes*, a multimedia, multi-platform, multi-layered slide production, now in it's second edition, brings this new technology to the teaching of nematology. The presentation, two years in production, is available for free (for educational purposes) download from the websites nematologists.org, ontaweb.org and nematode.net and focuses primarily on plant parasitic nematodes. Users of this presentation should be mindful of and convey to audiences the great diversity and immense importance of other members of this unique assemblage of animals grouped into the phylum Nematoda. Contributions of photographs or formatted data that improves existing sections or adds new sections on nematodes other than those that are plant parasites are most welcome. This presentation should NOT be considered a FINISHED product. Hopefully, with submissions from individuals interested in nematodes, it will evolve into an ongoing project that reflects the biological, ecological, and scientific knowledge accumulated about nematodes and produces an increased awareness of their vital roles in agriculture worldwide.

FUNCTIONAL ANALYSIS OF *GLOBODERA PALLIDA* SPRYSEC PROTEINS. **Mei[1,2], Y.Y., S. Mantelin[2], A. Haegeman[1,3], K. Wright[2], G. Gheysen[1] and J.T. Jones[2].** [1]Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium; [2]Cell and Molecular Sciences Group, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom; [3]Institute for Agricultural and Fisheries Research (ILVO), Burgemeester Van Gansberghelaan 96, box 1, 9820 Merelbeke, Belgium.

The potato cyst nematode, *Globodera pallida*, is the most economically important nematode to British agriculture and is one of the most destructive pathogens of potato. A lack of major resistance genes and the removal of most effective nematicides have led to an increasing need to better understand the molecular basis of the interaction between *G. pallida* and its host in order to develop sustainable control strategies. The interaction between the plant and the cyst nematode is mediated by effectors that are synthesized in the nematode oesophageal glands and injected into the host through a stylet. A large number of effector candidates were identified from *G. pallida* as a result of the genome sequencing project. Of particular note is the *SPRYSEC* gene family which includes more than 300 members. Many *SPRYSECs* are expressed in the dorsal gland cell of invasive stage juveniles indicating their potential roles in plant parasitism. We have identified *SPRYSEC* proteins that suppress plant defence. In addition, potential host target proteins have been identified for several *SPRYSECs* by yeast two hybrid screens. The focus of the current work is on linking the molecular interactions and the observed phenotypes.

CREATION OF *PASTEURIA PENETRANS* CLONAL LINES FOR STUDY OF GENETIC VARIATION IN *PASTEURIA* SPP. POPULATIONS RELATING TO HOST VIRULENCE. **Mekete[1], T., S. Joseph[1], T. Hewlett[2], C. Watrin[2] and L. Schmidt[2].** [1]University of Florida, Entomology and Nematology Department, 970 Natural Area Drive, Gainesville, Florida 32611, USA; [2]Syngenta Crop Protection, LLC 12085 Research Drive, Suite 185, Alachua, Florida 32615, USA.

The genus *Pasteuria* is comprised of gram-positive, endospore-forming bacteria, which are castrating parasites of nematodes and crustaceans. The aim of this project was to produce clonal isolates of *Pasteuria penetrans* via single-spore infection of the host, to allow investigation of genotypic variation in the population as it relates to host-parasite dynamics and to identify the role of individual genotypes to the overall virulence of the population. *Pasteuria penetrans* endospores conjugated with fluoroscein isothiocyanate (FITC) allowed critcal observance of attachment under epifluorescent microscopy. Single spore attachment was obtained in a suspension which contained $2X10^3$ spores/ml and 4000 *Meloidogyne incognita* juveniles (J2)/ml centrifuged at 6000g for 1 min at 25°C. Although endospore attachment per individual nematode varied from 1 to 4, 50% of nematodes were observed to be attached with a single spore. No endospore attachment was detected at a spore concentration of $2x10^2$ spores/ml. Centrifugation of the suspension containing $2x10^4$ and $2x10^5$ spores/ml provided a higher rate of attachment (>10 spores/nematode). Fourteen *P. penetrans* clonal lines resulting from the effort were screened for purity by Sanger sequencing analysis of 16s rDNA and house-keeping genes.

NEMATOLOGY TRAINING IN THE UNITED STATES OF AMERICA AND WHAT THE FUTURE HOLDS. **Melakeberhan, H.** Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA.

Over the last 50 years or so, nematology training in the United States of America has changed in many ways. The significance of nematodes on food security (as pests and as biocontrol agents) and on understanding basic biology and ecosystem functioning that impact global climate change continue to increase, but the numbers of university and

non-university nematology positions have been declining. While some of the decline in positions may reflect changes with time, a lot more needs to be done to change the trend by emphasizing the breadth and depth of what nematology has to offer to society at-large. This will require considering lessons from the past, anticipating global change far into the future and building a pathway for nematology to prosper in the changing times. Against this background, I will discuss the following points in this presentation: i) impact of the chemical era on training and disciplinary development; ii) growth of ecology, entomophilic and molecular nematology; iii) the need for integrating basic, applied, and traditional nematology, iv) adjusting sub-disciplinary domination and views for the greater good; v) taking academic success into the stock market; and vi) understanding local to national level policies impacting training and research funding.

INCREASING STUDENT PARTICIPATION TO ENSURE THE FUTURE OF NEMATOLOGY IN RAISING THE STANDARD OF LIFE IN DEVELOPING COUNTRIES STRUGGLING WITH ECOSYSTEM DEGRADATIONS. **Melakeberhan, H.** Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA.

In an increasing intertwined world, balancing food security, adaptation to climate change-driven problems, and improving standard of life and overall planetary ecosystem health present many challenges for all and special opportunities for nematology. Everybody wants to live in good conditions, but people in many developing countries have to get by every day by doing what they could to survive and to feed their families, even if it results in ecosystem destruction. Where ever ecosystem destruction may happen, its impact is global. Most of the resources and the know-how to solve the problems may be centered in the North, but the solutions have to be performed mostly in the South. As E. O. Wilson, towering global figure, reminds us ''*. . .the most diversity of organisms and the destruction of life exist in developing countries... and those nematodes representing four out of five multi-cellular animals on the planet and so sensitive to soil ecosystem changes offer so many opportunities for, among other things, understanding environmental changes..*'' (10/22/2007, Global View Series Lecture). None of these challenges can be addressed in sustainable ways without paying attention to soil degradation, which is a long-term problem, and without building expertise and capacity on the ground. These situations present unique opportunities for nematology to build a stronger North-South bridge that will lead to addressing many pressing global issues in tenable ways. Training students and developing two-way collaborative research and outreach programs are some of the mechanisms to develop solutions to the long-term problems. These issues and the role of the internet, biggest equalizer and enabler, will be discussed.

QUANTIFYING BIOLOGICAL BASIS OF SOIL HEALTH DEGRADATION IN SELECTED SUB-SAHARAN AFRICA SOIL GROUPS. **Melakeberhan[1], H., T. Schmidt[3], Z.T.Z. Maung[1], T. Teal[2], S. Yildiz[1], W. Kimenju[4], C. Kwoseh[5] and V. Saka[6].** [1]Agricultural Nematology Laboratory, Department of Horticulture; [2]Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA; [3]Departments of Microbiology and Immunology, and Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA; [4]University of Nairobi, P.O Box 30197, G.P.O, Nairobi, Kenya; [5]Kwame Nkrumah University of Science and Technology, PMB, UPO, Kumasi, Ghana; [6]Lilongwe University of Agriculture and Natural Resources, Lilongwe, P.O. Box 219, Malawi.

While the impact of terrestrial ecosystem degradation (TED) on sub-Saharan Africa (SSA) food security and loss of biodiversity is well known, the solutions primarily center on fixing soil physiochemical deficiencies through pH and fertilizer management. When there are structural deficits of organic carbon (%C) and nitrogen (%N), both dependent on biological processes to maintain healthy levels, there is a need to integrate soil biology into soil degradation management strategies. This requires quantifying the biological community structure and function that drives the soil food web, the nutrient cycling platform. As part of building a data base for developing scalable models across soil groups (orders) (the plates up on which different cultures stump their ecosystem change footprints), we investigated nematode and microbial communities in Ferralsol, Lithosol, and Nitosol soil groups under subsistence agriculture in Ghana, Kenya and Malawi. Analysis of soil samples from disturbed (agricultural) and undisturbed (natural vegetation) landscapes in two regions about 30 km (Ghana) to 300 km (Kenya and Malawi) apart shows: i) lower biodiversity in disturbed than in natural landscapes across soil groups and countries; ii) abundance of trophic groups varied by landscape and soil group while frequency of occurrence varied by soil group; iii) similar nutrient mineralization pathways and depleted soil food web across landscapes, suggesting naturally fragile soils; and iv) microbial and nematode communities distinctly separating by soil groups and countries, suggesting difference in biological structures. Implications of the results on understanding TED and integrating soil biology into SSA's soil degradation management strategies will be discussed.

MOLECULAR AND MORPHOLOGICAL CHARACTERISATION OF *MELOIDOGYNE HAPLA* POPULATIONS (NEMATODA: HETERODERIDAE) FROM ROSE GREENHOUSES IN ETHIOPIA. **Meressa[1,3], B., H. Heuer[2], H. Dehne[3] and J. Hallmann[1].** [1]Julius Kühn-Institut, Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Toppheideweg 88, D-48161, Münster, Germany; [2]Julius Kühn-Institut Federal Research Center for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12 D-38104, Braunschweig,

Germany; [3]Institute for Crop Science and Resource Conservation (INRES), Department of Phytomedicine, University of Bonn, Nußallee 9, D-53115 Bonn, Germany.

Meloidogyne hapla, considered mainly as a temperate nematode species, was found for the first time parasitizing rose plants in greenhouses in Ethiopia. A survey was conducted in greenhouses randomly selected from 12 farms distributed in six districts, during 2011 and 2012. Single species nematode cultures were established for each farm by propagating single egg masses on tomato cv. Moneymaker. Identification and characterization was based on molecular sequences of the 28S D2-D3 expansion segments within the ribosomal DNA and the region located between cytochrome oxidase unit II (*COII*) and the 16S rRNA gene of the mitochondria (mtDNA). In addition, LM and SEM images together with morphometric measurements of the female, male and J2 were compared with *M. hapla* descriptions. Most morphological characters of the female, male and J2 were similar to other descriptions, with exceptions in some morphometric values. The perennial pattern conforms to characteristics of the species in most aspects, but the vulva slit was shorter than the Hawaiian population. Morphometric differences were observed in both females and J2. Female spear length and J2 body length were greater than the population from East Africa and hyaline tail length was higher than that of the Hawaiian population. Phylogenetic relationships of the Ethiopian *M. hapla* with other related *Meloidogyne* species on the basis of both mtDNA and D2-D3 expansion segment sequence analysis revealed highly supported clades containing our isolates and other published isolates from different countries. The Ethiopian isolates shared 83-100% similarity values of D2-D3 sequence with isolates from China, Germany, Moldova and the Netherlands.

A NEW METHOD FOR STUDYING POPULATION GENETICS OF CYST NEMATODES BASED ON GENOTYPING BY SEQUENCING. **Mimee[1], B., P.Y. Véronneau[1], M.O. Duceppe[1], M. Jean[2], F. Belzile[2] and G. Bélair[1].** [1]Agriculture and Agri-Food Canada, Horticulture Research and Development Centre, 430 boul. Gouin, St-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada; [2]Université Laval, Faculté des Sciences de l'Agriculture et de l'Alimentation, Département de Phytologie, 2425 rue de l'Agriculture, Québec, Quebec, G1V 0A6, Canada.

Cyst nematodes are of great economic importance and infect the roots of several major crops, including cereals, rice, soybean and potatoes. They are responsible for billions of dollars of losses each year and a number of species are regulated pests. Plant resistance is the most effective management tool, but the existence of virulent populations can reduce its efficacy. Collecting information on population genetics is thus crucial for cyst nematodes control. However, current technologies using Single Sequence Repeats (SSRs) are time-consuming and expensive because they require numerous individual larvae and PCR runs. Also, the difficulty to obtain lots of markers limits their use for trait mapping (e.g. pathotypes or avirulence genes identification). Single nucleotide polymorphisms (SNP) are abundant and widespread genetic markers across the genome that has proven to be effective for analyses with nematodes. Recent advances in next-generation sequencing and bioinformatics now allow the development of powerful methods for population genetics studies. Here, we have taken advantage of the reproduction mode of cyst nematodes, resulting in a high genetic diversity within a single cyst, to develop a simple population genetics analysis pipeline based on genotyping by sequencing. The genome-wide allele frequencies of 26 populations of golden nematodes, from eight countries and representing the five known pathotypes, have been compared. This method allowed a clear separation of the pathotypes and a fine analysis of the genetic links among global populations. In addition of being powerful, this tool has proven to be very time and cost effective and could be used for other cyst nematode species.

NEMATODE DISTRIBUTION ON HORTICULTURAL CROPS IN VARAMIN, IRAN. **Mohammad Deimi[1], A.M. and A. Karami[2].** [1]Young Researchers and Elite Club, Takestan Branch, Islamic Azad University, Takestan, Iran; [2]Former student, Faculty of Agriculture, Islamic Azad University, Takestan, Iran.

Horticulture and fruit production represents 20% of the gross domestic product in Varamin, Iran. We present the distribution of plant-parasitic nematodes in horticultural crops from 25 localities in Varamin. Six soil sub-samples/1000 m2 and 12 sub-samples/ha at 30 cm depth were analysed. In tomato, pepper, eggplant, and lettuce 14 plants/ha were taken. In the samples analysed, a predominance of *Meloidogyne incognita* and *Meloidogyne javanica* (28%) were found in horticultural crops produced under cover. *Pratylenchus neglectus* was found in 78% and *M. incognita* and *M. javanica* in 17% of the samples, respectively. *Meloidogyne javanica, M. incognita, Ditylenchus dipsaci, P. neglectus* and *Pratylenchus penetrans* were found in lettuce fields. The genus *Longidorus* was also observed.

COMPATIBILITY AND VIABILITY OF *STEINERNEMA* AND *HETERORHABDITIS* SPECIES AT DIFFERENT CONCENTRATIONS OF PESTICIDES UNDER LABORATORY CONDITIONS. **Mohankumar[1], A., P. Sundararaj[1] and S.L. Hafez[2].**[1]Department of Zoology, Bharathiar University, Coimbatore, India; [2]University of Idaho, Idaho, USA.

Experiments were conducted under laboratory conditions (26 ± 2 °C) to investigate the compatibility of three chemical insecticides *viz*., Dimethoate 30% EC (65% a.i.), Dichlorovos 76% EC (92% a.i), Monocrotophos 36% SL (68% a.i) with the infective juveniles (IJs) of newly isolated entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) from the Western Ghats of South India, India. Five concentrations (0.5, 2, 4, 6 and 8 ml/l) of the respective pesticides were replicated five times

and 1000 IJs from each genera were added individually to the solution and viability was assessed microscopically at four hour intervals for 12 hours. Data indicated that there was a significant difference in survival rate of nematodes among the concentrations and also between the genera. *Steinernema* spp. was more tolerant to insecticides compared to *Heterorhabditis* spp. Two insecticides, dichlorovos and monocrotophos caused 100% mortality at the highest concentration of 6 and 8 ml/L. In general both the genera are compatible with all three insecticides at the lower concentration of 0.5, 2 and 4 ml/L.

SANTIZERS AS NEMATODE CONTROL AGENTS - COULD IT WORK? **Mohlala, R., M.S. Daneel, W.P. Steyn and G. Tefu.** Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa.

Nematodes cause serious problems on vegetable crops resulting in up to 20% yield losses if not properly treated. Due to the phasing out of class 1-nematicides such as aldicarb and methyl bromide, the agricultural industry is in urgent need of alternative chemicals, one such avenue is sanitizers. Many of these products can kill micro-organisms very effectively in water and on hard surfaces however, very little research has been done with sanitizing agents in soil environments. After successfully testing the efficacy of Agrigold (copper compound) and Sporekill (QAC), both sanitizers, in preventing egg hatching in the laboratory, greenhouse trials were conducted to test their efficacy for nematode control and growth enhancement on tomato. A complete randomized design was used and tomato plants were infested with 2000 *Meloidogyne* spp. eggs per 2 l plant bag. In the QAC trial, product A (wetting agent) was included after claims of nematicidal activity. Results showed that plant growth enhancement was significantly higher for Agrigold and product A compared to the untreated control. In the Agrigold trial *Meloidogyne* sp. numbers per g of roots were 265 – 323 in 1/500 Agrigold compared to 500 for the untreated control treatment. Product A and Sporekill provided similar results. Although growth was enhanced with all the products tested nematode numbers often were higher due to a better root system. It is clear that these products enhance plant growth but do not seem to be very effective in nematode control.

HOW TO CONTROL *MELOIDOGYNE CHITWOODI*. **Molendijk, L.P.G., J.H.M. Visser and G.W. Korthals.** Applied Plant Research, Wageningen UR, Edelhertweg 1, 8200 AK Lelystad, the Netherlands.

Since the 1980s *Meloidogyne chitwoodi* is an important topic in arable farming in the Netherlands. Its quarantine status and the quality damage on important cash crops as potato, carrots, black salsify and gladiolus cause a strong demand for implementing control strategies which can prevent yield losses and spread of this species as much as possible. In cooperation with farmer organisations and scientists a nematode control strategy was developed and implemented to control *M. chitwoodi* with a minimum use of nematicides. In this presentation some essential elements of the nematode control strategy will be illustrated. The focus will be on the design of strategic crop rotations with non-hosts or resistant varieties based on knowledge of the field conditions. Results on the risk of introducing *M. chitwoodi* in different soil types with infested seed potato tubers will be presented. Finally the inventory on the occurrence of different populations of *M. chitwoodi* in the Netherlands will be discussed. As a result of the cooperative activities of farmers, extension services, routine sampling agencies and nematologists the awareness on *M. chitwoodi* increased considerably during the last decade. We are optimistic that the successful integrated approach of controlling potato cyst nematodes can be repeated for *Meloidogyne* species.

CONSERVATION AND AUGMENTATION OF ENTOMOPATHOGENIC NEMATODES ON CITRUS FOR CONTROL OF FALSE CODLING MOTH. **Moore[1,2], S.D., A. Manrakhan[3], M. Gilbert[4], W. Kirkman[1], J.-H. Daneel[3], J. de Waal[5] and R.-U. Ehlers[6].** [1]Citrus Research International, PO Box 20285, Humewood 6013, South Africa; [2]Department Zoology & Entomology, Rhodes University, Grahamstown, South Africa; [3]Citrus Research International, PO Box 28, Nelspruit 1200, South Africa; [4]Citrus Research International, PO Box 2201, Matieland 7602, South Africa; [5]Dow Agrosciences, PO Box 355, Paarl 7620, South Africa; [6]e-nema, Klausdorfer Str. 28-36 24223 Schwentinental, Germany.

False codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is an important pest of citrus and other crops in sub-Saharan Africa. Control measures have traditionally focussed on the above-ground life stages (eggs, neonate larvae and adults) but have ignored the soil-borne stages (prepupa, pupa and eclosing adults). Several years of thorough and compelling laboratory trials with entomopathogenic nematodes (EPNs) indicated extreme susceptibility of FCM to EPNs. Conservation of naturally occurring *Heterorhabditis zealandica* Poinar (Rhabditida: Heterorhabditidae) through non-usage of a nematicide (cadusafos) resulted in dramatically lower fruit infestation by FCM. However, a couple of earlier surveys indicated that naturally occurring EPNs are found in a very small minority of agricultural soils. Consequently, large scale field trials targeting the soil-dwelling life-stages were conducted with relatively low doses of 10 and 20 infective juveniles (IJs)/cm^2. A single application of *H. bacteriophora* Poinar to a citrus orchard floor reduced FCM infestation of fruit by up to 81%. However, results were generally variable. As a result, an approach using regular (monthly) applications at 10 and even 5 IJs/cm^2 was investigated, with improved results.

PARACTINOLAIMUS MORUS N. SP. (DORILAYMIDA: ACTINOLAIMIDAE), A NEW NEMATODE FROM VENEZUELA. **Morales-Montero, P. and E. San-Blas.** Instituto Venezolano de Investigaciones Científicas, Centro de Estudios Botánicos y Agroforestales, Laboratorio de Protección Vegetal, Av. 8 entre Calles 79 y 80, Maracaibo, edo. Zulia, Venezuela, CP 4001.

Paractinolaimus morus n. sp., found associated with guava (*Psidium guajava*) plants were fixed in formaldehyde (5%) and mounted in glycerin for morphometrical and morphological characterization. *Paractinolaimus morus* n. sp. resembles *Paractinolaimus vigor* in general form and in ratios a, b and c. The new species differs in size and by the morula shape of the *pars dilatata* which is diagnostic of *P. morus*. Females are characterized by 1342 - 1955 μm body length, four large onchia and numerous denticles in the buccal cavity, odontostyle 20 – 26 μm long, reproductive tract amphidelphic and reflexed; both branches of equal length and vulva opening with longitudinal and transversal slits forming a cross. Tail conoid and ventrally arcuated. Males not common, comprising less than 0.5% of the population. General morphology except for reproductive system same as that of females, 8 to 9 ventromedian supplements in series and tail short and rounded. This is the first report of *Paractinolaimus* in Venezuela.

FIRST REPORT OF *DITYLENCHUS GALLAEFORMANS* (TYLENCHIDA: ANGUINIDAE) INDUCING GALLS ON *CLIDEMIA FENDLERI* (MELASTOMATACEAE) FROM VENEZUELA. **Morales-Montero[1], P., S. Flores[2], S. Subbotin[3,4] and E. San-Blas[1].** [1]Instituto Venezolano de Investigaciones Científicas, Centro de Estudios Botánicos y Agroforestales, Laboratorio de Protección Vegetal, Av. 8 entre Calles 79 y 80, Maracaibo, edo. Zulia, Venezuela, CP 4001; [2]Instituto Venezolano de Investigaciones Científicas, Centro de Ecología, Laboratorio de Ecología de Suelos, Km 11, Carretera Panamericana, Altos de Pipe, edo. Miranda, Venezuela, CP 1010A; [3]Plant Pest Diagnostics Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA; [4]Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia.

Several *Ditylenchus* species have been reported from Central and South America, but only one species of this genus, *D. dipsaci* parasitizing onion and garlic, has been found in Venezuela. In 2009, a neotropical nematode *Ditylenchus gallaeformans* attacking several species of invasive melastomatacean weeds was found in Brazil and Costa and later the description of this species was published. The nematode induces formation of gall-like structures on the foliage and inflorescences. The host range included nine Melastomataceae species (*Miconia ibaguensis*, *M. albicans*, *M. calvescens*, *M. coralline*, *M. latecrenata*, *M. mendoncae, Clidemia capitellata, C. hirta,* and *Leandra lacunosa*). *Ditylenchus gallaeformans* was found in a tropical rainforest in the central part of Cordillera de la Costa from Venezuela as a parasite of *Clidemia fendleri* (Melastomataceae) causing deformations and galls on inflorescences and abaxial surface of the leaves. Morphological, morphometrical and molecular characterization of this population is provided. The PCR-D2-D3-28S-RFLP diagnostic profile for *D. gallaeformans* generated by six restriction enzymes is presented. *Clidemia fendleri* being an endemic plant of Venezuela is a new host of *D. gallaeformans*. This finding is a first record of this nematode in Venezuela.

PINE WILT DISEASE, AND THE PINEWOOD NEMATODE: A WORLDWIDE ISSUE, A NEMATOLOGICAL CHALLENGE. **Mota[1,2], M.** [1]NemaLab/ ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal; [2]INIAV/Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, Av. da República, Quinta do Marquês, 2784-159 Oeiras, Portugal. mmota@uevora.pt.

Bursaphelenchus xylophilus, the pinewood nematode (PWN), and causal agent of pine wilt disease (PWD), was detected for the first time in 1999, in Portugal, Europe. In 2008, the PWN was detected in new forest areas in the centre of the country despite efforts developed by the national forestry and quarantine authorities to control the nematode and its insect vector (*Monochamus galloprovincialis*). The nematode has also recently been reported to be present in Spain and on Madeira Island. Circulation of non-treated wood and wood products may explain the spread of the nematode. Control strategies have been focused on the vector by using chemical traps, by cutting down symptomatic trees, heat-treatment of lumber, and monitoring of main roads and ports through which lumber and wood products are transported, by the Portuguese authorities. The nematode constitutes a threat to the rest of Europe, if proper measures are not taken by European governments. The same applies to other world regions which offer appropriate bio-ecological conditions such as susceptible pine species, a *Monochamus* sp. and ideal temperatures for disease development. The issue constitutes a major challenge to Nematology in regards to nematode (and insect) bio-ecology, pathogenicity, use of molecular biology in diagnostics and detection, histopathology, etc. Many gaps in the knowledge of this complex biological system persist. The involvement of bacteria, associated with the PWN in causing pine wilt, has been claimed. New quick detection methods and understanding of the nematode population dynamics are being developed. Nematode genomics may provide some insight to better understand the pathogenic effects caused inside the plant. Pathogenicity testing of susceptible pine species is imperative. A review of recent progress is hereby presented.

BIONEMATICIDE EFFECT OF SOME PLANT EXTRACTS ON *MELOIDOGYNE INCOGNITA* CONTROL ON TOMATO. **Mousa, E.M., M.E. Mahdy and R.A. Bakr.** Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Egypt.

Experiments were carried out to evaluate the nematicidal effect of some plant extracts against root-knot nematodes *Meloidogyne incognita* in tomato. Three different ornamental plants were used i.e. Oleander *(Nerium oleander),* Marjoram *(Origanum marjorana)* and Datura *(Datura stramonium)*. Nematode parameters were evaluated under laboratory and greenhouse conditions. Results obtained showed that plant extracts caused a great decrease of both egg hatching and larvae mortality under laboratory conditions. Tomato seedlings exposed to the treatment of plant extracts at the time of transplanting showed high reduction of both number of females and egg masses after two months of nematode inoculation. Number of galls was significantly reduced compared to the control. Concentrations of phenoloxidase and peroxidase enzymes in the infected plants were markedly increased by all treatments compared to untreated plants. Plant growth parameters were also enhanced compared to the control.

VOLATILE ORGANIC COMPOUNDS IN ROOT-KNOT NEMATODE (*MELOIDOGYNE* SPP.)–HOST INTERACTIONS. **Murungi[1], L.K., D. Coyne[2] and B. Torto[1].** [1]Behavioral and Chemical Ecology Department, International Centre of Insect Physiology and Ecology, PO Box 30772-00100 Nairobi, Kenya; [2]International Institute of Tropical Agriculture, P.O. Box 30772-00100, Nairobi, Kenya.

Crop losses due to root-knot nematodes (*Meloidogyne* spp.) in African vegetable smallholder farms range between 30 and 100%. Coupled with their high levels of incidence, they constitute a major constraint to vegetable crop production. Although olfaction is known to play a key role in the host seeking process in entomopathogenic nematodes, little is known about the mechanism of attraction between *Meloidogyne* spp. and their hosts. The ability of the infective stage of *Meloidogyne* spp. nematodes to detect host-specific odors likely enables them to locate and infect suitable hosts. We hypothesize that roots of perpetual spinach emit such volatile chemical attractants. This poster highlights our exploratory research to understand and identify olfactory cues mediating host plant-nematode interaction. Assays conducted to understand the *Meloidogyne* spp. nematode-perpetual spinach interaction and volatile organic compounds involved are also described. The implications of our current findings in relation to *Meloidogyne* spp. nematode management are discussed.

MANAGEMENT OF ROOT-KNOT NEMATODES-BACTERIAL WILT COMPLEX USING RESISTANT TOMATO VARIETIES IN COASTAL KENYA. **Muriuki[1], L.K., G.M. Kariuki[1], Z.M. Kinyua[2], R.K. Gathu and D.L. Coyne[3].** [1]Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya; [2]Kenya Agricultural Research Institute (KARI), P.O. Box 14733-00800 Nairobi, Kenya; [3]International Institute of Tropical Agriculture (IITA), c/o *icipe*, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya.

Tomato and peppers valuable vegetable crop in Kenya are severely affected by pests and diseases, especially root-knot nematodes (*Meloidogyne* spp.) and bacterial wilt (*Ralstonia solanacearum*), which are both associated with high yield losses. The objective of this study was to evaluate tomato and pepper germplasm with varying levels of resistance to root-knot nematodes and bacterial wilt, individually and as a complex. Tomato cv. Cal J, Kilele (Local checks), Hawaii, Okistu, N-UG (AVRDC) and pepper cv. PI201232, PBC384, PP0237-7502 (AVRDC), California wonders and Commandant (Local checks) were screened at Pwani University, Kenya. Three week old seedlings were transplanted and each inoculated with *Meloidogyne* spp. (500 J2) and *R. solanacearum* 10^7 cfu/ml either singly or as combined inocula, and assessed against a non-inoculated control, which contained sterile soil only. Level of resistance of each cultivar was rated based on plant growth and disease parameters. Significant differences were observed between treatments and the control. Reduction in growth parameters was highest when the two pathogens were inoculated together compared to when inoculated singly. California wonder was moderately susceptible compared to the other four pepper cultivars, which were moderately resistant to both pathogens. Tomato cv. Kilele and Okistu were moderately resistant and the other three cultivars were susceptible to both root knot nematodes and bacterial wilt. The study therefore shows that all pepper cultivars except California wonder pepper, and tomato cv. Kilele and Okistu can effectively be used in management of root–knot nematodes-bacterial wilt disease complex in coastal Kenya.

THE YAM NEMATODE, *SCUTELLONEMA BRADYS*, A NEW THREAT TO POTATO. **Mwamula[1], A.O. and N. Viaene[1, 2].** [1]Ghent University, Department of Biology, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Institute for Agricultural and Fisheries Research (ILVO), Plant, Crop Protection, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium.

The study investigated the possible threats of *Scutellonema bradys* on potato production by evaluating the host suitability and damage symptoms on five potato varieties grown in Europe and Africa. The probability of nematode entry, spread and establishment in Europe was also investigated through a pest risk analysis (PRA). Inoculation of potato with *S. bradys* in a pot test caused varying damage levels to the different potato varieties. The nematode infected both roots and tubers, resulting in the scaly surface appearance of tubers, brown root lesions and tuber rot. The number of nematodes recovered from roots, tubers and soil was greatest for the varieties Desiree, Spunta and Nicola (2-fold population increase), while reproduction was low on Diamant and Draga, where little or no increase was observed (reproduction factors 1.1 and 0.8, respectively). The PRA showed that the likelihood of entry of *S. bradys* from developing countries into the European Union (EU) is high due to

importation of contaminated yam tubers, although reaching a susceptible host is less probable. Establishment of the nematode is possible on a number of hosts including yam, potato and tomato, especially in the most southern parts of the EU. Nematode spread is likely through several pathways: tubers, peels and soil from infested areas or attached to equipmentor by travelers carrying plant parts and/or soil samples. The study demonstrated that *S. bradys* can damage potato and highlighted the need to reduce the risk of nematode entry and spread in the European Union region, as well as in Africa.

ECOLOGICAL ROLE OF BACTERIA ASSOCIATED WITH THE PINE WILT DISEASE SYSTEM. **Nascimento[1], F.X., C.S.L. Vicente[1], P. Barbosa[1], M. Espada[1], P. Vieira[1], K. Hasegawa[3] and M. Mota[1,2].** [1]ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Departamento de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal; [2]INIAV/Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal, Av. da República, Quinta do Marquês, 2784-159 Oeiras, Portugal; [3]Chubu University, Department of Environmental Biology, Chubu University 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan.

Mutualistic and beneficial relationships between nematodes and bacteria are present in nature, mostly occurring because of nutritional dependence and pathogen protection, and intrinsically related with the environment, the ecological conditions and the life stages of the nematode. Thirty-three years have passed since the first hypothesis suggesting a bacterial role in pine wilt disease that was initially thought to be caused only by the phytopathogenic nematode *Bursaphelenchus xylophilus*. In the late 1970s, researchers reported that bacteria associated with the pinewood nematode, *B. xylophilus*, could produce toxins that lead to pine wilt disease in pine seedlings. It was also suggested that a double vector system for pine wilt disease exists, where the bacteria were vectored by *B. xylophilus*, and *B. xylophilus* in turn vectored by an insect from the *Monochamus* spp. The specific involvement of bacteria in such complex disease is still controversial, although recently the number of studies focusing on the importance of bacteria has increased considerably. This review brings new insights on the role of bacteria in PWD.

SOURCES OF RESISTANCE TO ROOT-KNOT NEMATODES IN COWPEA GERMPLASM FROM SOUTH-EASTERN AFRICA. **Ndeve[1], A.D., W.C. Matthews[1], R.M. Chiulele[2], J.R. P. Santos[1], J.D. Ehlers[3] and P.A. Roberts[1].** [1]University of California, Department of Nematology, 3401 Watkins Drive, Riverside California 92521, USA; [2]Eduardo Mondlane University, Dep. of Crop Production, 3453 Julius Nyerere Ave, Building 1, Maputo 257, Mozambique; [3]University of California Riverside, Dep. of Botany and Plant Sciences, Riverside California 92521, USA.

Cowpea (*Vigna unguiculata* L. Walp) is an important source of food in Africa, and an relatively inexpensive source of proteins. However, nutritional and economic benefits of this crop are limited under infestation by root-knot nematodes, *Meloidogyne incognita* and *M. javanica*. *Rk*-genes, sources of resistance, have been identified and introgressed into some commercial cultivars. Broad-based resistance is the most efficient strategy to circumvent root-knot nematode damages on cowpea, and the known resistance is based on a complex set of genes, *Rk*, Rk^2 and *Rk3*. In a search for additional sources of broad-based resistance to RKNs, fifty-three Southeast African genotypes (land-races and accessions), comprising cowpea major gene pool 2, were screened for resistance to *Meloidogyne* spp. in the field, greenhouse and growth pouch tests. Data for root galling (GI), eggs per gram root (EgR) and egg masses per root system (EM), were analyzed using SAS statistical program version 9.0, following the Mixed Model Procedure. Results indicated significant ($P < 0.05$) differences in reaction to Root-knot nematodes. Eleven genotypes were consistently highly resistant to both species based on GI, EgW and EM parameters. The egg masses per root system parameter for *M. javanica* on some genotypes which was not significantly low. Genotypes FN-2-9-04, Var-3A, Var-11D, Namuesse-D, INIA-5A, Gile-k-local, Vita-7, FAEF-14-Inhaca-E, Muinana-Lawe and Massava-11 showed 50% or more reduction in galling and egg reproduction compared to the susceptible genotype, UCR-779. These resistance sources are being compared to known *Rk*-genes in allelism tests to determine their uniqueness and value in breeding.

BIOFUMIGATION WITH *BRASSICA JUNCEA, RAPHANUS SATIVUS* AND *ERUCA SATIVA* FOR THE MANAGEMENT OF FIELD POPULATIONS OF *GLOBODERA PALLIDA*. **Ngala, B.M., P.P.J. Haydock, S. Woods and M.A. Back.** Crop and Environment Sciences Department, Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom.

The potato cyst nematode, *Globodera pallida* is the most economically important nematode pest of potatoes in the UK, inflicting an annual cost of approximately £50 million to UK potato industry. Historically, farmers in the UK have relied on granular nematicides and synthetic fumigants to control *G. pallida*. Environmental concerns about the use of synthetic chemicals mean their future under reformed EU legislation is uncertain. Therefore, there is urgent need to search for sustainable replacements. Biofumigation, the suppression of soil borne pests and pathogens by biocidal compounds released when brassicaceous green tissues are disrupted, is increasingly being viewed as an effective and potential replacement to these fumigants. The present study examined *Brassica juncea*, *Raphanus sativus* and *Eruca sativa* on *G. pallida* in three field experiments conducted in Shropshire, UK. Samples of each *Brassica* species were analysed for their glucosinolates content just before incorporation. *Brassica juncea* significantly ($P = 0.03$) reduced *G. pallida* population while *R. sativus* prevented

further multiplication ($P_f/P_i \approx 1.0$) in experiment-1. In experiment-3, both *B. juncea* and *R. sativus* either treated with or without metconazole demonstrated significant partial and complete biofumigation on *G. pallida* encysted eggs. Glucosinolate concentrations varied significantly ($P < 0.001$) between the different plant parts (roots and shoots) as well as time of cultivation. A close correlation was observed between glucosinolate concentrations and mortality of *G. pallida* encysted eggs for summer cultivated brassicas. The results have demonstrated that *B. juncea* and *R. sativus* can play an important role in PCN management, particularly if they are included in an integrated pest management scheme.

DISTINCT PATTERNS OF PLANT GENE EXPRESSION REVEAL THE BASIS FOR AGRONOMIC FLEXIBILITY IN *M. HAPLA*. **Nielsen[1], D., V.M. Williamson[2], P. DiGennaro[3], and D. McK. Bird[1, 3].** [1]Bioinformatics Research Center, NCSU, Raleigh, North Carolina 27695, USA; [2]Department of Nematology, UC Davis, Davis, California 95616, USA; [3]Department of Plant Pathology, NCSU, Raleigh, North Carolina 27695, USA.

Field isolates of *Meloidogyne hapla* differ in their pathogenicity on particular crops, presumably as a consequence of genetic variation in the effector repertoire between nematode strains. To explore how genetic diversity in the pathogen influences host gene expression, we developed an extensive tool-kit for *M. hapla*, including whole genome sequence from 3 inbred, independent wild isolates, and a linkage map based on analysis of F2 progeny from a cross between strains LM and VW9. These *M. hapla* strains differ in key parasitic attributes, including resistance-breaking, gall size, number of feeding sites, and fecundity. We performed cross-species expression QTL (eQTL) analysis by mapping 2 billion ESTs, revealing nematode loci responsible for the changes in host gene expression. This eQTL approach quantifies the expression of all host and parasite genes, while simultaneously reporting exomic SNPs in *M. hapla* which we used for genotyping and mapping. Based on analysis of 98 F2 lines from the LM x VW9 cross, we have identified 127 plant genes whose expression is influenced by allelic variation at one or more parasite loci. We found that plant transcription factors were statistically over-represented, including many that are highly expressed in organs other than roots. This suggests that nematodes are usurping host signalling and that distinct strains activate different pathways. Remarkably, the *M. hapla* genome has tightly defined loci that influence expression of large numbers of plant genes. Reassuringly, although most of the nematode genes within a QTL are newly discovered, some have been previously implicated in host-parasite signalling.

THE EFFECT OF INCREASED TEMPERATURES ON POPULATIONS OF POTATO CYST NEMATODES. **Niere, B.** Julius Kühn-Institut, Institute for National and International Plant Health, Messeweg 11/12, 38104 Braunschweig, Germany.

Temperature has a great influence on the development of nematodes. At higher temperatures, nematode population in general are expected to develop faster or have more generations. This will lead to higher final population densities and consequently will have a negative impact on crop yield. During the next decades projections of climate change for north-western Europe predict a mean air temperature increase by +2°C by 2050 and up to +4°C by 2100. In this region, potatoes is an important crop and production in some areas is affected by the potato cyst nematodes. The effect of an increase in mean temperature on the development of different populations of *Globodera pallida* and *Globodera rostochiensis* was studied in climate chamber experiments using a standard and an increased temperature setting. The mean temperature in the climate chambers differed on average 3°C over the course of the experiments. In these experiments, potato genotype was added as an additional factor. The experiments followed a protocol for resistance testing of potato cultivars. Nematode multiplication rates on the varieties Désirée, Hansa, Amanda, Innovator, Aveka, Pallina, Eurobona and Seresta were determined and the relative susceptibility of these varieties towards Ro1, Pa2 and Pa3 populations was assessed under different temperature conditions. Although both potato cyst nematodes species are reported to have different temperature requirements, all populations developed well at higher temperatures. Results will be presented and discussed in the light of expected temperature changes in the future.

AN ASSESSMENT OF CURRENT EUROPEAN UNION LEGISLATION ON THE CONTROL OF POTATO CYST NEMATODES. **Niere, B. and E. Pfeilstetter.** Julius Kühn-Institut, Institute for National and International Plant Health, Messeweg 11/12, 38104 Braunschweig, Germany.

Potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, are harmful organisms of potato and quarantine regulations apply in most countries. In the European Union (EU), measures are specified in the ''Plant Health Directive'' (2000/29/EC) and the ''Control Directive'' (2007/33/EC). Both Directives focus on regulating pathways for the introduction and spread of potato cyst nematodes. The ''Control Directive'' specifies the measures that need to be taken in order to determine their distribution, to prevent their spread and to control them. New elements such as an annual survey to determine their distribution were introduced. Other measures, like the use of resistant varieties have been specified to address the problem of virulence variation in potato cyst nematodes. Because of the EU restrictions on chemical use, resistant varieties are considered one of the most important control measures. Overall, the new EU legislation has brought a higher level of harmonization in official measures. Additional pathways for the spread of nematodes have been addressed. Now, processors of potatoes need to have officially approved waste disposal procedures for, e.g., residual soils. Since the Control Directive only addresses ''European populations'' of potato cyst nematodes, the provisions of the Plant Health Directive needed to be

updated to align with the requirements of the Control Directive and to maintain the level of protection against the introduction of virulent populations from South America threatening potato production in the EU. The phytosanitary measures and recent changes will be presented and an assessment given on their relevance in controlling these pests.

CONTROL OF PLUM SAWFLIES (*HOPLOCAMPA MINUTA* AND *HOPLOCAMPA FLAVA*) BY ENTOMOPATHOGENIC NEMATODES. **Nježić[1], B. and R-U. Ehlers[2].** [1]University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, 78000 Banja Luka, Bosnia and Herzegovina; [2]e-nema GmbH, Klausdorfer Strasse 28-36, 24223 Schwentinental, Germany.

Plum sawflies (*Hoplocampa minuta* and *Hoplocampa flava*) are among the most important pests of European plum (*Prunus domestica*, L.) worldwide. In organic production, no control measures are available. In conventional production synthetic insecticides are available, but application is at petal fall when bees can still be present in an orchard. Since sawflies spend part of their life cycle in the soil the use of entomopathogenic nematodes (EPN) to control soil dwelling stages was considered. Efficacy of three species of entomopathogenic nematodes *Steinernema feltiae, Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were tested against plum sawflies larvae and adults. EPN efficacy against larvae was tested in laboratory and field conditions. Laboratory assays were performed against 1, 10, 20 and 45 days old larvae. Mortality of 1 day old larvae was 92-100 %, whereas no mortality was observed for older larvae. In a field trial an area under the plum treeś canopies was treated with nematodes, just before first larval fall to the soil was anticipated. The following year, before adult emergence from the soil, the treated areas were covered by insect proof nets. White sticky plates were placed under the net to catch emerging adult sawflies. In a separate trial adult plum sawflies were targeted. Trees were completely covered by insect proof nets. Nematodes were applied on the soil surface before adult insect emergence was anticipated. A reduction of 90-98 % in fruit infestations was recorded in treatments with nematodes compared to non-treated control plots. This is the first report of successful control of plum sawflies with EPN.

SUSCEPTIBILITY OF BLUEBERRY CULTIVARS TO REPLANT DISEASE ASSOCIATED WITH *CRICONEMOIDES ORNATUM*. **Noe, J.P., G.B. Jagdale, W.T. Holladay and P.M. Brannen.** University of Georgia, Athens, Georgia 30602, USA.

Blueberry (*Vaccinium* spp.) replant disease (BRD) is characterized by poor growth, yellowing, stunting, and severely reduced yields in replanted areas, and its incidence is correlated with high numbers of *Criconemoides ornatum*. We evaluated five cultivars of each of Rabbiteye (Brightwell, Ochlocknee, Powder Blue, Premiere, Vernon) and southern highbush (Emerald, Farthing, Rebel, Star, Legacy) types for their resistance/tolerance to BRD in two fields in Appling and Bacon Counties, Georgia. Six replicate plots of each cultivar (n = 4 plants) were arranged in a randomized-complete-block design and planted in 2011. Nematode populations in each plot were assessed in May and October 2013. Plant vigor, plant volume, and percent plant survival were recorded in October 2013. *Criconemoides ornatum* population densities increased between May and October for all cultivars, but increases were greatest for highbush cultivars. Population densities of *M. ornatum* were higher and plant growth parameters, including plant survival, were lower on highbush than on rabbiteye cultivars. A combined-site comparison showed that highbush had 5-6 times higher population densities of *C. ornatum* and 4-17 times lower plant growth parameters than rabbiteye cultivars, suggesting that blueberry replant disease is more severe on southern highbush. Differences were observed in plant growth and survival among the five southern highbush cultivars. Opportunities may exist within germplasm to improve tolerance to blueberry replant disease through cultivar development.

CURRENT NEMATICIDE OPTIONS AND THEIR INTEGRATION WITH IPM STRATEGIES. **Noling, J.W.** University of Florida, IFAS, Citrus Research & Education Center, Lake Alfred, Florida 33850, USA.

Chemical management options have undergone considerable change in the past decade due mainly to increasing regulatory scrutiny and labelled use restrictions. To overcome some of these challenges and to better target application, variable-rate application systems for all types of nematicides have been developed to prescriptively apply nematicides in a site-specific, variable-rate manner. New chemical products, both under development and those which have completed government registration, illustrates the new emphasis on safer, reduced toxicity biorational nematicidal products such as seed treatments which reduce human and environment impacts by avoiding direct application to soil. The recent loss of methyl bromide has contributed to the current coformulated use of different soil fumigants to expand the spectrum of soil pest control for many high value fruit and vegetable crops. Fumigants like 1, 3-Dichloropropene and MITC generating products continue to play an essential role for nematode control in a variety of lower value vegetable and agronomic crops. As soil fumigants become more and more regulated and their use restricted, a variety of different approaches to soil disinfestation are being considered. These include methods and timings of chemical application, emission reduction technologies, as well as integration of other pest management techniques, particularly for weeds which serve as alternative hosts to nematodes. Successful adoption of any nematode management options will depend on cost, return, ease of use, and effectiveness of the different strategies.

SORPTION OF FLUENSULFONE, A POTENTIAL NEMATICIDE FOR CONTROL OF THE POTATO CYST NEMATODE, *GLOBODERA PALLIDA*. **Norshie, P.M., I. Grove and M.A. Back.** Nematology and Entomology Group, Crop and Environment Research Centre, Harper Adams University, Newport, Shropshire, TF10 8NB, UK.

Two laboratory batch experiments determined sorption of fluensulfone (a new nematicide of the fluoroalkenyl group) by six arable soils from potato fields in England. The experiments studied the sorption of the technical grade and a 15% granular formulation and were part of a larger study to evaluate fluensulfone efficacy for control of the potato cyst nematode, *Globodera pallida*. In Experiment 1, sorption as a function of soil type was determined as per Freundlich sorption constant K_F obtained from isotherms over four initial concentrations (1.25, 2.5, 5.0 and 7.5 mg L^{-1}). Experiment 2 compared the kinetics and extent of sorption of the technical grade and the granular product in order to determine effects of the formulation on sorption. The results showed that both soil type and form of fluensulfone significantly influenced the extent of sorption. However, sorption was low across the soils. In experiment 1, the K_F and sorption normalised to soil organic carbon K_{FOC}, ranged from 0.72 – 1.72 mg Kg^{-1} soil and 63.9 – 114.6 mL Kg^{-1} soil respectively. In experiment 2, the formulation did not influence the kinetics of sorption but decreased K_D by four times, and this was ascribed to limited availability of fluensulfone in solution for uptake. Both experiments identified soil organic matter as the soil factor to have influenced fluensulfone sorption. It is suggested that sorption may not limit availability of fluensulfone in the soil solution for nematicidal activities and that the nematicide could be used for control of *G. pallida* in a wide range of soils.

INTERACTION BETWEEN THE NEMATICIDE NIMITZ AND HERBICIDES USED IN SUGARCANE PRODUCTION AND *PRATYLENCHUS ZEAE*. **Novaretti[1], W.R.T., E. Benetti[2], F.M.L. Silva[2], R.J.F. Duarte[2] and A.M. Reis[3].** [1]ANNA Laboratório – Piracicaba, São Paulo, Brasil; [2]Milenia Agrociências – Londrina, Paraná, Brasil; [3]Usina Diana – Avanhandava, São Paulo, Brasil.

The occurrence of negative interactions in sugarcane resulting from the joint application of carbamates nematicides and herbicides, which have substituted ureas in its chemical composition, has been demonstrated in several research studies, resulting in typical symptoms of phytotoxicity. The typical symptoms of phytotoxicity were lleaves that became yellowed and dry. The symproms may vary from moderate to intense.. The objective of this experiment was to evaluate the possible negative interaction among Nimitz nematicide and the different herbicides treatments used at sugarcane planting. Theexperimental design was a randomized block with split plots in a field naturally infested by *Pratylenchus zeae,* in a level considered medium for the crop. The treatments consisted of Tebutiurom 500 SC at 2.4 L / ha; Metribuzim 480 SC at 4.0 L / ha; Hexaron 600 WG (Diuron 468 + Hexazinone 132) at 2.0 kg / ha, Tebutiurom 500 SC at 2.4 L / ha plus Diuron 800 WG at 2.0 kg / ha and hand weeded. The sub-plots treatments were: control without nematicide, Nimitz 2.0 and 4.0 L / ha and Carbofuran 350 SC at 7.0 L / ha. All nematicide treatments significantly reduced the population numbers of *P. zeae*, with percentage reduction ranging from 36.67 to 98.64%, depending on the treatment and dosage applied. Regarding productivity, the values obtained at harvest did not show negative interactions among the Nimitz product and the herbicides. The control of nematodes leads to production increases of more than twenty tons of sugarcane per hectare.

AN AUDIT AND HOST STATUS ASSESSMENTS OF PLANT-PARASITIC NEMATODES AND WEEDS IN SUBSISTENCE AGRICULTURE, WITH REFERENCE TO *MELOIDOGYNE* SPECIES. **Ntidi[1, 2], K.N., L. Bronkhorst[1], H. Fourie[2] and A.H. McDonald[2].** [1]Agricultural Research Council-Grain Crops Institute (ARC-GCI), Private Bag X1251, Potchefstroom 2520, South Africa; [2]North-West University, School of Environmental Sciences and Development, Private Bag X6001, Potchefstroom 2520, South Africa.

Plant-parasitic nematodes not only infect and damage agricultural crops. A variety of weed species serves as reservoirs for nematode pests and also competes with crop plants for the available soil water and nutrients. A nematode survey was conducted on 67 localities in the nine provinces of South Africa to establish which nematode genera and species are predominant on the economically important weed species in small-scale agricultural areas. Fourteen weed species were, furthermore, screened for their host suitability to *Meloidogyne javanica* and *Meloidogyne incognita* race 2, respectively, in two independent greenhouse trials. Each seedling was inoculated with ± 5 000 root-knot nematode eggs and second-stage larvae (J2) 14 days after planting and nematode evaluations were done 56 days later. Twenty-nine plant-parasitic nematode species belonging to 15 genera, as well as two fungus-feeding nematode genera and one species were identified from soil and root samples of weeds collected from 67 localities situated in subsistence production regions of South Africa. Host status assessments indicated that three of the fourteen weed species evaluated, maintained high numbers of both *M. javanica* and *M. incognita* eggs and J2/root system and had RF-values >1, indicating susceptibility.

FIELD APPLICATION OF ENTOMOPATHOGENIC NEMATODES FOR THE CONTROL OF CODLING MOTH (*CYDIA POMONELLA*) IN APPLE AND PEAR ORCHARDS. **Odendaal, D., M.F. Addison and A.P. Malan.** Department of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa.

Codling moth,*Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a key pest of apples and pears throughout South Africa. Concerns over the environmental impact, widespread dispersal of resistant populations of codling moth, and the sustainability of synthetic pesticides, have encouraged the development and use of alternative pest management technologies within an integrated pest management (IPM) strategy. Entomopathogenic nematodes (EPN) are lethal pathogens of insects and have the potential to play an important role in an IPM programme. The impact of inundative semi-field application of commercially available EPN and the effect of environmental conditions on the mortality of diapausing codling moth larvae were investigated in an apple orchard. Codling moth larvae, reared under diapausing conditions, were used to culture infective juveniles *in vivo* for use in the different trials. Wire-mesh cages filled with apple tree bark and 20 last-instar codling moth larvae were used as the containment method. For each treatment, eight trees, in a randomised design, were used. Cages dipped in different nematode species and concentrations were kept moist, while temperature and moisture levels were recorded for 24 h after application. Mortality by infection with EPN was confirmed by dissection, four days after application. *Steinernema yirgalemense,* at five different concentrations, were investigated as well as *Steinernema feltiae* and *Heterorhabditis bacteriophora*. *Steinernema yirgalemense* caused the highest level of mortality of codling moth larvae, however, no significant difference was found between the *S. yirgalemense* concentrations investigated.

THE NEW NEMATICIDE NIMITZ (FLUENSULFONE): ITS ADVANTAGES AND LIMITATIONS. **Oka, Y.** Nematology Unit, Gilat Research Center, Agricultural Research Organization, Mobile Post Negev 85280, Israel.

Fluensulfone, a new nematicide of the fluoroalkenyl group, has proven very effective in controlling root-knot nematodes, *Meloidogyne* spp. Advantages of fluensulfone are; low toxicity to mammals and non-target organisms, irreversible nematicidal activity against second-stage juveniles of *Meloidogyne* spp. in contrast to currently used nematicides, even motile juveniles that had been treated with fluensulfone and rinsed in water became immobile with time and thirdly, high systemic activity in plants. A single foliar spray of peppers with a fluensulfone solution at 3.0 g L^{-1} prior to inoculation reduced the galling index by 80% and the number of nematode eggs by 73 to 82% of controls. The reduction in these parameters by fluensulfone was much higher than that obtained with other nematicides at the same concentration. No evidence of enhanced biodegradation or cross-biodegradation of fluensulfone by other compounds in the laboratory. Repeated soil application of fluensulfone did not reduce the nematicidal activity of fenamiphos or cadusafos, and repeated applications of these nematicides did not lower the nematicidal activity of a subsequent application of fluensulfone. Lastly, no evidence of occurrence of fluensulfone-resistant *Meloidogyne javanica* in the laboratory. Limitations of fluensulfone are; phytotoxicity to some crops and no or very weak systemic activity to nematodes inside plants. Foliar spray or soil drenching to pepper after inoculation with *Meloidogyne incognita* did not inhibit nematode development inside roots. Further no or very weak activity to some migratory nematodes. *Aphelenchoides besseyi*, *Aphelenchoides fragariae*, *Bursaphelenchus xylophilus* and *Ditylenchus dipsaci* were highly tolerant at concentrations effective to *Meloidogyne* species.

NEMATODE FAUNA OF A PERMANENTLY SUBMERGED PADDY FIELD. **Okada[1], H. and S. Niwa[2].** [1]National Institute for Agro-Environmental Sciences, 3-1-3, Kan'nondai, Tsukuba city, Ibaraki 305-8604, Japan; [2]Tomakomai Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Takaoka, Tomakomai 053-0035, Japan.

Few studies have reported the nematode fauna in paddy fields, although these fields are recognized as biodiversity hotspots in Asia. We have reported seasonal dynamics of nematode communities in common types of paddy fields in Japan, which are temporarily submerged in a rice growing period (TSP). Here, we report the nematode fauna of a paddy field which is permanently submerged throughout a year (PSP). This is an unusual situation for rice cultivation, but we consider it a model habitat to investigate the nematode fauna of freshwater wetlands in Japan. In our previous comparison with an adjacent upland rice field, we found a unique nematode community in TSP. We also found that the major taxa of upland field, *Filenchus, Acrobeloides* and Rhabditidae occurred even in TSP, and that they are likely to be reproduced in a drained period. In our current study, we hypothesized that these taxa would not occur in PSP, because PSP is submerged throughout a year. We also hypothesized that PSP would have some characteristic taxa which prefer permanently submerged conditions. To examine these hypotheses, we took soil samples in May, Aug. and Nov. in 2007 and 2008 in PSP and adjacent terrestrial habitats (ATH) as a reference site. As we hypothesized, *Filenchus, Acrobeloides* and Rhabditidae hardly occurred in PSP, although they did occur in ATH. We found, however, other taxa occurred commonly in both PSP and ATH. We also suggest *Paraplectonema* and *Paraphanolaimus* are characteristic in PSP to prefer permanent submergence.

HOST CHEMICAL COMPONENTS ATTRACT THE PHORETIC NEMATODE *CAENORHABDITIS JAPONICA*. **Okumura[1], E., R. Ozawa[2], T. Yoshiga[3] and Y. Takeuchi[1].** [1]Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake, Sakyo, Kyoto 606-8502, Japan; [2]Center for Ecological Research, Kyoto University, Hirano 2-509-3, Otsu, Shiga 520-2113, Japan; [3]Department of Applied Biological Sciences, Saga University, Honjo 1, Saga 840-8502, Japan.

The bacteriophagous nematode *Caenorhabditis japonica* forms a phoretic and necromenic association with the subsocial burrower bug, *Parastrachia japonensis*. *Caenorhabditis japonica* dauer larvae (DL) are specifically found on the body

surface of females. In the previous study, we found that DL are attracted to hexane extracts of the body surface components of *P. japonensis* in a species-specific manner. In this study we conducted GC-MS analysis of the hexane extracts from *P. japonensis* females and bioassays to identify the attractants in the extracts. Hexane extracts were prepared by washing *P. japonensis* body surface with hexane. We detected four *P. japonensis*-specific chemicals to be used in a chemoattraction assay. Chemoattraction assay was done on a 6-cm NGM plate with 1-cm diam. test and control spots near the edge of the plate. After 9 μl of 10, 1, 0.1, and 0.01 μl/ml diluted chemical in hexane and hexane alone was applied onto test and control spots, respectively, DL were inoculated onto the center of the plate and the number of nematodes on each spot was counted every 10 min for 1 hour. Among the chemicals tested, DL were attracted to octadecane and (*E*)-2-hexenal, and the most effective concentration was 1 μl/ml. Sauer larvae attracted to the chemical remained on the spot as when they did on the host hexane extracts. This data suggest that *C. japonica* DL may recognize both octadecane and (*E*)-2-hexenal derived from body surface of *P. japonensis* as host-specific chemicals.

THE DIVERSITY OF ROOT KNOT NEMATODES AND IMPLICATIONS IN CROP PRODUCTION. **Onkendi[1], E., A. Mongae[2] and L. Moleleki[1].** [1]University of Pretoria, Department of Microbiology and Plant Pathology; [2]McCain Foods, Pretoria, South Africa.

Root knot nematodes (RKN) or *Meloidogyne* spp pose a significant threat to crop production globally. Yield losses, rejection of produce and transmission of RKN infected planting material are some of the factors that ultimately lead to major losses to the growers. With the on-going withdrawal of chemical nematicides from the market, it is likely that an increase in yield losses due to RKN can be anticipated. It is therefore imperative that the threat of RKN affecting various crop plants is accurately evaluated. In this study, the diversity of RKN infecting potato plants in different potato growing regions in South Africa was evaluated. To this end, the intergenic region (IGS), 28S D2-D3 expansion segments within the ribosomal DNA (rDNA) together with the region between the cytochrome oxidase subunit II (COII) and the 16SrRNA gene of the mtDNA were targeted for PCR amplification, sequencing and phylogenetic analysis. The three tropical species; *M. javanica*, *M. incognita* and *M. arenaria* were identified as the dominant species, occurring in almost every region sampled. *Meloidogyne hapla* and *M. enterolobii* occurred in Mpumalanga and KwaZulu–Natal respectively while *M. chitwoodi* was isolated from two growers located within the Free State. Implications of the growing numbers of RKN in the rhizosphere as potential synergy between RKN and secondary pathogens in crop production are discussed.

A NEW SPECIES OF *LAIMAPHELENCHUS* (TYLENCHIDA: APHELENCHOIDIDAE) FROM SERBIA. **Oro, V.** Nematology Laboratory, Institute of Plant Protection and Environment, T. Drajzera 9, 11000 Belgrade, Serbia.

Species of the genus *Laimaphelenchus* are very small nematodes, usually less than 1 mm in length. The use of a scanning electron microscope (SEM) can provide more detailed information about morphology of this group of nematodes as scanning electron microscopy may reveal structures and morphological patterns that cannot be seen with an optical microscope, which may be helpful in the taxonomy of *Laimaphelenchus* spp. Some morphological characters are so small that their dimensions are measured in nanometers. An undescribed species of *Laimaphelenchus* was found in Belgrade, Serbia. Nematodes from glycerol were transferred to ethanol, dried at critical point, gold coated and scanning electron micrographs taken with a Jeol JSM-6460 LV SEM. In addition to the characters that define the genus *Laimaphelenchus* the presence of cephalic papillae and inner labial papillae (sensillae) were observed. The new species has a unique tail bearing four pedunculate tubercles with 10-12 finger-like protrusions. The role of the protrusions is explained. In addition to morphological characterization, the molecular characterization of the species was based on 18S, 28S and COI molecular markers.

BROAD HOST RANGE OF THE COCKROACH-GUT PARASITIC NEMATODE *LEIDYNEMA APPENDICULATA*. **Ozawa[1], S., C.S.L. Vicente[1, 2], P.G. Koehler[3] and K. Hasegawa[1].** [1]Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University, Kasugai, Aichi 487-8501, Japan; [2]ICAAM, Departamento de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal; [3]Department of Entomology and Nematology, University of Florida, Building 970, Natural Area Dr., Gainesville, Florida 32611, USA.

Normally, the relationship between host and parasite is highly specific and its balance has been established over their long evolutionary history. The thelastomatid parasitic nematodes have been reported from many species of cockroaches. Four strains of the smoky brown cockroach, *Periplaneta fuliginosa,* has been found to be infected with only one nematode species, *Leidynema appendiculata,* in Japan. However, nothing is known about parasitic nematodes in *P. fuliginosa* of other countries, or the host specificity of *L. appendiculata*. We investigated the parasitic nematode in *P. fuliginosa* UF that has been cultured in the University of Florida for more than 60 years, and found it still infected with only *L. appendiculata*. Interestingly, the Surinam cockroach, *Pycnoscelus surinamensis,* distributed in Japan as food for pet reptiles, and the Turkestan cockroach, *Blatta lateralis,* were also infected with *L. appendiculata*. Subsequently, *L. appendiculata* eggs were collected and used to artificially infect various cockroach species. We found the Japanese native cockroach *Periplaneta japonica*, *Blattella nipponica* and American cockroach *Periplaneta americana* were artificially infected with this nematode. Infections of *L. appendiculata* have been reported in several *Blattaria* species including lab cultured *P. americana* in the USA, but we

haven't seen the infection of this nematode in *P. americana* in Japan so far. Further studies are needed but now we hypothesize that the original host of this nematode is *P. fuliginosa*. We also propose that *P. fuliginosa* are responsible for the distribution and subsequent infection of a variety of hosts by *L. appendiculata*.

MITOCHONDRIAL HAPLOTYPES FOR IDENTIFICATION OF TROPICAL ROOT-KNOT NEMATODE SPECIES AND LINEAGES. **Pagan[1], C., D. Coyne[2], G. Kariuki[3], R. M.D.G. Carneiro[4] and V.M. Williamson[1].** [1]Dept. of Plant Pathology, Univ. of California, Davis, USA; [2]International Institute of Tropical Agriculture (IITA), c/o icipe, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya; [3]Dept. of Agricultural Science and Technology, Kenyatta University, Nairobi, Kenya; [4]EMBRAPA - Recursos Genéticos e Biotecnologia, C. P. 02372, 70849-979 Brasilia, DF, Brazil.

The asexually-reproducing root-knot nematodes (RKN; *Meloidogyne* spp) are widespread and damaging pests in tropical and sub-tropical regions throughout the world where they are often not identified to species level. The mitochondrial genome due to its uniparental inheritance is a useful tool for comparing and identifying these closely related hybrid species. Previously developed primer pairs that together amplify two mitochondrial DNA sequences that span a non-coding spacer and part of the adjacent large subunit rDNA were used to amplify DNA from well-characterized voucher samples including 15 different RKN species. Based on amplification-product sizes and restriction enzyme digestion patterns, *Meloidogyne javanica*, *M. enterolobii*, *M. incognita* could be distinguished and assigned unique mitochondrial haplotypes. Additional key species, including *M. hapla* and other sexually reproducing species were also resolved by this procedure. *Meloidogyne arenaria* isolates did not group as a single haplotype, consistent with other reports of diversity within this species. DNA extracted from a juvenile, single female or egg mass can be used for this assay. Ethanol-preserved females or egg masses can also be used allowing samples to be stored and shipped for analysis. This protocol is a rational strategy for initial characterization of RKN species especially in regions where sampling has been limited such as Sub-Saharan Africa. In addition, the DNA sequence of the amplified fragments, especially of the mitochondrial rDNA, is informative regarding the relationship between species, including those not previously identified, based on maternal lineage.

SYNTAXIN 31 FUNCTION IN *GLYCINE MAX* RESISTANCE TO THE PLANT-PARASITIC NEMATODE *HETERODERA GLYCINES*. **Pant[1], S.R., P.D. Matsye[1], B.T. McNeece[1], K. Sharma[1], A. Krishnavajhala[2], G.W. Lawrence[2], V.P. Klink[1].** [1]Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762, USA; [2]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, USA.

A *Glycine max* syntaxin 31 homolog (Gm-SYP38) is expressed in *Heterodera glycines*-induced syncytia undergoing an incompatible interaction. The observed Gm-SYP38 expression is consistent with prior gene expression analyses that identified the alpha soluble NSF attachment protein (Gm-alpha-SNAP) resistance gene because homologs of these genes physically interact and function together in other genetic systems. Syntaxin 31 protein resides on the *cis* face of the Golgi apparatus. It binds alpha-SNAP-like proteins, but has no known role in resistance. Gm-alpha-SNAP overexpression induces Gm-SYP38 transcription, making it reasonable to test the function of Gm-SYP38. Overexpression of Gm-SYP38 rescues *G. max*$_{[\text{Williams 82/PI 518671}]}$, genetically *rhg1*$^{-/-}$, by suppressing *H. glycines* parasitism. In contrast, Gm-SYP38 RNAi in the *rhg1*$^{+/+}$ genotype *G. max*$_{[\text{Peking/PI 548402}]}$ increases susceptibility. Overexpression of both Gm-alpha-SNAP and Gm-SYP38 induces the transcriptional activity of the cytoplasmic receptor-like kinase *BOTRYTIS INDUCED KINASE 1* (Gm-BIK1-6) which is a family of defense proteins. BIK1-like genes are known to anchor to membranes through a 5' MGXXXS/T(R) *N*-myristoylation sequence. Gm-BIK1-6 had been identified previously by RNA-seq experiments as expressed in syncytia undergoing an incompatible reaction and its overexpression rescues the resistant phenotype. In contrast, Gm-BIK1-6 RNAi increases parasitism. The analysis demonstrates a role for syntaxin 31-like genes in resistance that until now was not known.

IDENTIFICATION OF RESISTANCE TO CEREAL CYST NEMATODE *HETERODERA FILIPJEVI* IN WINTER WHEAT GENOTYPES THROUGH ASSOCIATION MAPPING. **Pariyar[1], S.R., A.A. Dababat[2], S. Siddique[1], G. Erginbas[2], A. Morgounov[2], J. Leon[3] and F.M.W. Grundler[1].** [1]Institute of Crop Science and Resource Conservation, Molecular Phytomedicine, University Bonn, D-53115, Bonn, Germany; [2]International Maize and Wheat Improvement Centre (CIMMYT), Ankara, Turkey; [3]Institute of Crop Science and Resource Conservation, Plant Breeding, University Bonn, D-53115, Bonn, Germany.

The aim of this study was to identify quantitative trait loci)/genes conferring resistance against the cereal cyst nematode *Heterodera filipjevi* in wheat. Cyst number per plant was determined in 161 winter wheat genotypes in two consecutive years in growth room trials. Susceptibility was very low in 1%, low in 17%, medium in 37%, high in 34% and very high in 11% of the genotypes. Four genotypes (NUDdakota, ECOnomka, KATea and LANtian 12) with very low and low susceptibility, respectively, were selected and nematode infection, development, and reproduction were analyzed in detail. The infection rate compared to highly susceptible cv. Bezostaya 1 was significantly lower and nematode development inside roots was significantly delayed in NUD, ECO, KAT and LAN at 2, 5, 10 and 15 dpi. The number of cysts per root system was also significantly lower in NUD, ECO, and LAN. However, cyst size was significantly smaller only in LAN and ECO. All

populations were genotyped with an Illumina 90K SNP iSelect wheat chip at TraitGenetics, Germany, and association between molecular markers and quantitative trait loci was analyzed. Twenty eight loci associated with *H. filipjevi* development were identified in which 11 of them were associated with low nematode susceptibility, while 17 were associated with very high susceptibility. In addition, six markers were located on chromosome 1A where quantitative trait loci regions associated with *Heterodera avenae* resistance had been detected in previous studies. Future work will focus on identifying the genes conferring resistance to *H. filipjevi* and revealing their mode of action.

CHROMADOREAN NEMATODE PHYLOGENY REVISITED BASED ON COMPARATIVE ANALYSIS OF COMPLETE MITOCHONDRIAL GENOME SEQUENCES. **Park[1], J.-K., J. Kim[1], S.-H. Lee[1], T. Kim[1] and S.A. Nadler[2].** [1]Program in Cell Biology and Genetics and Department of Parasitology, College of Medicine, Chungbuk National University, Cheongju 361-763, South Korea; [2]Department of Entomology and Nematology, University of California, Davis, California 95616, USA.

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 based on SSU sequences have been challenged by phylogenetic hypotheses based on complete mitochondrial genomes. We revisited some phylogenetic issues for chromadorean nematodes based on comparative analyses of complete mitochondrial genomes sampled from major representatives of chromarodean infraorders. The resulting mitochondrial genome tree for nucleotide and amino acid sequence data including 12 protein-coding genes depicted the following relationships, only some of which were inconsistent with SSU phylogeny: (1) non-monophyly of nematode clade III; (2) non-monophyly of Panagrolaimonorpha, (3) the nested position of Diplogasteromorpha within the Rhabditomorpha clade, (4) non-monophyly of Tylenchomorpha (independent origins of plant parasitism within chromadorean nematodes), (5) sister relationship between Rhigonematomorpha and Ascaridomorpha, (6) monophyly of Aphelenchoidea. Phylogenetic relationships among the major chromadorean orders revealed from analysis of mtDNA sequence are generally consistent with results of mitochondrial gene order comparison, with the exception of *Strongyloides stercoralis Heterorhabditis bacteriophora,* and *Ascaridia* spp. Some relationships remain contradictory between mtDNA and nuclear rDNA trees. These conflicting relationships require reappraisal using both combined analyses of different data sets, and application of new data from additional nuclear genes. It is anticipated that additional taxon sampling for mitochondrial genome sequences will provide a wealth of information on mitochondrial genome evolution and data for developing phylogenetic hypotheses for chromadorean nematodes.

RAPID DETECTION OF THE CEREAL CYST NEMATODE (*HETERODERA AVENAE)* USING LOOP-MEDIATED ISOTHERMAL AMPLICATION. **Peng[1], D., X. Xu[1,2], H. Peng[1] , W. He[1] and D. Jiang.** [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China; [2]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China.

Cereal cyst nematode (*Heterodera avenae*) is the most important plant-parasitic nematode on cereal crops in the wheat producing areas of China. In this study, a rapid and highly sensitive assay and diagnostic tool for the identification of *H.a avenae* was developed using loop-mediated isothermal amplification (LAMP). The LAMP assay targeted RAPD fragments of *H. avenae*. Three LAMP primers were designed and specificity of the LAMP assay was confirmed using different nematode species including *Heterodera filipjevi, Heterodera goettingiana, Heterodera elachista*, *Heterodera glycine, Heterodera latipons, Meloidogyne javanica* and *Meloidogyne arenaria.* The detection threshhold of the LAMP assay was as low as 10^{-2} and 10^{-4} of single juvenile and cyst DNA, and the detection sensitivity of the LAMP method for *H. avenae* DNA is 100 times higher than normal PCR-based detection methods. The LAMP amplifications could be observed visually after the addition of SYBR Green I and the lateral flow dipstick.. LAMP assay could be utilised for the detection of nematodes in wheat roots infected by *H. avenae*. The LAMP assay developed in this study is highly effective, easy to perform and readily adaptable for the early identification and monitoring of *H. aenae* on diseased seedlings in the field.

INTEGRATED MANAGEMENT OF CEREAL CYST NEMATODE *HETERODERA AVENAE* - A CASE STUDY EFFECT OF SEED-COATINGS TO CONTROL CEREAL CYST NEMATODE ON WHEAT IN CHINA. **Peng[1], D., R. Hao[1,2], W. Huang [1], C. Liu[1,2], H. Li[2] and H. Li [3].** [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, People's Republic of China; [2]Key laboratory of Monitoring and Management of Disease and Insects, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, People's Republic of China; [3]Grassland Science Institute of Gansu Agricultural University, Lanzhou 730070, People's Republic of China.

Cereal cyst nematodes (CCN, *Heterodera avenae* and *H. filipjevi*) are the most economically important plant-parasitic nematodes on cereal crops in China, occurring in 16 provinces. Estimated yield losses were 18%-35% in Henan, 11-18% in Hebei, and 15%-28% in Qinghai province. One generation of *H. avenae* is completed per growing season. *Heterodera avenae* is the dominant species in the wheat production areas. Control of CCN on wheat by means of six seed-coatings

(Gannong seed coating I, Gannong seed coating II Gannong seed coating III, abamectin seed coating AV1, abamectin seed coating AV2 and 5.76% emamectin benzoate), were evaluated in this study. Wheat seeds were coated with the different treatments before sowing and the numbers of cysts in soil and wheat yield were evaluated after harvesting. The results showed that the numbers of cysts dropped significantly after seed-coating. The highest reduction in cyst numbers were 56.0%, 53% and 47% in the treatment by Gannong seed coating III (1:35), Gannong seed coating I (1:50) and Gannong seed coating II (1:35), respectively. The wheat yield increased by 37.6%, 19.4%, 17.9% and 17.7%, respectively for Gannon seed coating III (1:35), Gannon seed coating I (1:50), Gannon seed coating II (1:35) and Gannon seed coating I (1:35). The self-patented Gannong seed coating III not only has the better efficacy for the control of CCN, but also has characteristics of environmental safety, lower toxicity, labor and cost saving, which is suitable for widely application in practical disease control.

TRANSCRIPTIONAL ANALYSIS OF CEREAL CYST NEMATODE *HETERODERA AVENAE* AT THREE DIFFERENCE STAGES THOUGH RNA SEQUENCING. **Peng, H., G. Wang, J. Cui, W. Huang, W. He, D. Peng.** State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.

The cereal cyst nematode, *Heterodera avenae* is a major nematode pest on cereal crops worldwide and causes significant economic yield losses in many countries; however the molecular characterization of its plant-parasitism has been limited. In this study, the transcriptomes were sequenced from three difference stages of *H. avenae* including pre-parasitical J2, J4 and white female by Illumila Hiq 2000. Over 47348000, 45186000 and 45260000 reads were generated from pre-parasitical J2, J4 and white female separately. After assembling the reads, 66,962, 43,953 and 62,697 contigs with an average length of 1546, 1029 and 1068 bp were selected for further analyses, respectively. Homology searches revealed that 48.8% - 62.2% of unigenes of *H. avenae* were similar to known genes. 5671, 6244 and 6961 unigenes were announced separately using Gene Ontology; 1824, 2029 and 2275 unigenes were mapped in KEGG biochemical pathways. The main purpose of this study was to identify the effectors of *H. avenae* using different approaches. 203 clusters with similarities to effectors from other plant parasitic nematodes were identified within the data. On the other way, 2323 clusters had signal peptides but no transmembrane domain were identified, these genes that might include novel effectors. Finally, the transcriptomes were used to identify possible target genes for RNA interference (RNAi)-based control strategies.

A WAX ESTER PROMOTES COLLECTIVE HOST FINDING IN THE DAUER LARVA OF *PRISTIONCHUS PACIFICUS*. **Penkov[1], S., A. Ogawa[2,4], U. Schmidt[3], D. Tate[2], V. Zagoriy[1], S. Boland[1], M. Gruner[3], D. Vorkel[1], J-M. Verbavatz[1], R. J. Sommer[2], H-J. Knölker[3] and T.V. Kurzchalia[1].** [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; [2]Department for Evolutionary Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany; [3]Department of Chemistry, Technische Universität Dresden, Dresden, Germany; [4]Laboratory for Developmental Dynamics, RIKEN Quantitative Biology Center, Kobe, Japan.

Survival of nematode species depends on how successfully they disperse in their habitat and in finding a new host. Under harsh environmental conditions many free-living nematodes stop development after the second larval (J2) stage and enter into a stage called the dauer larva. For dispersal, dauer larvae of multiple nematodes have evolved a specific behaviour called nictation or waving: worms stand on their tails and wave the body allowing for attachment to larger animal vectors. Nictation of individuals and attachment to insects under laboratory conditions is also described in the nematode *Pristionchus pacificus*. This behavior is crucial for *Pristionchus* since, under natural conditions, these nematodes rely on a tight association with scarab beetles, called necromenic. The dauer larvae invade the host, wait for its natural death, and then resume development by feeding on microorganisms that grow on the host carcass. Herein we report that *Pristionchus pacificus* nematodes synthesize a dauer stage-specific, extremely long-chain polyunsaturated wax ester that we name nematoil. Dauer larvae secrete nematoil to their body surface as an adhesive lipid that promotes the congregation of multiple individuals into large tower-like structures called dauer towers. The formation of the dauer towers is part of a novel host finding strategy, collective nictation, a process in which the whole dauer towers wave, presumably to maximize the chance of attaching to a new host organism.

TESTING THE MONOPHYLY OF THE SUPERFAMILY DOLICHODOROIDEA *SENSU* SIDDIQI, 2000 (TYLENCHIDA): EVIDENCE FROM THREE RIBOSOMAL GENES. **Pereira[1] , T., L. Caixeta[2], M. Mundo-Ocampo[3], J. Cares[2] and J. Baldwin[1].** [1]Department of Nematology, University of California, Riverside, California 92521, USA; [2]Department of Phytopathology, University of Brasilia, DF 70743-060, Brazil; [3]CIIDIR-IPN, Unidad Sinaloa, Mexico.

The superfamily Dolichodoroidea *sensu* Siddiqi, 2000 is comprised of four families, 30 genera, and about 450 species. Within Dolichodoroidea, the family Telotylenchidae is the most representative with four subfamilies, 20 genera, and having *Tylenchorhynchus* as the most specious and widely distributed genus. The extant classification proposed by Siddiqi (2000) is exclusively morphological-based and current molecular phylogenies are based upon a few representatives. Specimens were collected worldwide including Brazil, Argentina, Mexico, USA, Vietnam, and Iran. Morphological identification is based on

LM and SEM. The monophyly of the superfamily Dolichodoroidea is tested based on three ribosomal genes (18S, ITS, and 28S). Preliminary results showed the non-monophyly of the superfamily Dolichodoroidea regardless of the gene used. For instance, the genus *Tylenchorhynchus* showed a strongly supported clade with 16 different sequences. However, some *Tylenchorhynchus* sequences also grouped with other genera (*e.g. Quinisulcius*, *Macrotrophurus*, and *Dolichodorus*). The subfamily Merliniinae was monophyletic, however not related with Telotylenchinae. Instead, this group was placed as a sister taxon of Psilenchidae. Phylogenetic trees based on ITS and 28S genes showed some similarities: a large *Tylenchorhynchus* clade, a sister relationship between Merliniinae and Psilenchidae, the monophyly of *Dolichodorus* and *Belonolaimus*, and the polyphyly of *Tylenchorhynchus*. On the other hand, the 18S gene had no resolution to resolve most of the relationships within Dolichodoroidea. These results showed partial agreement with the classification provided by Siddiqi (2000). Nevertheless, the relationships at lower taxonomic levels need to be further evaluated. Such findings will be further improved by including additional taxa and genes.

INTRA- AND INTER-SPECIFIC GENETIC VARIATION IN THE GENUS *CEPHALENCHUS*: IMPLICATIONS FOR SPECIES DELIMITATION. **Pereira[1], T., K. Chang[1], M. Mundo-Ocampo[2] and J. Baldwin[1].** [1]Department of Nematology, University of California, Riverside, CA 92521, USA; [2]CIIDIR-IPN, Unidad Sinaloa, Sinaloa, Mexico.

Molecular phylogenetics of Tylenchina *sensu* De Ley and Blaxter, have been heavily biased to economically important groups of plant parasites. Conversely tylenchs such as fungal feeders (i.e. Tylenchidae), not directly associated with crop losses have been under represented in phylogenies. Broader taxon sampling of these groups is crucial to more fully understanding the evolution of plant parasitism in nematodes. Herein, molecular and morphological data from eight *Cephalenchus* populations from Brazil, USA, Mexico, Canada, and Vietnam were used to evaluate the phylogenetic position of the genus, traditionally (i.e. based on morphology) classified within the Tylenchidae Örley, 1880. Using a multilocus approach (ribosomal: 18S, ITS, 28S; mitochondrial: COI), levels of genetic variation were compared within and between these populations and discussed with respect to implications for species delimitation. Preliminary results demonstrated diverse levels of intraspecific variation among populations (0.1% to 10.5% for 28S and 2.1% to 15% for ITS). Generally, for both genes, interspecific genetic variation was higher than intraspecific variation; however, one population from Brazil (BRZ-01) showed high levels of intraspecific variation, comparable to those found between geographically divergent populations. Findings suggest that sequence comparison (% similarity) may be inadequate to define species affinities but rather hypothesis of monophyly must be tested to assess the validity of species boundaries as well as to recognise natural groups at higher taxonomic ranks.

SELECTION FOR RESISTANCE TO *MELOIDOGYNE* SPP. IN *COFFEA ARABICA* ACCESSIONS UNDER GREENHOUSE AND FIELD CONDITIONS. **Peres[1], A.C.J., A.S. Jorge Junior[2], J.K.A. Mattos[1], V.R. Correa[2,3], S.M.L. Salgado[4] and R.M.D.G. Carneiro[2].** [1]Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, 70.910-970 Brasília, DF, Brazil; [2]Embrapa Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-970 Brasília, DF, Brazil; [3]Universidade de Brasília, Dept. de Fitopatologia, 70910-900 Brasília, DF, Brazil; [4]EPAMIG, 36.570-000 Viçosa, MG, Brazil.

Root-knot nematodes are amongst the most economically important plant-parasitic nematodes infecting coffee in Brazil, particularly, *Meloidogyne paranaensis* and *Meloidogyne incognita*. The objective of this study was to identify coffee materials obtained from the Coffee Germplasm Bank (EPAMIG), resistant to *M. paranaensis* and *M. incognita* race 1, which have shown promising production under field conditions. Seedlings of 18 different accessions, a resistant (cv. IPR-100) and a susceptible (cv. Mundo Novo) cultivar, were inoculated under greenhouse conditions. Nematode reproduction factor was used to infer coffee responses (resistance/susceptibility) to nematode infection. Five accessions from crossing between Catuaí Vermelho x Amphillo MR 2161, one accession from Catuaí Vermelho x Amphillo MR 2474, two accessions from Timor Hybrid UFV 408-01 and the resistant control cv. IPR-100 were resistant to *M. incognita* race 1. Four accessions from crossing Catuaí Vermelho x Amphillo MR 2161, one accession from Timor Hybrid UFV 408-01 and the cv. IPR-100 were resistant to *M. paranaensis*. Field testing with parental genotypes showed that plants originated from Catuaí Vermelho x Amphillo MR 2161 or Amphillo x H. Natural MR 36-349 progenies were resistant and highly productive, while plants that originated from progenies Catuaí Vermelho x Amphillo MR 2474 , Timor Hybrid UFV 408-01 (treatment 8) and Catuaí Vermelho x Amphillo MR 2161 were resistant and with good yield. Overall, materials from treatments 3, 5, 8, 14, 19 and 20 were resistant to both *M. paranaensis* and *M. incognita* race 1 under greenhouse/field conditions and showed promising agronomic traits to be used in coffee breeding programs.

SURVEY AND REVISION OF NEMATODES INHABITING NORTH AMERICAN MILLIPEDS. **Phillips, G. and E.C. Bernard.** University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology Building, Knoxville, TN 37996-4560 USA.

Nematodes that parasitize millipeds are commensal kleptoparasites. Thelastomatidae and Rhigonematidae are two common families that inhabit the intestine of North American millipeds. In North America, 16 nematode species have been

recognized from millipede fauna. The primary goal of this research is a comprehensive taxonomic analysis of these nematodes and their specific host-parasite relationships with millipeds. In dissections undertaken so far, 269 millipeds from 6 orders (15 species) have yielded 0–417 nematodes. Thelastomatids are typically encountered in the distal hindgut while rhigonematids are mostly observed in the proximal hindgut at the pyloric region. Polydesmid millipeds usually contain the most nematodes. In these millipeds, the nematodes may be so concentrated in the pyloric area that the intestine appears completely occluded, yet milliped movement and health appear unaffected. Spirobolid millipeds, such as *Narceus americanus*, yield the largest rhigonematid nematodes, some reaching 10.2 mm in length. Rhigonematids typically are more numerous but thelastomatids are more diverse, with at least ten species found so far. Some millipeds, such as some parajulids and platydesmids, are devoid of nematodes while others are heavily infested. Nematodes found in the milliped order Spirostreptida (*Choctella cumminsi* and *Orthoporus ornatus*) clearly show that thelastomatids are more prevalent than rhigonematids. At least two nematode taxa have been collected that are not yet classifiable to family. Milliped width and nematode presence appear to be correlated; millipeds less than 2 mm wide in cross-section do not contain nematodes.

NEMATODE INFESTATION, AFLATOXIN CONTAMINATION AND *ASPERGILLUS* SPP. BIOMASS IN GROUNDNUTS FROM SMALL-HOLDER FARMERS IN KWAZULU-NATAL. **Phokane[1], S., D. Fourie[2], E. Ncube[1] and B.C. Flett[1,2].** [1]Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa; [2]Unit of Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa.

Groundnut (*Arachis hypogaea* L.) is an important food source due to the high essential fatty acid, protein and energy content of the kernels. Nematodes pests are often detected in groundnuts and often create infection sites for fungal pathogens. The aim of this study was to quantify nematode infestation, aflatoxin contamination and *Aspergillus* spp. biomass in groundnut kernels from small-holder farmers in northern KwaZulu-Natal, South Africa. Thirty kernel samples were collected after harvest from farmer's fields at five localities. Nematodes were extracted using the Bearman-tray method, while aflatoxin contamination was analysed using the LC-MS technique. Aflatoxin producing *Aspergillus* spp. were quantified using real-time PCR. Four plant-parasitic nematode genera were identified, namely: *Ditylenchus africanus*, *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. Hull and kernel samples from Manguzi and Mbazwana had the highest plant-parasitic nematode infestation levels, while no nematodes were present in either hulls or kernels obtained from Vryheid. *Ditylenchus africanus* and *Meloidogyne* spp. had the highest frequency of occurrence in both hull and kernel samples that were collected at Manguzi and Mbazwana. Population levels of the latter genera were 12 and 22 times higher in the hulls compared to those in the kernels, respectively. *Pratylenchus* spp. were present in hulls from all localities, while *Helicotylenchus* spp. were present in very low numbers in kernels only from Manguzi. Aflatoxin contamination was not detected in any of the groundnut samples; however, low levels of aflatoxin-producing *Aspergillus* spp. biomass, ranging from 0.0068 to 11.46ng/µl, were detected in samples from Manguzi and Mbazwana.

IMPLEMENTATION OF A HIGH THROUGHPUT REAL-TIME PCR METHOD FOR POTATO CYST NEMATODE DETECTION IN SCOTLAND. **Pickup, J., A. Reid, Y. Cole and C. Longley.** Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh EH12 9FJ, UK.

In the three years since 2010, when the European Directive 2007/33/EC on the control of PCN came into force, implementation of the new statutory measures, particularly the new harmonized soil sampling rate, we have seen a three fold increase in the number of samples processed at SASA. To manage this increase with the same staffing resources, SASA has had to overhaul the traditional diagnostic methods we used previously (i.e. cyst extraction by Fenwick can and diagnosis by visual examination). A new system has been developed for the collection and processing of soil samples, extraction of DNA from float material and detection of PCN by high throughput real-time PCR. The whole process is controlled and monitored by a bespoke software package (Seed Potato Unified Data System or SPUDS). Approximately 18,000 samples are analysed each year, with the incidence of PCN positive samples remaining similar to that determined by visual examination. As the number of samples taken per field has increased, the area of land recorded as infested with PCN has consequently increased by over two-fold.

NEMATODES AS A THREAT TO THE FRENCH FRY PROCESSING INDUSTRY. **Pieterse, B.J.** McCain Foods (SA), P.O. Box 1023, Delmas, 2210, South Africa.

McCain Foods (SA) is committed to supply safe and healthy food to customers and strives towards limiting the impact of their operations on the environment. Most of the potatoes needed for processing are supplied by local potato growers. McCain Foods (SA) also grows approximately 400 ha of potatoes. The french fry industry in South Africa is very competitive. Finished products have to be produced and processed at a cost lower than that of imported products. This implies limiting the cost of production on-farm and also keeping cost of processing as low as possible. Pesticides represent one of the major costs of production. Apart from the cost factor McCain Foods (SA) is also aware of environmental impacts and strive towards application of less active ingredients per ha. Efficiency in the factory is determined by quality factors such as dry

matter content and tuber defects such as nematode damage. Equally important is the yield obtained in terms of the finished product. Processing nematode invested tubers means that the factory process has to be adjusted to peel more tuber tissue than normal. With severe infection and deeper tissue damage it may be necessary to use mechanical peeling instead of steam peeling and so resulting in even less yield as well as increased cost of production. The processing industry, french fry production in this case, urgently needs improved nematode control solutions to be competitive in the world and in so doing contribute to the economy of the country by supplying safe and cost effective products to the consumer.

ENDOPHYTES, TISSUE CULTURE PLANTS, ORGANIC MATTER AND FALLOW: THE BEST STRATEGY TO MANAGE PLANT PARASITIC NEMATODES IN BANANAS. **Pocasangre, L.E.** Tropical Crops, EARTH, University, Costa Rica.

It is well established that after black Sigatoka, plant parasitic nematodes are the main constrain in banana production in the tropic and subtropics. The most important nematodes associated with banana are: *Radopholus similils*, *Helicotylenchus multicinctus* and *Meloidogyne incognita*. Currently the conventional way to manage plant parasitic nematodes in bananas is with two or three applications of nematicides, which can cost between 300-450 USD/ hectare/ year. However even with this treatment the majority of banana plantation still have plant parasitic nematodes population of over 10000 plant parasitic nematodes/100 g roots.. On the other hand, there is a increased pressure from consumers and supermarkets to eliminate or reduce the application of nematicides due to the contamination of groundwater, rivers, oceans and most important the risk of health problems for field workers. Wholefood supermarkets are buying sustainable banana fruit produced in EARTH University. In the new ratin policy or standard, nematicides like vydate and herbicides are forbidden. Field trials conducted in the commercial banana plantation of EARTH University had shown that the use of tissue vulture plants (TCP) protected with endophytes as well as adding 6 tonnes of compost base on rachis reduced the population of plant parasitic nematodes as well as improved the vigor of the plantation. We also demonstrated three years of nearly eradicate *R. similis* and populations of less than 1000 nematodes /100g of roots were recorded. Research to establish protocols for the use of tissue culture plants, endophytes, compost and fallow as control methods for plant parasitic nematodes in banana production is needed.

POTENTIAL USES OF TRADITIONAL MEDICINAL PLANTS AS ALTERNATIVE CROPS IN SOIL WITH HIGH LEVELS OF *MELOIDOGYNE* SPECIES. **Pofu[1], K.M. and P.W. Mashela[2].** [1]Agricultural Research Council, Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria, 0001, South Africa; [2]School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa.

Momordica balsamina (Cucurbitaceae) has various traditional medicinal uses in South Africa. Preliminary field observations when *M. balsamina* plants were inter-planted with tomato (*Solanum lycopersicum*) plants for repelling whiteflies, suggested that the former were not infected by *Meloidogyne* species, while the latter were heavily infected. Two separate experiments were therefore, conducted to determine the nematode-resistant potential of *M. balsamina* to *Meloidogyne incognita* and *Meloidogyne javanica*. Seven treatments, *viz*. 0, 250, 650, 1 050, 1 450, 1 850 and 2 250 eggs and second-stage juveniles of each nematode species were arranged in a randomized complete block design, with 12 replications. At 56 days after inoculation, the reproductive factor values at all levels of inoculation for each species were less than one, while nematode infection had no effect on yield components of *M. balsamina*. In plant-parasitic nematology, when RF values are less than unity and nematode infection has no effect on growth of the test plant, the nematode-plant relation is resistant. In conclusion, *M. balsamina* was resistant to *M. incognita* and *M. javanica* and could therefore be used as an alternative crop for managing population densities in smallholder farming systems.

MANAGING *MELOIDOGYNE* SPECIES IN WATERMELON PRODUCTION USING INDIGENOUS NEMATODE-RESISTANT ROOTSTOCKS IN SOUTH AFRICA. **Pofu[1], K.M. and P.W. Mashela[2].** [1]Agricultural Research Council, Vegetable and Ornamental Plant Institute, Private Bag X293, Pretoria, 0001, South Africa; [2]School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa.

An inter-generic grafting procedure which eliminated incompatibility challenges between *Citrullus* and *Cucumis* genera was developed. Two separate micro-plot experiments, one on *Citrullus* cv. 'Congo' onto *Cucumis* inter-grafts and the other on *Citrullus* cv. 'Charleston Gray' on *Cucumis* inter-grafts were initiated to determine the performance of the inter-grafts with respect to: (1) *Cucumis* nematode-resistant rootstocks retaining their nematode-resistance status to *Meloidogyne incognita* and (2) *Citrullus* cultivars as scions retaining their growth potential. The three treatments, *viz*. (i) un-grafted watermelon, (ii) watermelon-on-*Cucumis africanus* and (iii) watermelon-on-*Cucumis myriocarpus*, were each inoculated with 1 000 eggs and second-stage juveniles of *M. incognita*. Relative to untreated controls, grafting cv. 'Congo' on *C. africanus* and *C. myriocarpus* reduced RF values by 92-93%, while for cv. 'Charleston Gray' the reduction was by 94-96%. Similarly, root galls were reduced by 94-96% and 90-98% for cv. 'Congo' and cv. 'Charleston Gray', respectively. Generally, treatments had no effect on performance of watermelon inter-grafts. In conclusion, *Cucumis* seedling rootstocks retained their non-host status capabilities to *M. incognita*, while scions of the two *Citrullus* cultivars retained their growth potential when grafted on *Cucumis* species.

USE OF CLIMEX® BIOCLIMATIC MODELS TO IDENTIFY *MELOIDOGYNE ARTIELLIA* AND *DITYLENCHUS GIGAS* RISK PRONE AGRO-ECOLOGICAL REGIONS IN THE WORLD. **Poole[1], M.C. and S. B. Sharma[2].** [1]Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth WA 6151, Australia; [2]Murdoch University 90 South Street Murdoch WA 6150, Australia.

The British root knot nematode, *Meloidogyne artiellia* and stem nematode, *Ditylenchus gigas* are economically important nematode species. Host range for *M. artiellia* includes cereals, legumes and crucifers and is known to cause severe losses to chickpea. Compared with *Meloidogyne incognita* and *Meloidogyne javanica*, *M. artiellia* currently has limited distribution, confined mainly to the Mediterranean and European regions. Compared with *Ditylenchus dipsaci, D. gigas* has a limited host range and geographical distribution. In this study bio-climatic models were developed using CLIMEX® bioclimatic software to identify climatic regions suitable for *M. artiellia and D. gigas* to establish. The bioclimatic models for *M. artiellia* and *D. gigas* indicated that suitable areas existed in several countries in Africa, Asia, Europe, North America, Oceania and South America. The bio-climatic models suggest that both nematode species have the potential to adversely impact host crop production in many agro-ecological regions where they have not yet established. At least eight countries in Africa, ten countries in Asia and four countries in South America have climatic conditions suitable for *M. artiellia*. Similarly, at least two countries in North America, six in South America, seven in Africa, ten in Asia, eleven in Europe and two in Oceania have climatic conditions suitable for *D. gigas*. The introduction of *M. artiellia* and *D. gigas* to these new geographical regions is highly likely in absence of effective biosecurity measures for pathways that have the potential to introduce nematode species. The paper highlights the need for identifying nematode pest free regions and maintaining their freedoms by implementing appropriate nematode surveillance strategies for early detection of nematode incursions and preparedness to implement eradication responses.

TWO NEW *STEINERNEMA* SPECIES FROM THE ICORNUTUM GROUP FROM ZULIA STATE (VENEZUELA). **Portillo, E., P. Morales-Montero and E. San-Blas.** Instituto Venezolano de Investigaciones Científicas, Centro de Estudios Botánicos y Agroforestales, Laboratorio de Protección Vegetal, Av. 8 entre Calles 79 y 80, Maracaibo, edo. Zulia, Venezuela, CP 4001.

During a survey of entomopathogenic nematodes in Zulia state (northwestern Venezuela), two populations of the genus *Steinernema* were isolated from soil samples. *Steinernema* sp. (cachirí strain) was isolated from cultivated pastures whereas *Steinernema* sp. (LPV474 strain) was isolated from a transitional fallow land. The presence of two horn-like papillae in the lip region, indicate that both species belong to the 'bicornutum-group' of *Steinernema* spp. For taxonomic studies, 10 *Galleria mellonella* were exposed to infective juveniles, first and second generation males and females were collected. For morphological and morphometric studies, 20 specimens from different stages of the nematodes were heat killed at 60 °C for 2 minutes; fixed in TAF and mounted in glycerine. The two populations were compared with the other nematodes from the ''bicornutum group''. *Steinernema* sp. (cachirí strain) differs from *Steinernema riobrave, Steinernema yirgalemense* and *Stienernema pakistanensis* in spicule and gubernaculum length and in number and shape of genital papillae. The species also differs from *S.riobrave* in the presence of vela on spicule and from *S. pakistanensis* in the absence of mucrons. *Steinernema* sp. (LPV474 strain) can be distinguished from *Steinernema bicornutum* by the absence of mucrons, spicule length and shape, tail length and gubernaculum length. These two species represents the first reports of *Steinernema* from Venezuela.

DEVELOPING EFFECTIVE CONTROL STRATEGIES TO CONTROL THE CITRUS NEMATODE, *TYLENCHULUS SEMIPENITRANS*, IN SOUTHERN AFRICAN CITRUS ORCHARDS. **Pretorius, M.C. and C. Kotze.** Citrus Research International, PO Box 28, Nelspruit, 1200, South Africa.

Tylenchulus semipenetrans infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus orchards with yield losses of up to10% recorded. Multiple commercial applications of these compounds proved to be highly effective in reducing nematode populations in orchards. The use of several of these toxic compounds have, however, globally become under pressure. The search for alternative control methods to effectively control citrus nematodes is a priority in the Southern African citrus industry. Pre-plant fumigation of replant soils with methyl bromide during the 1980's was highly effective in reducing nematode numbers in citrus orchards and also increased yields and fruit size. Available commercial fumigants and post-plant treatments were done on a replant orchard at Crocodile Valley Citrus Co. with the treatments being 50% methyl iodide and 50% chloropicrin methyl bromide; metam-natrium; 1.3 dichloropropene, 1.3 dichloropropene/chloropicrin; Furfural; Nematode egg stimulant + nematicide and Cadusafos. Initial results indicated that the female nematode counts in the fumigated treatments were significantly lower, decreasing between 81 and 98% compared to the untreated control. Tree height and stem diameter of the fumigated treatments (276 cm) increased significantly compared to the unfumigated treatments (254 cm) and untreated control.Two non-toxic, post-plant products were also evaluated with the Bayer product (BCS AR 83685 SC 500), reducing the female population counts by a minimum of 70% after only one application. Certain dosages of the Makhteshim product (Nimitz (MCW-2) also effectively reduced the female population numbers by 72%. The need to seek cost effective alternatives for the South African citrus industry is imperative for future research.

HOST STATUS OF COMMERCIAL WHEAT AND SUNFLOWER CULTIVARS TO *MELOIDOGYNE* SPP. **Pretorius[1], M., S. Steenkamp[2], H. Fourie[1] and A.H. Mc Donald[1].** [1]North-West University, Unit of Environmental Sciences and Management, Private Bag X6001, Potchefstroom, 2520 South Africa; [2]Agricultural Research Council-Grain Crops Institute, Private Bag X1252, Potchefstroom, 2520 South Africa.

Meloidogyne spp. cause extensive damage to a number of crops because of their well-adapted feeding mechanism, their high reproductive potential and wide host ranges. Although producers still rely heavily on chemical control to manage these nematode pests, reliable but toxic nematicides has been taken off the market. The future of the remaining nematicides is also uncertain. Host plant resistance is an alternative management tool that can assist in keeping root-knot nematodes below damage threshold levels. Twenty commercially-available sunflower and 29 wheat cultivars were tested in greenhouse trials against *Meloidogyne incognita* and *Meloidogyne javanica*, respectively, to determine their host status to these two root-knot nematode species. A susceptible tomato cv. Floradade was included in both trials as a susceptible standard. Parameters used to select for resistance included reproduction factors, egg-laying female indices and number of eggs and J2 per root system. None of the sunflower cultivars tested showed resistance to *M. javanica*. All the wheat cultivars tested exhibited resistance to *M. incognita*. The latter screenings will be repeated during the next growing season to confirm resistance of these wheat cultivars to *M. incognita*.

EVALUATION OF A BIOLOGICAL SOIL CULTURE AS A POTENTIAL ORGANIC AGENT FOR MANAGING ROOT-KNOT NEMATODES. **Pretorius, M.S.A., W.J. van Aardt, A.H. Mc Donald, C. Jansen and H. Fourie.** Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520.

Extensive research efforts are underway to identify novel agents for the management of *Meloidogyne* spp. that parasitize and damage a range of crops. The effect of various filtered and non-filtered concentrations of a soil-derived organic and biological compound, Soil Bio-Muti (SBM), was investigated on the motility, respiration and reproduction of *Meloidogyne incognita* in separate experiments. All SBM concentrations both filtered and non-filtered, reduced J2 motility significantly ($p \leq 0.05$) from 3h to 24h after exposure compared to the tap water control. Significant interactions existed for ELF indices, egg and J2 numbers/root system in terms of the treatments, filtered and non-filtered entries 30 days after J2 that had been exposed to the SBM concentrations had been inoculated on roots of susceptible tomato seedlings. Furthermore, a progressive decrease in J2 respiration occurred as the SBM concentrations in which J2 where suspended, increased. Results of this study suggest that SBM has inhibiting effects on J2 motility and oxygen consumption as well as reproduction of *Meloidogyne incognita*. Follow-up studies will be conducted to identify the biological agents and/or their secondary metabolites that adversely affected *M. incognita* in studies done to date. Field testing is also envisioned since this may shed more light on the mechanism of the causing agents that could render an alternative and environmentally safe product for use in the management of root-knot nematodes.

IPM TO CONTROL ROOT KNOT NEMATODES ON TOMATO IN BEIJING OF CHINA. **Qiu, J., T. Liu and W. Liu.** Institute of Plant & Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing China 100097.

Control of *Meloidogyne incognita* using an IPM approach was examined in this study.The treatments were Microfume plus metham-sodium, granules made by raised broth of *Paecilomyces lilacinus*, the concentrate raised broth of *Aspergillus niger*-Y61 or sesame dregs, alone, or combined with lythidathion granules or abamectin granules. Treatments were applied by bunch irrigation before the transplanting of tomato seedlings. Also, resistant varieties of tomato such as Xian Ke series, X.K 5, X.K 6 and X.K 8, were used to be the contrast, since they have resistance to root knot nematode in protected cultivation. The number of root knots, juveniles and egg masses/100g soil were counted. The best treatment was fumigation followed by *P. lilacinus* granules or *A. niger*-Y61 broth or sesame dregs respectively; plus lythidathion granules or abamectin granules. The result showed the root knot numbers were reduced from 61% to 87%; the J_2 numbers were reduced from 85.33% to 87.11%; reduced rate of egg masses over 81.47%. Our assay indicated that the most effective control for root knot nematode (*M. incognita*) on tomato is to use resistant varieties of X.K5. The use of chemical pesticides can be reduced significantly from 10% to 50% by the combination of nematocide lythidathion granules plus *P. lilacinus* granules or sesame dregs or *A. niger*-Y61broth.

INTENSITY OF DROUGHT STRESS ON SUGARCANE - *MELOIDOGYNE INCOGNITA* PATHOSYSTEM. **Quintela[1], M.P., E.M.R. Pedrosa[1], M.M. Rolim[1] and A. Chaves[2].** [1]Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Recife, 52171-900, Pernambuco, Brazil; [2]Estação Experimental de cana-de-açúcar do Carpina, Universidade Federal Rural de Pernambuco, Carpina 55810-000, Pernambuco, Brazil.

This study aimed to evaluate early sugarcane development and activity of catalase and ascorbate peroxidase enzymes under different intensity and lasting of drought stress associated with *Meloidogyne incognita* parasitism. An experiment was carried out in a greenhouse using micro propagated seedlings of sugarcane RB92579 variety, for 90 days. Experimental design was completely randomized in factorial arrangement 7 (drought stress -56% of pot water capacity (PWC) for 30 days, 56% PWC for 60 days, 56% PWC for 90 days, 22% PWC for 30 days, 22% PWC for 60 days, 22% PWC for 90 days and the

control, 90% PWC) × 2 (*M. incognita* density – 0 and 20.000 eggs per pot) with four replications. Increases in time and intensity of drought stress decreased *M. incognita* reproduction and rate of sugarcane leaf area development, but increased ascorbate peroxidase activity. The longer the drought, the slower was post-stress plant recovery. Catalase activity was not affected by drought or nematode stress. The most stressful conditions provided the highest reductions on plant height, stalk diameter, shoots and root dry biomass, as well on biomass allocation to shoots, but it increased biomass allocation to the root system and the root/shoot ratio.

ROOT-KNOT NEMATODE EFFECTORS TARGET HOST CELL NUCLEUS TO ESTABLISH FEEDING CELLS. **Quentin, M., L. Zurletto, M. Jaouannet, M. Magliano, M.N. Rosso, E. Danchin, P. Abad and B. Favery.** INRA-Université de Nice-Sophia Antipolis-CNRS, UMR 1355-6243 Institut Sophia Agrobiotech: 400 route des Chappes F-06903 Sophia Antipolis France.

Root-knot nematodes are obligate endoparasites able to infect almost all cultivated plants worldwide. These nematodes maintain, for weeks, a biotrophic relationship with their hosts by inducing the differentiation of root cells into hypertrophied and multinucleate feeding cells. Nematode effectors produced in the esophageal glands and injected within host cells through the stylet certainly play a role during infection to manipulate plant cell morphogenesis and physiology, and promote nematode establishment. Blocking the activity of parasitism gene products involved in the success of infection would offer specific alternatives to control nematodes in the field. In this regard the nematode effectors are particularly attractive targets. In a search for new nematode effectors, we used transcriptome analysis and comparative genomics to identify *Meloidogyne incognita* genes encoding proteins potentially secreted in the host tissue during the early steps of infection. We identified candidate parasitism genes that were specifically expressed in early parasitic stages, encoded predicted secreted and nuclear localized proteins. We localized the expression of several candidate parasitism genes in the pharynx, the intestine or specifically in the esophageal glands of parasitic juveniles. Among these new effectors identified, we demonstrated the secretion of MiEFF1 by the nematode *in planta* and immunolocalised the secreted MiEFF1 in the nuclei of giant-cells. As functional analyses and characterization of their plant targets progress, the roles of these *M. incognita* nuclear effectors are currently elucidated. Here we present these effectors and the hypothesis for their roles with regards to the unique feeding behaviour of these pests.

SPATIAL DISTRIBUTIONS OF NEMATODE TAXA IN ARABLE AND (SEMI-)NATURAL FIELDS. **Quist[1], C., P. Mooyman[1], S. van den Elsen[1], D. Brus[2], C. Mulder[3], A. Termoshuizen[4], J. Bakker[1] and J. Helder[1].** [1]Laboratory of Nematology, Wageningen University, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands; [2]WUR-Alterra, Soil Geography, Droevendaalsesteeg 3, 6708 PB Wageningen, The Netherlands; [3]National Institute for Public Health and the Environment (RIVM), A. van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands; [4]BLGG Research, Binnenhaven 5, 6709 PD Wageningen, The Netherlands.

Because of their abundance, their trophic diversity and their relatively straightforward extractability, nematodes have a potential as a proxy for the biological condition of soils and sediments. So far the potential of this group is underexploited for technical reasons; the microscopic analysis of nematode assemblages is labour intensive and requires extensive expertise. With the availability of a relatively large molecular framework, it became possible to design molecular assays that allow for the quantitative analysis of individual taxa against complex DNA backgrounds. The availability of high throughput tools to monitor nematode assemblages makes it possible to investigate the spatial distribution of nematode taxa at field level. Experts are aware that some nematodes occur in patches whereas others are more or less evenly distributed over a field. In order to assess the degree of patchiness of individual nematode taxa, twelve fields (4 from each marine clay, river clay and sandy soils) of a single hectare each were investigated in great detail. Using a sampling grid optimized for geostatistic analysis, 96 composite samples were taken from each of these fields. Each of these samples were analysed with 24 molecular assays (corresponding to 23 nematode taxa and one internal control). Preliminary results, visualized as semi-variograms and surface maps, show a great variation in distribution patterns not only on the basis of nematode taxa but also based on soil type. The data that will be presented are essential for the design of scientifically sound sampling schemes for agricultural and natural soil at hectare scale and above.

POST-PLANT NEMATODE CONTROL IN QUEEN PINEAPPLE PLANTINGS IN HLUHLUWE, SOUTH AFRICA - THE STORY AFTER ALDICARB. **Rabie, E.C., and B.W. Mbatha.** ARC-ITSC, Hluhluwe Research Station, P.O. Box 194, Hluhluwe 3960, South Africa.

Nematodes can be considered the most damaging pest of Queen pineapples cultivated in northern KwaZulu-Natal. Although more than 30 nematode species were found associated with pineapples in South Africa only *Meloidogyne javanica* and *Pratylenchus brachyurus* are of economic importance, off which the latter is the most damaging, especially on sandy soils. Control practices prior to planting include fallowing, crop rotation, application of soil amendments and chemical control. Chemical control of nematodes is the primary control method applied in Queen pineapple cultivation in South Africa. Preplant control is essential when a nematode population is already present. The only option for post plant control is the

application of systemic nematicides due to the migratory endoparasitic character of *P. brachyurus*. The necessity of post plant control will be determined by the length of the crop cycle and the residual effect of the nematicide used for preplant control. Until recently nematode control was mainly done by preplant soil fumigation followed by the application of fenamiphos, oxamyl of aldicarb as post-plant treatment. The combination of fumigation and aldicarb gave the best control with the additional effect of mealybug and redmite control. Aldicarb was withdrawn from the market in 2011. The application of other granular nematicides into the plant funnel/heart was investigated for post-plant nematode control. If applied just before or after rain, this application method is found more effective as well as environmentally safe due to the minimal contact with the environment and lessened possible contact with the operators.

PERSPECTIVES ON GLOBAL REGULATION AND STEWARDSHIP OF SOIL FUMIGANTS. **Racke, K. and J. Busacca.** Dow AgroSciences, Crop Protection R&D, 9330 Zionsville Road, Indianapolis, Indiana 46268 USA.

Soil fumigants play a critical role in global agriculture for the effective management of nematodes and other soil-dwelling pests in many high value crops. As regulatory requirements and societal expectations for crop protection chemicals have evolved, manufacturers and growers have had to adapt and innovate to maintain access to an effective toolbox of products. With respect to soil fumigants, regional regulatory re-evaluation programs as well as the global phase-out of methyl bromide under terms of the Montreal Protocol have significantly impacted production practices and created additional opportunities for change. What has been the past experience and what is the future outlook for soil fumigants in such an evolving landscape? This paper will review significant regulatory developments related to soil fumigants, with particular emphasis on global availability of key products and impacts of major re-evaluation programs such as U.S. re-registration and the EU review. Key factors for effective navigation of regulatory change will be examined including new data generation, advanced human health and environmental assessments, practices to manage emissions, and the importance of assessing agronomic benefits and grower practices as part of the re-evaluation process. The overarching importance of effective product stewardship practices for maintenance of fumigant products will also be examined. Examples of regulatory requirements and stewardship practices will be drawn from experiences with the fumigant 1,3-dichloropropene (1,3-D), the active ingredient in Telone® soil fumigants.

DEVELOPMENTAL PLASTICITY CORRELATES WITH ACCELERATED MORPHOLOGICAL EVOLUTION IN DIPLOGASTRID NEMATODES. **Ragsdale, E.J., V. Susoy and R.J. Sommer.** Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, 35 Spemannstreet, 72076 Tübingen, Germany.

Developmental plasticity has been proposed as a vehicle for phenotypic diversification in animals and plants. However, few studies test the morphological potential of plastic traits in a phylogenetic framework. Here, we test the evolutionary consequences of developmental plasticity in the nematodes of Diplogastridae, which include species with moveable teeth, a predatory lifestyle, and in many cases stomatal dimorphism. We have studied the evolution of diplogastrid feeding-structures by analyzing changes of form and complexity, particularly in the context of a robust molecular phylogeny. In our analysis, stomatal dimorphism was supported to be an ancestral feature of Diplogastridae. The origin of the plasticity coincided with the appearance of predatory teeth and immediately preceded a radiation of complex feeding-forms. Comparative morphometric analyses show that developmental plasticity is associated with a shift to increased rates of phenotypic evolution. Furthermore, the fixation of a single phenotype has coincided with a further increase in disparity of stomatal shape. Thus, a macroevolutionary ''pulse'' of plasticity and the subsequent fixation of forms have allowed evolutionary novelties, increased morphological complexity, and rapid morphological exploration following a release from developmental constraints.

COST EFFECTIVE SOLID-STATE PRODUCTION OF ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE). **Ramakuwela[1], T., J. Hatting[1], M.D. Laing[2] and S. Hazir[3].** [1]ARC-Small Grain Institute, Bethlehem, 9700, South Africa; [2]School of Agricultural, Earth and Environmental Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa; [3]Department of Biology, Faculty of Arts and Sciences, Adnan Menderes University, 09010 Aydin, Turkey.

Production of entomopathogenic nematodes (EPN) for large scale commercial application has been restricted to developed countries because of high capital input and running costs associated with liquid fermentation. In developing countries, production will probably be achieved using *in viv*o culturing in insects, or by using *in vitro* solid culture on a small to medium scale. This study focused on optimising solid culture production of an indigenous *Steinernema* sp. for insect biocontrol purposes. Six low cost production media were evaluated at optimum temperature. Medium 6 produced the highest yield of IJs (781 678±221 IJs) / 5g medium, the highest level of live IJs (>84%) and the lowest number of adults (<10%) remaining in the medium at the time of harvest (28 days). Infective juvenile length did not affect virulence against last instar larvae of *Galleria mellonella*, which was >90% in all experiments. The optimum storage temperature was determined by incubating IJs in 0.1% formalin solution at 5 temperatures over 84 days. Survival was highest and most stable at 15°C, ranging from 84-88%. Storage in a sponge formulation at a concentration of 2.5 million IJs improved survival by 6% compared to aqueous storage at 15°C. Furthermore, EPN storage in a sponge at 25°C, after a period of low temperature (15°C) storage for 84 days, did not

have a detrimental impact on IJ survival and infectivity (87 and 95%, respectively). An estimated retail price (R90.61) of this *Steinernema* sp. was considerably lower than the market price for other *Steinernema* species, ranging from R271.50 to R458.55.

TESTING OF NEW NEMATICIDES FOR USE IN THE SOUTH AFRICAN SUGAR INDUSTRY. **Ramouthar, P.V., N. Ntshobeni , U. Pillay and S.D. Berry.** South African Sugarcane Research Institute, Private Bag X02, Mt Edgecombe 4300, South Africa.

Due to its efficiency, Temik (aldicarb) was the most widely used nematicide in the South African sugarcane industry. News of its withdrawal highlighted the overuse of this chemical within the industry and this prompted the industry not only to look for a replacement for Temik but also to support continuous research into alternative products. This study deals with the testing of a wide range of products for use in the South African sugar industry. The first stage of testing was conducted using pot trials. Fifteen products were tested and the range included chemical and biological products, liquids and granules and registered and unregistered products. Results from the pot trials were encouraging and based on these results, four products were chosen for field testing. These products contained the active ingredients abamectin, cadusafos, fenamiphos and a compound yet to be revealed by the manufacturer at varying rates. Treatment with the test nematicides showed a 25-92% reduction in plant parasitic nematode numbers in the roots of the plant crop trial at six months and a 46-91% reduction in the ratoon trials. A 12-89% reduction in thrips numbers, currently a major pest in the South African sugar industry, was also noted for one of the products. Registration trials for this product are thus currently in progress. Research for more alternatives is still continuing to reduce over reliance on a single chemical. Further exploration of the biologicals is also a key focus with the promotion of environmentally friendly sustainable farming within the industry.

STUDY OF NEMATODES IN THE RHIZOSPHERE OF CITRUS ORCHARDS IN SOUTHEAST OF IRAN. **Rashidifard[1], M., E. Shokoohi[1], A. Hoseinipour[1] and S. Jamali[2].** [1]Plant Protection Department, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; [2]Plant Protection Department, College of Agriculture, Guilan University, Rasht, Iran.

Citrus orchards are located in the south and north of Iran. The citrus industry is an important part of the national economy of Iran. Plant parasitic nematodes cause losses of approximately 10-30% of the world's citrus orchards .During a survey on the biodiversity of plant parasitic nematodes in citrus orchards of the Shahdad region in Kerman province (southeastern Iran), 50 samples were collected from the rhizosphere of citrus trees during 2011 and 2012. Nematodes were extracted using the modified Baermann funnel method, fixed, transferred to glycerin and mounted on permanent slides. The nematodes were identified using morphological and morphometrical diagnostic characters, using a light microscope fitted with a drawing tube. Thirteen nematode species, belonging to 10 genera including *Agamermis* spp., *Alaimus* sp., *Aphelenchus avenae, Hemicriconemoides chitwoodi, Diphtherophora* spp., *Nanidorus minor, Pratylenchus neglectus, P. scribneri, Psilenchus* cf. *aestuarius, P. hilarus, P.* cf. *klingleri* and *Tylenchorhynchus agri* were found from this survey. In addition, *Tylenchulus semipenetrans* was recovered in most of the soil samples studied.

ASSESSING HOST-PLANT GENE EXPRESSION DURING INFECTION OF SYNCYTIUM-FORMING NEMATODE *ROTYLENCHULUS RENIFORMIS*. **Redding[1], N., P. Agudelo[1] and C. Wells[2].** [1]School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson, South Carolina, USA 29634; [2]Department of Biological Sciences, Clemson University, Clemson, South Carolina, USA 29634.

Reniform nematode, *Rotelynchulus reniformis*, is a semi-endoparasite capable of infecting more than 300 host plant species in tropical, subtropical, and warm temperate regions. Its mode of parasitism is a complex process whereby female nematodes penetrate host roots and introduce effectors that lead to the formation of feeding sites. Physiological changes in host cells cause them to fuse together into a multinucleated mass called a syncytium from which the nematode feeds. Our long-term goal is to identify vulnerable points in the process of syncytia formation that can be disrupted to halt the nematode life cycle and protect soybean plants. The objective of the current study was to identify genes that are differentially expressed during the early stages of syncytium formation in soybean. A split root system allowed us to inoculate half the roots of each plant with *R. reniformis* while the rest of the root system remained uninfected. Illumina RNA-seq was performed on infected and uninfected root tissue harvested from three replicate plants at three, six, nine and twelve days post-inoculation. Resulting sequence data were assembled with the Cufflinks pipeline, annotated with Blast2GO, and used to compare infected and uninfected soybean transcriptomes with JMP-Genomics. From the resulting list of differentially-expressed genes, we have identified several candidates believed to be important in syncytia formation. These genes will be examined in additional functional analyses and *in situ* hybridization studies to confirm their role in syncytium development.

DISCOVERY OF A NEW BIORATIONAL NEMATICIDE. **Rehberger, L., B. Belkind, G. Venburg and R. Eldridge.** Valent BioSciences Corporation, 6131 Oakwood Road, Long Grove, Illinois 60047, USA.

A novel and highly effective biorational nematicide recently discovered by Valent BioSciences Corporation shows significant activity against plant parasitic nematodes in laboratory, greenhouse and small-plot field tests. VBC90017 is a

patented formulation of two biorational nematicidal active ingredients that synergistically controls nematodes and reduces nematode damage. VBC90017 offers potential timing and applications flexibility for the grower, showing good nematode and galling control from both pre-plant and post-plant applications. Results from a range of laboratory, greenhouse and research small-plot experiments are discussed.

VOTIVO®: INNOVATION DRIVEN, NEMATODE PROTECTION AND PLANT HEALTH BENEFITS. **Riggs[1], J., W. Andersch[2], U. Pluschkel[2] and K. Bugg[1].** [1]Bayer CropScience US, Research Triangle Park, NC, USA; [2]Bayer CropScience AG, Alfred-Nobel-Straße 50, D-40789 Monheim am Rhein.

Bacillus firmus I-1582 is a natural occurring, non-transgenic spore-forming bacterium developed by Bayer CropScience for suppression of nematode damages by seed-treatment (Poncho/VOTiVO) or by soil-application (Flocter®, Nortica®). VOTiVO formulations are sold as fluid suspensions or wettable powders demonstrated to have a shelf-life of more than 2 years. The spores of the bacteria also remain active on the seeds for an extended period of time, under storage conditions conducive for seed viability. Seed treatment with Poncho/VOTiVO led to a colonization of the root-surfaces immediately after seed germination by the bacteria. The bacteria grow and multiply by using the organic compounds, excreted by the plants. Root exudates are considered to be involved in the orientation of various nematode species towards their host plants. Spores of *Bacillus firmus* I-1582 are also able to colonize on nematode eggs and perforate the egg-shell, ceasing the development of the juveniles. Seed treatment with VOTiVO demonstrates enhanced vigor and growth of seedlings, resulting in increased root & shoot-masses in comparison to the control plants. *Bacillus firmus* is known for its ability to form the phytohormone indol-acetic acid (auxins) or to solubilize phosphorous from soil constituents leading to a more vigorous seedling growth. In corn, the performance of VOTiVO® has been evaluated in combination with the insecticidal seed treatment Poncho. Field trials demonstrated that Poncho/VOTiVO is capable to reduce plant damages from a wide range of nematodes. In the majority of field trials, Poncho/VOTiVO® treated seeds produced higher yields, 5-6 bu/acre (314-377 kg/ha).

ROOT-KNOT NEMATODE RESISTANCE GENE STACKING IN BREEDING LINES USING HIGH-THROUGHPUT GENOTYPING. **Roberts[1], P.A., B.L. Huynh[1], T.J. Close[2] and W.C. Matthews[1].** [1]Department of Nematology, University of California, Riverside, California 92521, USA; [2]Department of Botany & Plant Sciences, University of California, Riverside, California 92521, USA.

As an advancement over breeding for nematode resistance based solely on infection assays and field screens to phenotype and select breeding progenies, high-throughput genotyping platforms are becoming available for comprehensive genome-wide marker selection schemes. Genomewide marker coverage based on SNP marker polymorphism in biparental marker-assisted recurrent selection (MARS) and marker-assisted recurrent backcross (MABC) populations has been applied to breeding for resistance to multiple root-knot nematode (*Meloidogyne*) species in the grain legume cowpea (*Vigna unguiculata*). QTL analysis and genetic mapping were used to identify the locations of resistance trait determinants in the cowpea genome. Foreground selection for marker haplotypes in the QTL regions carrying the positive resistance alleles was combined with background selection for elite plant type using evenly spaced genomewide markers. Using this approach, breeding lines with broad-based resistance were developed which carry resistance genes effective against avirulent and virulent isolates of *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne arenaria*, and which combine resistance to both root-galling and nematode reproduction (egg production). Co-selection for resistance to Fusarium wilt, which can forms a disease complex with root-knot nematodes, also has been achieved using the SNP-marker technology. A configurable workflow developed as a Breeding Management System by the CGIAR-Generation Challenge Program for applying the molecular-based breeding tools of this system is being used in the advancement of MARS and MABC progenies to develop nematode resistant varieties.

CUBA: TWO DECADES WORKING ON INTEGRATED NEMATODE MANAGEMENT IN AGRICULTURAL CROPPING SYSTEMS. **Rodríguez[1], M.G., E. Fernandez[2], L. Hidalgo-Díaz[1], R. Cuadra[3], J.M. Draguiche[4], H. Gandarilla[5] and I. Castro-Lizazo[6].** [1]National Centre for Plant and Animal Health (CENSA).P.O.Box 10. San José de las Lajas. Mayabeque Province. Cuba; [2]Plant Health Research Institute (INISAV), Calle 110 #514. Playa Municipality Havana, Cuba; [3]Reasearch Institute for Tropical Agriculture (INIFAT), Havana, Cuba; [4]Villa Clara Province Plant Health Laboratory, Carretera de Maleza Santa Clara Villa Clara, Cuba; [5]National Quarantine Laboratory. National Centre for Plant Health, Agriculture Ministry, Cuba; [6]Agricultural University of Havana (UNAH), Mayabeque Province, Cuba.

The agriculture policy in Cuba has changed since the last decade of the twentieth century, and today co-existing urban/periurban agriculture and different production systems are present. Plant parasitic nematodes (mainly *Meloidogyne* spp., *Radopholus similis* and *Pratylenchus* ssp.), represents some of the most harmful pests in horticultural, coffee and fruits systems. The quarantine service is responsible for surveillance in international borders and for sampling the most important crops annually. Several tactics has been developed and introduced in these areas, such as crop rotation, trap crops, biodisinfection and biological control agents, accompanied by capacity building of stakeholders. Several strains of *Trichoderma*

spp., *Bacillus thuringiensis* and commercial biological products such as KlamiC® (*Pochonia chlamydosporia* var. *catenulata*) and Hebernem® (*Tsukamurella paurometabola*), have showed good results in different conditions and are included in some integrated nematode management systems in farms, organic gardens and governmental agricultural enterprises. Grafting tomatoes, different preventive and hygienic alternatives and biological control agents are using in crops under shelter conditions, and the use of chemical nematicides is only authorized in high infections. In soil biodisinfection, several organic materials have been used, such as native plants, crops debris, and by-products from sugar and rum industries, among others. Trap crops, use of healthy plants, extraction of nematode susceptible weeds, biological control, and management of non susceptible crops are the most common alternatives in organic gardens. In governmental agricultural enterprises of banana, tuber crops and vegetables they use mainly tillage, healthy seeds, resistant varieties, biological control and crop rotation. A summary of research and results will be presented.

DEVELOPING A BIOLOGICAL CONTROL AGENT FOR MOLLUSCS IN SOUTH AFRICA. **Ross, J.L. and A.P. Malan.** Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

European molluscs (slugs and snails) have become significant pests in South Africa, affecting agronomy, horticulture and viticulture. Current methods for controlling molluscs rely on chemical molluscicides, such as metaldehyde and carbamate compounds. Both these compounds are poisonous to a 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 to investigate a method of biological control. To date the most successful commercial method for the biological control of molluscs in Europe is the nematode *Phasmarhabditis hermaphrodita*. This nematode is mass produced by BASF (formally Becker Underwood UK Ltd) and sold under the trade name of Nemaslug®. The product works by releasing infectious nematode larvae that seek out molluscs and enter through natural openings. Once inside the mollusc, bacteria associated with the nematode are released which stop the mollusc from feeding. Death usually follows 4-21 days after nematode infection. The Agricultural Pest Act 36 of 1947 prohibits the introduction of exotic animals into South Africa. Therefore no method of biological control currently exists for molluscs in South Africa. In this study we present a systematic survey of mollusc-parasitic nematodes in South Africa, and the development of an indigenous isolate for mass production.

ANALYSIS OF VIRAL SEQUENCES WITHIN SOYBEAN CYST NEMATODES. **Ruark, C.L., T.L. Sit, S.R. Koenning and S.A. Lommel.** Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27607, USA.

Viruses of nematodes have been understudied yet may have an important influence on nematode ecology. Soybean cyst nematode, *Heterodera glycines*, is a leading cause of soybean pathogenesis and dramatically suppresses soybean yield in North Carolina. Recently, four RNA viruses were reported in three highly inbred lines of *H. glycines* originating from Illinois. The objective of our research was to determine if North Carolina soybean cyst nematodes were also a reservoir for these viruses. Total RNA was extracted from cysts and second-stage juveniles of four inbred North Carolina soybean cyst nematode lines (OP20, OP25, OP50 and a race 2 greenhouse isolate). Total RNA was also extracted from cysts of recently collected wild soybean cyst nematode populations from North Carolina fields. PCR products of up to 500 base pairs were generated (with viral-specific primers based on published Illinois sequences), cloned and sent for sequence analysis. All four of the inbred *H. glycines* lines were positive for a soybean cyst nematode-specific nyavirus (ScNV) while three of the four contained a soybean cyst nematode-specific phlebovirus (ScPV). Sequence variation among inbred North Carolina strains was less than the differences with published Illinois sequences. Some field isolates of *H. glycines* were positive for both ScNV and ScPV. However, in at least one field population, neither of these viruses could be amplified. More sequence variation existed in field populations of *H. glycines* as compared to inbred lines. Soybean cyst nematode rhabodovirus (ScRV) and soybean cyst nematode tenuivirus (ScTV) detected in Illinois H. glycines populations were not found in any of the assayed North Carolina populations. Better understanding of nematode viruses may lead to a novel avenue of soybean cyst nematode biocontrol.

NEMATODE FOOD WEB DIVERSITY AND STRUCTURE. **Ruess, L., R. Cutando and K. Wagner.** Humboldt-Universität zu Berlin, Institute of Biology, Ecology Group, Philippstraße 13, 10115 Berlin, Germany.

Nematode assemblages in soil reflect substrate texture, climate, biogeography, organic inputs and anthropogenic disturbance. Nematodes use a wide range of resources including bacteria, fungi, roots or microfauna, and these diverse biological interactions result in a key role in soil food webs. Shifts in forest management affect food webs distinctly as the proportions of readily decomposable to recalcitrant compounds are altered by tree species identity and diversity. Such resource diversity at the base of the soil food web is highly relevant for population dynamics and trophic complexity, and thus consumer diversity that influences ecosystem functioning. Besides tundra biomes temperate forests are the largest soil C stocks but only recently tree species diversity and identity effects on belowground compartments came into focus. The impact on the nematode microfood web was investigated in: i) a large scale study on increased land use intensity, i.e. from natural to managed forest stands, and ii) a tree diversity experiment with six species representing different litter quality (ash, lime, beech, oak, spruce, pine) and

arranged in five diversity levels (1, 2, 3, 5, 6 species mixtures). In this diversity manipulation study, nematode communities showed only weak interactions with tree diversity but distinct relation to the presence of specific species. In the observational study, nematode faunal analysis revealed disturbance by land use as well as regional effects (e.g. local climate, soil type) on food web diversity and structure. These changes, within and across trophic levels, likely shape decomposition processes and in turn energy flow in belowground food webs.

POPULATION DYNAMIC OF *MELOIDOGYNE FALLAX* IN POTATO CROPS AND MULTIPLICATION IN STORED TUBERS. **Rulliat, E., E. Treuillier, F. Ollivier, A. Buisson, C. Sarniguet, C. Prigent and L. Folcher.** ANSES-Laboratoire de la Santé des Végétaux-Unité de Nématologie, Domaine de la Motte au Vicomte 35653 Le Rheu- France.

In the European Union, *Meloidogyne fallax* Karssen, 1996 and *Meloidogyne chitwoodi* Golden, O'Bannon, Santo and Finley, 1980 are registered as quarantine nematodes according to Directive 2000/29/EC. Within the context of monitoring those quarantine pests, an experiment was conducted with the aim of studying (i) the level of contamination of potato tubers by *M. fallax* on ultra-early harvested crops and (ii) the capacity of *M. fallax* to develop within stored tubers. In that respect, a follow-up of the populations dynamic was undertaken on tubers from their initiation to the harvest time i.e. 98 days after planting. The nematological analyses combined both morphobiometrical and molecular techniques. As a result, the findings proved that even if the potatoes were harvested very early, this did not prevent the tubers from being infected by *M. fallax*. Second stage juvenile (J2) infected the developing tubers. These juveniles developed inside the tubers to adult females which can be detected a few weeks before harvest. Even if the level of *M fallax* infestation was limited at harvest time, the production of a new generation inside the stored tubers can be observed. Interestingly, this result confirms a well reported phenomenon in *Meloidogyne* biology but constitutes the first report of this fact for *M. fallax*.

EFFICIENCY OF CHEMICAL TREATMENT OF MELON SEEDS ON THE CONTROL OF *MELOIDOGYNE JAVANICA*. **Saliba[1], H.C.R., C.T. Borges[1], R. Navroski[1], I. Lima-Medina[2], J.T. Schafer[1], V. Meneghello[1] and C.B. Gomes.** [1]Faculdade de Agronomia, Universidade Federal de Pelotas, Campus Universitário s/n C. P. 354, Pelotas/RS. Brazil; [2]Embrapa Clima Temperado, C.P. Postal 403, Pelotas/RS, Brazil.

Root-knot nematodes are a problem pest of melon in Brazil. Considering the lack of resistant cultivars in the Brazilian market and the shortage of information about seed chemical treatment to control this pest, the aim of this study was to evaluate the efficiency of seed treatment for *Meloidogyne javanica* control in melon plants using a mixture of nematicide, fungicide and insecticide. Melon seeds cv. Gaucho Redondo treated with Thiabendazole 485 SC (4,8 ml) and Thiamethoxan at 4,8 ml, 0,66 ml and 1,63 ml/100 kg of seeds, respectively, were sown in polystyrene trays. Twenty days after seeding, each plant was transferred to a pot containing a mixture of sterilized soil and a substrate at a proportion of 3:2. Subsequently, each plant was inoculated with 5,000 eggs and second stage *Meloidogyne javanica* juveniles.Twenty-five days after the inoculation each plant was evaluated for the number of galls, second stage juveniles and eggs/root systems. Subsequently the nematode reproduction factor (RF) was determined to estimate the efficiency of seed chemical control of *M. javanica*. The melon seed treatment did not reduced the number of galls on the melon roots; however the use of chemical products reduced the *M. javanica* RF by 40% compared to the control.

SPATIAL DISTRIBUTION OF POTATO CYST NEMATODES IN THE NILGIRI HILLS OF SOUTH INDIA. **Saranya[1], C., P. Sundararaj[1] and S.L. Hafez[2].** [1]Department of Zoology, Bharathiar University, Coimbatore, India; [2]University of Idaho, Idaho, USA.

Potato cyst nematodes (*Globodera pallida and Globodera rostochiensis*) are major nematode pests, causing 80 percent yield loss on potato crop in Nilgiri hills of Tamilnadu, India. An intensive survey was conducted in 13 potato growing villages of Nilgiri Hills to find out the distribution and occurrence of potato cyst nematodes. Nematodes were extracted from a total of 117 soil samples collected from potato fields using standard methods. In total, , 3548 mature cysts were recovered, of which 1075 were viable. Potato cyst nematode incidence was highest at Nanjanad village (61.3%) followed by Ithalar village (56.5%).The lowest incidence was observed in Balacola village (24%) followed by Ebbanadu (14.3%) village. Altitude, temperature and potato crop monoculture practices are the common factors that increase the abundance of potato cyst nematodes at the Nanjanad and Ithalar villages.

DEVELOPMENT, GENES AND MORPHOLOGY IN THE PHYLUM NEMATODA. **Schierenberg, E., C. Kraus, M. Kroiher, P. Schiffer, J. Schulze.** Cologne Biocenter, University of Cologne, Germany.

Nematodes, which are thought to date back into the Precambrian, exhibit a body plan that has remained surprisingly uniform, probably due to the unique construction principle with a single-chamber hydroskeleton. In contrast, the genetic and genomic variance between different *Caenorhabditis* species was found to be unexpectedly high. It is development that ties together these two disparate phenomena. To trace the evolutionary changes that took place in the long history of this phylum we started a comprehensive comparative survey of embryogenesis in a variety of phylogenetic branches leading to hatching juveniles with their taxon-specific morphological peculiarities using 4-D microscope recordings. Applying bioinformatic

tools we have begun to analyse genomes and transcriptomes generated in different laboratories including our own and relate these data to the developmental features. Likewise we found major differences on the genome/gene expression level and with respect to cellular behaviour particularly between basal and more derived species but also between closely related representatives. These differences appear to be more fundamental during the first half of embryogenesis than the second half where a nematode is formed from a ball of cells. Our data indicate that the evolution of development within the phylum Nematoda went from indeterminate early cleavage without initial polarity to invariant cell lineages with early establishment of polarity. Such dramatic changes require modifications in the cell-specification processes and the underlying molecular mechanisms. Many genes that are shared between more basal nematodes and non-nematode outgroups are absent in the model system *Caenorhanditis elegans* and its close relatives. Thus, rather different ways of how to construct a nematode must have been established during evolution without corresponding reflection on the morphological level.

THE EFFICACY OF CROP GUARD (A.I. FURFURAL) AS AN IN-SEASON SOIL TREATMENT FOR THE CONTROL OF NEMATODES ON VINES AND STONE FRUIT IN SOUTH AFRICA. **Scholtz, R., E. Buntting and A. Steyn.** Illovo Sugar Ltd, South Africa, PO Box 31003, Merebank, 4059, Durban, South Africa.

With the phasing out of toxic nematicides and the use of more environmental friendly pesticides as in the agricultural sector internationally, the need for safer pesticides has been developed. Crop Guard® (a.i. furfural) is a natural product derived from various plant sources. Sugarcane bagasse is an important raw material used in the production of furfural. Illovo Sugar Ltd. is a leading sugar and furfural producer in Africa and has developed and commercialized Crop Guard® as an in-season soil treatment for the control of nematodes in soil under South African field conditions. Crop Guard® is currently registered on 18 different crops in South Africa and has also been granted a yellow band registration during 2013. Vine trials were executed at Nylstroom (Modimolle) in the Limpopo Province of South Africa. Treatments were made during the spring and autumn root flush periods of the vines. Results indicated that over three consecutive seasons, 50+25+25 l/ha rate resulted in much lower Criconema (Ring nematode) numbers than the control treatment. A peach trial was executed at Robertson in the Cape Province of South Africa. Applications were made during the spring and autumn root flush periods. Resulting data indicated that the 50+25+ 25 l/ha gave the best results in terms of yield and control on *Criconema* (Ring nematode) and *Helicotylenchus* sp. (Spiral nematode).

THE EFFICACY OF PROTECT (A.I. FURFURAL) AS A PRE-PLANT SOIL TREATMENT FOR THE CONTROL OF NEMATODES ON PINEAPPLES AND STRAWBERRIES UNDER FIELD CONDITIONS IN SOUTH AFRICA. **Scholtz[1], R., E. Buntting[1], A. Steyn[1], E. Rabie[2] and I. Meintjies[3].** [1]Illovo Sugar Ltd, South Africa, PO Box 31003, Merebank, 4059, Durban, South Africa; [2]Agricultural Research Council, Institute for Tropical and Subtropical Crops, Nelspruit, South Africa; [3]Agricultural Research Council, Vegetable and Ornamental Plant Institute, Roodeplaat, South Africa.

With the phasing out of Methyl Bromide as a general soil sterilant as well as the banning of certain nematicides in the agricultural sector of many first world countries, and shortly in third world countries too, the need for safer pesticides has developed. Protect® (furfural a.i.) is a natural product derived from various plant sources. Sugarcane bagasse is an important raw material used in the production of furfural. Illovo Sugar Ltd. is a leading sugar and furfural producer in Africa and has developed and commercialized Protect® (furfural a.i) as a soil treatment for the control of certain nematodes and fungi in soil under South African field conditions. A pineapple trial was executed at the Hluhluwe Research Station of the Agricultural Research Council- Instititute for Tropical ands Subtropical Crops, KwaZulu-Natal. Results indicated that a pre-plant rate of 400 l/ha Protect® seven days before plant, resulted in significantly lower population numbers for *Pratylenchus brachyurus* and *Meloidogyne* spp., than the untreated control. Harvesting at two years after plant showed higher yields than for the untreated control, or similar yields as the standard EDB/Telone treatments. A strawberry trial was executed in sandy soil at Cullinan, Gauteng, South Africa. Results indicated that a pre-plant rate of 400 l/ha Protect®, seven days before plant, resulted in significantly lower numbers for *Meloidogyne sp*. than the untreated control. Yield results showed significantly higher fruit mass per ha than for the untreated control or similar yields as the standard MBr/Telone treatments.

EFFECT OF DEFENCE ELICITORS CHITOSAN, BION® AND METHYL JASMONATE ON ROOT-KNOT NEMATODE *M. INCOGNITA*. **Schouteden[1], N., C. Vos[2], A. Elsen[3], B. Panis[4] and D. De Waele[1,5].** [1]Laboratory of Tropical Crop Improvement, Department of Biosystems, KU Leuven, Willem de Croylaan 42 – box 2455, 3001 Leuven, Belgium; [2]Centre of Microbial and Plant Genetics, Kasteelpark Arenberg 20 - box 2460, 3001 Leuven, Belgium; [3]Bodemkundige Dienst van België, Willem de Croylaan 48, 3001 Leuven, Belgium; [4]Bioversity International, Willem de Croylaan 42 – box 2455, 3001 Leuven, Belgium; [5]School of Environmental Sciences and Develpoment, Noth-West University, Private Bag X6001, 2520 Potchefstroom, South Africa.

Root-knot nematodes, *Meloidogyne* spp., cause substantial damage to most economically important crops. Over the recent years the use of many nematicides has been prohibited due to their harmful impact on the environment and human health. An alternative to control root-knot nematodes is the application of defence elicitors. Defence elicitors are components that induce

systemic resistance in the plant and can thus be applied in nematode management. We tested the elicitors chitosan, Bion® (active component acibenzolar-S-methyl) and methyl jasmonate in different concentrations for its controlling activity towards root-knot nematodes. The tomato plants were grown in a mixture of sand and potting soil and sprayed with elicitors in different concentrations. Two or three days after spraying, the plants were inoculated with freshly hatched second stage juveniles of *Meloidogyne incognita* Malaysia and 10 days after inoculation (DAI), 21 DAI and 56 DAI plants were harvested, plant parameters assessed and the gall index was determined. In addition, the roots were collected and stained with acid fuchsin to visualize the nematodes microscopically. In another experiment egg masses and amount of eggs were assessed after 35 DAI in two tomato cultivars. Results show a minor influence of the defense elicitors against *M.incognita*. Implications of our results and future perspectives will be discussed.

MOLECULAR STUDY OF AN ISOLATE OF *STEINERNEMA FELTIAE* FILIPJEV, 1934 FROM IRAN, BASED ON ITS RDNA SEQUENCE POLYMORPHISM. **Sediqi[1], E., E. Shokoohi[1] and J. Karimi[2].** [1]Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; [2]Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

A survey of entomopathogenic nematodes was conducted in southeastern Iran. During this study, an isolate belonging to the species *Steinernema feltiae* was recovered in the Lalezar region, Kerman province. The ITS rDNA was amplified and sequenced. Molecular information based on sequence of the ITS rDNA confirmed this isolate as *S. feltiae*. The Nblast result showed 8 nucleotides differences between our isolate and the same species belong to Israel and Iran respectively. The phylogenetic relationship of the species studied based on Maximum Likelihood placed this isolate of *S. feltiae* closed to other populations of *S. feltiae* from Israel (KC571264, KC571263, KC571261) and Iran (JF920962, JF920964). Pairwise genetic distance using Maximum Composite Likelihood showed no genetic variation between different populations of *S. feltiae* from Iran.

BIODIVERSITY OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.) FROM MAJOR TOMATO GROWING AREAS OF ETHIOPIA. **Seid[1,3], A., W.M.L. Wesemael[2,3], W. Decraemer[3,4], T.M. Mekete[5] and C. Fininsa[1].** [1]Haramaya University, College of Agriculture and Environmental Sciences, School of Plant Sciences -Crop Protection Unit, P.O. Box 138, Dire Dawa, Ethiopia; [2]Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit-Crop Protection, Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium; [3]Ghent University, Department of Biology, Nematology Unit, Ledeganckstraat 35, B-9000 Ghent, Belgium; [4]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium; [5]University of Florida, Entomology and Nematology Department, Bldg.970, Natural Area Drive, Gainesville, FL 32611, USA.

The objective of the study was to investigate the biodiversity of *Meloidogyne* spp. in major tomato growing areas of Ethiopia. Samples were collected from major tomato growing areas based on the elaborated agro-ecological zones of Ethiopia. Tomato farms and gardens in 40 different localities mainly from Rift Valley, Upper Awash and Eastern Hararghe were sampled during January to March 2013. A total of 212 randomly chosen fields were visited and 212 composite soil samples from the rhizosphere (0-25cm deep) of tomato and 123 root samples (approximately 200g each) were collected separately. Samples were collected from ten to fifteen plants per field (1/2 ha) in a zig-zag fashion. Sampling sites range from greenhouse tomato producers to small scale farms. Root Gall Index (RGI) and Egg Mass Index (EMI) were determined based on a 0-5 scale. DNA was extracted from second stage *Meloidogyne* juveniles (J2s) and PCR was performed using species-specific primers as well as primers that amplify the Intergenic Spacer Region (IGS) and 28S regions of the rDNA. PCR products were then sequenced bi-directionally. A 100% incidence of *Meloidogyne* spp. was detected from 47% of the samples.. An EMI and RGI of 5 were recorded from 40% of the samples. The use of species-specific primers indicated that 90% of the samples were infested by both *M. incognita* and *M. javanica*. Sequencing of the 28S region of rDNA and species-specific primers also confirmed a first report of *M. hapla* in Ethiopia at one sampling location 'Zeway' (1640 meter above sea-level).

HISTORY AND INDUSTRIAL DEVELOPMENT OF THE ''RAIN OF DEATH'' (*PASTEURIA* SPP.). **Sekora, N. and T. Hewlett.** Syngenta Crop Protection, Pasteuria Bioscience Laboratory, Alachua, FL, USA.

Pasteuria species are obligate parasites of many invertebrates, including plant-parasitic nematodes. The potential of *Pasteuria* spp. as biological control agents has been studied since the first plant-parasitic nematode-suppressive soil was observed in the 1970's. Quantities of *in vivo Pasteuria penetrans* spores sufficient for pot and microplot trials (2×10^9) could be produced from ground root tissue containing infected root-knot nematodes (*Meloidogyne* spp.). This method was used in 1996 to successfully control *Meloidogyne arenaria* populations in the soil of the Land Pavilion at EPCOT Center, Disney World. However, the production of enough *in vivo Pasteuria*-laden material to treat more than a few acres was determined to be too expensive to be practical. Many attempts to culture *Pasteuria* spp. *in vitro* were met with failure until 2004, when a small startup company, Entomos, was able to grow *Pasteuria penetrans* in small volumes of artificial medium. Shortly thereafter, the method was refined to produce large quantities of *in vitro P. penetrans* was developed using fermentation in

500 mL volumes by the same group, now at Pasteuria Bioscience. Syngenta Crop Protection, having acquired Pasteuria Bioscience in 2013, now has the ability to produce 50,000 L of *in vitro Pasteuria* spp. for use as a commercial nematicide. Production at this scale has yielded enough *P. nishizawae* to use as a soybean seed treatment against *Heterodera glycines* on more than three million acres in 2014. Further development will continue on additional *Pasteuria* species and will target species of *Meloidogyne*, *Pratylenchus*, and *Heterodera* in the near future.

INCREASE IN SEVERITY OF FUSARIUM WILTS IN CUCURBITACEOUS PLANTS CAUSED BY *MELOIDOGYNE INCOGNITA*. **Seo, YunHee and Young Ho Kim.** Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea.

Root-knot nematode (*Meloidogyne* spp.) and fusarium wilt fungi cause severe damage to cucurbitaceous plants including oriental melon in Korea. Although shintozoa (*Cucurbit maxima* x *Cucurbit moschata*) resistance to fusarium wilt has been widely used as a stock for grafting oriental melon cultivars, the disease is still prevalent in major oriental melon-growing areas. Thus, experiments were conducted to examine the reasons for the current prevalence of the fusarium wilt in oriental melons. Twenty-nine *Fusarium* isolates obtained from oriental melon fields were inoculated on shintozoa, cucumber and oriental melon, in which most *Fusarium* isolates were more virulent to oriental melon and cucumber than to shintozoa. In this test, wilt severity was increased more in plants by the combined inoculation of both *Meloidogyne* and *Fusarium* than by the inoculation of fusarium isolates alone. Light microscopy also showed extensive destruction of vascular tissues with poor giant cell formation by the infection of both pathogens, while no or little extensive destruction of vascular tissues occurred by the infection of either pathogens alone. However, the formation of tyloses, which is responsible for the wilt symptom expression, was frequently observed in the oriental melon and cucumber but not in shintozoa infected with either *Fusarium proliferatum* or both pathogens. All of these results suggest that root-knot nematodes may be mostly responsible in the current increases of fusarium wilt severity in oriental melon cultivation in Korea, although effects of changes in pathogen virulence on the disease severity were not totally ruled out.

MOLECULAR ADAPTATION MECHANISMS IN THE ANTARCTIC NEMATODE *PANAGROLAIMUS DAVIDI*. **Seybold[1], A., C. Marshall[1], D. Wharton[2] and P. Dearden[1].** [1]Department of Biochemistry, University of Otago, Dunedin, New Zealand; [2]Department of Zoology, University of Otago, Dunedin, New Zealand.

The Antarctic nematode *Panagrolaimus davidi* tolerates desiccation and freezing and is the only animal known to survive intracellular ice formation. Although physiological mechanisms of this extreme adaptation are partly revealed, mechanisms on the molecular level remain largely unknown. Recent studies identified genes up-regulated during desiccation such as late embryogenesis abundant proteins, heat shock proteins, antioxidants and trehalose genes. Furthermore, the presence of ice-active proteins has been suggested by the observation of recrystallization inhibition and hexagonal ice crystal shape. To identify the genes involved in this extreme adaptation we are currently testing high throughput RNAi techniques, such as feeding and soaking. To investigate whether *P. davidi* is accessible to RNAi via feeding, the embryonic lethal genes Pd-rps-2 and Pd-dhc as well as the blister gene Pd-duox-42 were tested and the expression level was confirmed by quantitative PCR. While Pd-rps-2 treated samples showed a significant increased lethality compared to the control samples, there was no significant difference at the expression level. To investigate weather *P. davidi* is more accessible to RNAi via soaking, neurostimulants and desiccation supported soaking techniques were tested. A significant downregulation of Pd-rps-2 was obtained if samples were exposed to desiccation prior to soaking. We are currently testing RNAi on candidate genes such as trehalose and late embyogenesis abundant genes in order to identify the function of these genes during exposure to desiccation and freezing.

STATUS OF ENTOMOPATHOGENIC NEMATODES AS BIO-CONTROL AGENTS IN PAKISTAN. **Shahina, F. and K.A. Tabassum.** National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan.

The study of entomopathogenic nematodes (EPNs) in Pakistan have been initiated in the 1990's. More than 7000 soil samples were collected from different climatic regions of Pakistan. Up till now extensive work has been carried out on the isolation, distribution taxonomy, biology and efficacy of EPNs and as a result three new species viz., *Steinernema pakistanense*, *Steinernema asiaticum* and *Steinernema maqbooli* were described while seven known species *Steinernema abbasi, Steinernema siamkayai, Steinernema feltiae, Steinernema carpocapsae, Steinernema litorale*, *Heterorhabditis indica* and *Heterorhabditis bacteriophora* were first records for Pakistan. The genetic diversity and phylogenetic analysis of all isolates of *Steinernema* and *Heterorhabdiis* were investigated on the basis of ITS regions of rDNA, 12S rRNA mitochondrial gene, D2D3 regions and 28S rDNA. One-hundred-and-forty sequences were submitted to Genbank and a genomic library was constructed. Pathogenicity and efficacy trials of indigenous EPN isolates have also been done successfully with positive and promising results of more than 100 insect pests of different crops. Because of the output of these studies the Intellectual Property Organisation of Pakistan granted twelve national patents. There is a dire need to focus further research on these EPN isolates to explore and exploit their potential as an alternative to pesticides in Pakistan, especially in IPM programmes.

BREEDING A SUPER NEMATODE FOR ENHANCED INSECT PEST SUPPRESSION. **Shapiro-Ilan[1], D.I. and B.J. Adams[2].** [1]USDA-ARS, SE Fruit and Tree Nut Research Laboratory, Byron, GA 31008 USA; [2]Department of Biology and Evolutionary Ecology Laboratories, Brigham Young University, Provo, UT, USA.

Entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* are important regulators of natural insect populations, and are used commercially as biological control agents for pest suppression. Successful biocontrol applications depend on the introduced organism having an array of beneficial traits such as virulence, host-finding, environmental tolerance etc. Thus biocontrol potential can be improved by enhancing these traits. Approaches to strain improvement include discovery, selection, hybridization, transgenics or a combination thereof. These methods have been successfully applied to a number of entomopathogenic nematode species. In addition to enhancing traits, trait stability is another factor that is critical for biocontrol success. Beneficial traits can deteriorate during repeated culturing in laboratory or industrial settings. Deterioration of various traits has been reported in entomopathogenic nematodes. The cause of trait change was found to be genetically based (at least in part) and inbreeding depression was implicated as a significant contributing factor. Recently the creation of homozygous inbred lines was found to deter the negative repercussions of trait change during serial culture. Inbred lines can be generated in the laboratory through serial self-fertilization (heterorhabditids) or sibling mating (steinernematids). Additionally, for heterorhabditids only, multiple inbred lines can be automatically generated in liquid culture because the nematodes cannot mate in the liquid media. Generation of multiple versus single inbred lines for commercial development each has advantages and disadvantages. Selected inbred lines and beneficial trait improvement programs offer a substantial advancement in biocontrol potential for entomopathogenic nematodes.

SPATIAL AND TEMPORAL VARIATION OF INTERTIDAL NEMATODE COMMUNITIES AFTER THE DEEPWATER HORIZON OIL SPILL. **Sharma[1], J., P.M. Brannock[2], H. Bik[3], K.M. Halanych[2], and W.K. Thomas[3].** [1]Department of Biology, University of Texas at San Antonio, San Antonio, Texas 78249, USA; [2]Department of Biological Sciences, Auburn University, Auburn, Alabama 36849, USA; [3]Hubbard Center for Genome Studies, University of New Hampshire, Durham, New Hampshire 03824, USA.

The composition of free-living nematode communities at five intertidal sites in northeast Gulf of Mexico was studied at intervals over two years to examine their role in recovery of biotic communities in benthic sediments after contamination by the Deepwater Horizon oil spill. The diversity and composition of the nematode fauna in contaminated sediments was compared to that of pre-spill sediments. Over 6000 individuals from 70 genera representing 20 families were recovered. The generic richness of nematodes in sediments immediately following the spill decreased but gradually increased after 12 months. The pre-spill intertidal samples showed high evenness without dominance of a single species while immediately after the spill the Thoracostomopsidae and Desmodoridae families were dominant. These observations are confirmed by metagenomic analyses of nematode communities that show a significant decline in abundance of nematodes following the oil spill, with a corresponding increase and overwhelming dominance, of fungal taxa previously associated with hydrocarbons. The vertical distribution of the nematode community showed that the lower depths of 3-10cm had greater generic richness and evenness as compared to the upper 0-3cm although there was variation in species composition between the two depths. The dominance of scavenger and predatory nematode taxa following the oil spill suggests that they may utilize the abundant fungi in post-spill sediments. The re-colonization of nematodes on beaches after an oil spill suggests that they are important in recovery of biotic communities in contaminated sediments.

RESTORING ECOSYSTEM SERVICES OF URBAN SOILS: USE OF NEMATODE COMMUNITY AS A BIOINDICATOR TO REESTABLISH MISSING LINKS IN SOIL FOOD WEBS. **Sharma, K.[1] and P.S. Grewal[1,2].** [1]Environmental Science Graduate Program, Ohio State University, OARDC, Wooster, Ohio 44691, USA; [2]Entomology and Plant Pathology Department, University of Tennessee, Knoxville, Tennessee, 37996, USA.

Utilizing urban vacant land for growing food crops can promote access to healthy food in low income neighborhoods. However, soil health and quality are major concerns for urban agriculture in post-industrial cities. Replacing existing soil with top-soil from a neighboring area is the most common solution applied, during which no attention is given to the health of the soil organisms being transported. Nematodes form an important and diverse component of the soil food web, with presence across multiple trophic levels, offering the most comprehensive insight into the structure and function of food webs. Previous studies showed that due to intensive disturbance, nematode food webs in urban areas had significantly lower structural complexity and lacked higher trophic guilds (predatory and omnivorous species), thus severely limiting the ecosystem services potential of the soil. We tested a *new* approach to improve the soil food web structure in targeted vacant lots, to restore the full spectrum of ecosystem services provided. We hypothesized that the missing nematode trophic guilds (along with associated soil organisms) in the soil food web can be transplanted/reestablished using small soil cores (9cm dia, 5cm deep) when coupled with specific organic amendments. Field trials of core transplantation into turf grass plots (1.5 m X 1.5 m) resulted in a significant increase in the structure index over a period of 6 weeks. Positive results from this study could eliminate the need for large-scale topsoil replacements in urban gardens and will pave the way for establishment of sustainable and safe urban food production systems.

NEMATODE QUARANTINE AND BIOSECURITY RELATED TRADE AND GLOBAL FOOD SECURITY ISSUES. **Sharma[1], S.B., C. Magnusson[2], J.J. Chitambar[3] and U.C. Kodira[4].** [1]Murdoch University, 90 South Street, Murdoch Western Australia 6150, Australia; [2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Høgskoleveien 7, N-1432 Ås, Norway; [3]California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, California 95832-1448, USA; [4]USDA-APHIS-PPQ, Plant Health Programs, 4700 River Road, Riverdale, Maryland 20737, USA.

Plant-parasitic nematodes cause economic losses of over 14% in developing countries. Many of the known and highly-damaging species of nematodes are relatively localised in distribution. The consequences of globalisation including advances in transport technologies have raised significant biosecurity concerns, and surge in nematode dispersal risk accompanied by climate change is likely to create new opportunities for nematode populations to move and establish in new geographical regions. Nematode quarantine and biosecurity capabilities are generally lacking in the food insecure developing countries. Nematode quarantines and stringent regulatory measures have been successful in prevention and containment of highly damaging nematodes such as the potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida)* and pine wood nematode (*Bursaphelenchus xylophilus*). Currently, *G. rostochiensis* is regulated as a quarantine pest by 118 countries, while *G. pallida* is regulated by 81 countries. There is every probability for these species to gain entry into non-infested regions as populations below 500,000 cysts per hectare cannot be detected reliably. The pine wood nematode is endemic to North America and has spread to Japan, China, Korea, Portugal and Spain. A global approach to nematode quarantine and biosecurity is needed to minimise international and intercontinental invasion of nematode pests. Policy development and implementation of effective pre-border, border and post border biosecurity measures are needed. Safeguarding of food production regions from invasive exotic plant-parasitic nematodes is a must to achieve food security for the present and future generations. Case studies on regulatory control of potato cyst nematodes and pine wilt nematodes will be presented.

RELATIONSHIP BETWEEN *BELONOLAIMUS LONGICAUDATUS, TRICHODORUS OBTUSUS,* AND TURFGRASS ROOTS. **Shaver[1], B., P. Agudelo[1] and B. Martin[2].** School of Agricultural, Forest, and Environmental Sciences, Clemson University.[1]114 Long Hall Clemson, SC 29365; [2]Pee Dee Research and Education Center 2200 Pocket Rd. Florence, SC 29506.

Natural in-field variability of turfgrass root growth and nematode density makes it difficult to determine effects of nematicide or fungicide treatments applied to manage nematodes or fungal infections that result from nematode feeding. Over the past two years we have analyzed over 1000 soil cores (5-cm diameter by 20-cm depth) from trials on hybrid bermudagrass (*Cynodon dactylon* X *C. transvaalensis*) infested with *Belonolaimus longicaudatus* or from 'Empire' zoysiagrass (*Zoysia japonica*) infested with *Trichodorus obtusus.* Treatments included three different nematicides, a fungicide, and nematicide and fungicide combinations. A 100 cm^3 soil sample from each soil core was collected and assayed for nematode density using centrifugal sugar flotation. Roots in each soil core were then washed free of remaining soil, dried for five days at 70°C, and incinerated at 525°C for 3 hours in order to determine dry root weights. To more fully understand the relationship between nematode feeding and root growth, we regressed dry root weight with nematode density. In a separate nematicide trial, we compared the variability of root weights to root architecture. Soil cores (5-cm diameter by 20-cm depth) were collected and roots washed free of soil, scanned on a flatbed scanner, and analyzed for root architecture. Afterwards, root weights were evaluated as described above. Data shows that root architecture may be a better indicator of nematode damage than root weights. Results of the regression analysis will be helpful in understanding treatment effects of this and future nematicide trials.

ADAPTED TECHNIQUE FOR EXTRACTING NEMATODES FROM CARROTS. **Shubane, A. and M. Marais.** National Collection of Nematodes, Biosystematics Programme, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa.

It has always been difficult to correlate the symptoms of carrots (*Daucus carota* subsp. *sativus*) infested with *Meloidogyne* species and the number of nematodes observed. A technique was adapted to successfully extract root-knot nematodes from carrots. The carrots, including all secondary roots, were peeled with a vegetable peeler; the peels and the secondary roots were cut into 2 mm strips, placed in tap water and macerated in a blender at medium speed for 45 seconds. Ten millilitres of 4 % NaOCl was added to the suspension and aerated with an aquarium pump for 72 hours. After aeration the suspension was poured through a tower of 1000 μm -, 150 μm-, 45 μm - en 38 μm sieves, and thoroughly washed with a strong water stream. The macerated carrot pieces collected on the top sieve were discarded and the rest were washed in centrifuge tubes to which 5 mℓ kaolin was added and centrifuged for 7 min at 3500 rpm. The sample was then centrifuged with a sugar solution (450 mℓ in 1 ℓ of water) for 3 min at 3500 rpm. After washing the nematode supernatant through a 38 μ sieve to remove all traces of sugar, the sample was ready to determine the population numbers of both plant-parasitic and free-living nematodes. This technique was also successfully used to extract nematodes from beetroot, potatoes, peanut seeds, and peanut hulls.

POSITIONING NEMATOLOGY RESEARCH AND DEVELOPMENT THROUGH NIESA. **Sibanda[1], Z., H. Talwana[2], W. Wanjohi[3], W. Kimenju[4], K.G. Davies[5,6], R.H. Manzanilla-López[5], C. Masawe[7] and N. Luambano-Nyoni[8].** [1]Goldengro (pvt) Limited, P.O. Box MP 1306, Mount Pleasant, Harare Zimbabwe; [2]Department of Crop Science, Faculty of Agriculture, Makerere University, P.O. Box 7062, Uganda; [3]Agricultural Science and Technology, Kenyatta University, P.O. Box 43844 – 00100; Nairobi, Kenya; [4]Faculty of Agriculture, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya; [5]Department of AgroEcology, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom; [6]School of Life and medical Sciences, University of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB, United Kingdom; [7]Ministry of Agriculture and Food Security, Tanzania; [8]Sugarcane Research Institute, P.O. Box 30031, Kibaha Pwani, Tanzania.

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 .

NINJA: AN AUTOMATED CALCULATION SYSTEM FOR NEMATODE-BASED BIOLOGICAL MONITORING. **Sieriebriennikov[1], B., H. Ferris[2], R.G.M. de Goede[3].**[1]Nematology Unit, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Department of Entomology and Nematology, University of California, Davis, CA 95616, USA; [3]Department of Soil Quality, Wageningen University, P.O. Box 47, NL-6700 AA, Wageningen, The Netherlands.

Nematodes are important bioindicators of soil health and ecosystem services. Index systems based on life-cycle properties, feeding habits, biomass and metabolic activity have been developed for analysis and interpretation of nematode assemblages. We have developed an R code, NINJA (Nematode INdicator Joint Analysis), to calculate indices automatically. The code is compiled in html and distributed over the web. It has a user-friendly interface accessible via: http://spark.rstudio.com/bsierieb/ninja/. The only input requirement is a table with the taxonomic inventory of the nematode assemblage. The output includes tables and graphs which contain values for: Maturity Index, Maturity Index 2-5, Plant-parasitic Index, Channel Index, Enrichment Index, Structure Index, total biomass, and the composite, enrichment, structure, herbivore, fungivore, bacterivore, predator and omnivore metabolic footprints as well as proportions of different feeding types and c-p classes amongst herbivore and free-living forms. For all measures but the percentages, means and standard deviations are calculated per treatment or sampling site and accompanied by corresponding p-values. The tool is supported by a database, stored on the server, which contains life-history characteristics, c-p (coloniser-persister) assignments, feeding habits and body masses of most soil nematodes. The tool permits adjustment of default feeding habits based on the user's observation and experience. The NINJA tool is free, flexible and user-friendly; it greatly simplifies nematode faunal analysis and protects against calculation errors. Data interpretation is enhanced by graphs and primary statistical treatment. Intended future expansion of the database will include sequence accession numbers that will enable processing data based on molecular identification.

UPLAND COTTON GERMPLASM LINES WITH INTROGRESSED RESISTANCE TO THE RENIFORM NEMATODE. **Sikkens[1], R.B., K.S. Lawrence[1], D.W. Schrimsher[2], S.R. Moore[3] and D.B. Weaver[4].** [1]Entomology & Plant Pathology, Auburn University, Auburn, Alabama, USA; [2]Southern Agronomists, AGRI-AFC, Decatur, Alabama, USA; [3]Research & Development Scientist, Syngenta, Monroe, Louisiana, USA; [4]Crop, Soil and Environmental Sciences, Auburn University, Auburn, Alabama, USA.

Over the past decade a concerted effort was made to develop host plant resistance to reniform nematode (*Rotylenchulus reniformis*) in upland cotton (*Gossypium hirsutum*). Since 2007 this effort has resulted in the release of several germplasm lines with introgressed resistance to reniform nematode. Currently four groups of released germplasm lines can be distinguished based on their source of resistance and developmental background: (a) the LONREN group, with reniform nematode resistance derived from *Gossypium longicalyx*, (b) the BARBREN group with reniform nematode resistance derived from wild accession GB-713 of *Gossypium barbadense*, (c) the M713 group, also with resistance derived from the GB-713 accession but with a different developmental trajectory, and (d) the MT2468 group, with reportedly moderate levels of *R. reniformis* resistance derived from the photoperiodic primitive race accession TX2468 of *G. hirsutum*. During 2013 we evaluated the potential of 13 lines from these four groups, including three lines of the LONREN group, two lines of the

BARBREN group, five lines of the M713 group and three lines of the MT2468 group. Conventional cultivars FM966 and SG747 were included as *R. reniformis* susceptible controls. Our studies were conducted in the field, on adjacent plots with and without reniform nematode present, on reniform nematode infested outdoor microplots, and in the greenhouse. Results indicate that germplasm lines BAR 41 of the BARBREN group and all five M713 lines combine great *R. reniformis* resistance with excellent field tolerance.

SURVEILLANCE STRATEGIES FOR EARLY DETECTION OF NEMATODE INCURSIONS. **Singh[1,2,3], S.K., M. Hodda[2], N.C. Banks[1,2,4] and Rajan[5].** [1]Cooperative Research Centre for Plant Biosecurity, Bruce, ACT, 2617, Australia; [2]CSIRO Ecosystem Sciences, Canberra, ACT, 2601, Australia; [3]Graham Centre for Agricultural Innovation (an alliance between Charles Sturt University and the NSW Department of Primary Industries) Wagga Wagga NSW, 2678, Australia; [4]School of Veterinary and Life Sciences, Murdoch University, Murdoch 6150, Australia; [5]Crop Science Division, Indian Council of Agricultural Research, New Delhi 110114, India.

The spread of plant parasitic nematode species worldwide has increased over the last two decades and as a result the damage they cause to global crop production. Due to shortage of resources, most countries still depend upon traditional quarantine system of on border examination only. New developments in quarantine systems are being implemented with the involvement and active roles being played by the World Trade Organisation and International Plant Protection Convention. Now member countries carry out pest risk analysis, based upon pre export information/ field data to assess and minimise threat. Listing pests already present in a country and especially knowing what pests are not there through surveys; in territories of that country are the most critical and indispensable data requirements. Recent developments on biosecurity measures designed to prevent the entry and establishment of exotic species, are one of the most cost effective and environmentally friendly means of averting the negative impacts. Plant-parasitic nematodes due to their microscopic size, low probability of detection and diverse range of pathways represent a major challenge for the implementation of biosecurity measures worldwide. Surveillance strategies are an essential part of the plant biosecurity continuum and can greatly aid in the early detection of nematode incursions. In this paper, the design of surveillance strategies is illustrated using examples of potato cyst (*Globodera* spp.) and pine wood (*Bursaphelenchus* spp.) nematodes. Methods for prioritization of exotic nematode species; spatial mapping of risks; and targeted sampling taking into consideration seasonal cropping patterns and nematode population growth are discussed.

GLOBAL BIOSECURITY RISKS FROM THREE PLANT-PARASITIC NEMATODE SPECIES TO MAIZE, RICE AND WHEAT, USING CLIMEX MODELLING. **Singh[1,2,3,4], S.K., D.J. Kriticos[1,3], G.J. Ash[2] and M. Hodda[1,4].** [1]CSIRO Ecosystem Sciences, Canberra, ACT, 2601, Australia; [2]Graham Centre for Agricultural Innovation (an alliance between Charles Sturt University and the NSW Department of Primary Industries) Wagga Wagga, NSW, 2678, Australia; [3]Cooperative Research Centre for National Plant Biosecurity, Bruce, ACT, 2617, Australia; [4]CSIRO Biosecurity Flagship, Canberra, ACT, 2601, Australia.

Climate suitability is one of the most important factors affecting the chances of a translocated species establishing in a foreign location. CLIMEX models were developed to estimate the eco-climatic suitability and growth potential of three plant-parasitic nematodes; *Heterodera zeae, Hirschmanniella oryzae* and *Meloidogyne graminicola*, globally. The models were carefully parameterised based on the species phenology and their global distributions extracted from peer-reviewed literature. To simulate the different field conditions under which these species occur, the models included rain-fed and irrigation scenarios. Except for a few occurrences, the known geographical distributions for all three species were in accord with their projected potential distributions and substantiated by available experimental and field observations. Maps of host crops (maize, rice, wheat) and irrigation areas globally were compared with projected growth index maps of *H. zeae*, *H. oryzae* and *M. graminicola* and countries at risk are identified. All three species have the potential to expand their distribution range in countries where they are not present yet but have favourable eco-climatic and growth index values. Under irrigation scenarios, much greater areas globally are conducive for the growth of these three species than under rain-fed conditions. When assessing the establishment potential and biosecurity risks from exotic species, presence of modified conditions such as irrigation need to be taken into consideration.

THE EFFECT OF SPIROTETRAMAT ON *ROTYLENCHULUS RENIFORMIS* INFECTING PINEAPPLE. **Sipes, B.** University of Hawaii, 3190 Maile Way, Honolulu, Hawaii, 96822, USA.

Spirotetramat is a lipid biosynthesis inhibitor used to control sucking pests in a wide range of crops. The compound has been beneficial in the management of plant-parasitic nematodes in several woody perennial crops. The objective of these tests were to determine if spirotetramat controlled damage in pineapple (*Ananas comosus*) caused by *Rotylenchulus reniformis*. In a greenhouse experiment, pineapple crowns were dipped in water or a solution of spirotetramat (730 mlMovento/ha or 7.9 ml/10 l water) with each plant receiving the equivalent of 16 ml/plant. The crowns were planted in 20-cm diameter pots filled with a 1:1 sterile soil-sand mix and inoculated with 5,000 mixed life stages of *R. reniformis*. One month later, half the plants dipped in spirotetramat were treated with a foliar application of 7301ml Movento/ha. The pineapples were destructively

harvested 9 months later. Spirotetramat treated plants had lower numbers of nematodes compared to the untreated pineapple (48% and 44% lower), although plant growth was similar. A similar experiment was established in a commercial pineapple field. At 9 months after planting, pineapple D-leaf weights were similar among the same treatments. Where no preplant nematicide was applied, the spirotetramat dip and foliar application tended to increase D-leaf weight (35 g vs 33 g). Populations of *R. reniformis* in the field were similar regardless of the spirotetramat treatments received. Spirotetramat application affected nematode development on pineapple in the greenhouse and may lessen nematode damage in the field.

MODELLING OF TEMPERATURE DEPENDENT DEVELOPMENT OF TROPICAL ROOT-KNOT NEMATODE SPECIES. **Širca, S., P. Strajnar, M. Knapič, U. Žibrat and G. Urek.** Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia.

Root-knot nematodes are poikilothermic organisms and their development is generally temperature dependent. In a previous experiment in growth chambers we estimated that *Meloidogyne ethiopica* required 67, 48 and 36 days to complete the reproduction cycle, at mean daily temperatures of 18.3, 22.7 and 26.3°C, respectively. The base temperature (Tb) for *M. ethiopica* was established in a pot experiment at 14°C. At 13.9°C, the species was not able to reproduce. This data we then used to build a model for *M. ethiopica* development using Michaelis-Menten regression models. The extrapolated model suggests a minimum of approximately 40 DAI's (at 24°C to 30°C), while a stop in development is indicated at about 13.9°C (at DAI = 365). The model proved to be very useful for predicting *M. ethiopica* population dynamics. Additionally, we evaluated the differences among linear regression models of temperature – development rate relationships of six *Meloidogyne* species. The relation between temperature and development time appears to be linear only at temperatures several degrees Celsius above the base temperature. In fact, for the six species included in this study, linear models can be considered as applicable only at temperatures above 19°C. At lower temperatures, development rate decreases exponentially, not linearly. Our results suggest that linear models fail to accurately indicate minimum required time for postembryonic development. We argue that Michaelis-Menten or other non-linear models should be used for assessing thermal requirements of ontogenetic development.

ULTRASTRUCTURE OF SPERM DEVELOPMENT IN THE GENUS *DITYLENCHUS*. **Slos[1], D., P. Ensafi[1], M. Claeys[1], V.V. Yushin[2], W. Decraemer[1] and W. Bert[1].** [1]Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; [2]A.V. Zhirmunsky Institute of Marine Biology, FEB RAS, Vladivostok, Russia.

The ultrastructure of the spermatogenesis in *Ditylenchus arachis* and *Ditylenchus dipsaci* has been studied. The general pattern of spermatogenesis is recognized as 'rhabditid', a pattern which is rather conserved throughout the order Rhabditida. Spermatozoa of *Ditylenchus* represent an aberrant type of male sperm because of the absence of an axoneme and acrosome, a characteristic shared with other nematodes. The sperm development includes the formation of complexes of fibrous bodies (FB) and membranous organelles (MO) which appear in the spermatocytes. These complexes start to dissociate in separated MO and FB in the spermatids. Immature spermatozoa are unpolarised cells with a centrally located nucleus surrounded by spherical fibrous bodies and MO located at the periphery. In both species, the spermatheca contains chains of mature spermatozoa consisting of amoeboid bipolar cells subdivided in a pseudopod devoid of organelles and a main cell body. The main cell body consists of a centrally located nucleus lacking a nuclear envelope, many mitochondria and MO. The MO are connected to the plasmalemma and open to the intercellular exterior via a pore. Hence, our data unequivocally show that MO are present in *Ditylenchus*, contrary to what has been described before also for *D. dipsaci*, and thus showing the typical rhabditid pattern. Although both species have a similar spermatogenesis, *D. dipsaci* differs from *D. arachis* in having mature spermatozoa in the male testis, a character which is, to our knowledge, not observed before. This is a further indication that the well-studied developmental mechanisms in the informative model *Caenorhabditis elegans* cannot simply be extrapolated to other rhabditids.

PEAT AS A SUITABLE GROWTH MEDIA AND CARRIER FOR *DACTYLELLA OVIPARASITICA*. **Smith Becker, J., H. Witte, and J.O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

Dactylella oviparasitica is a facultative parasitic ascomycete of several endoparasitic nematodes. Various botanical materials such as ground corn cob, ground walnut shells and a commercial preparation of reed sedge peat (BioAPT) were tested for their ability to serve as carrier media for the introduction of the hyperparasite into soil. Hyphal growth of *D. oviparasitica* was inhibited by walnut shells and corn cob caused severe plant stunting when added as a soil amendment at 1% w/w. BioAPT peat supported fungal growth without affecting seedling growth. Grown aseptically on BioAPT peat, the fungus remained viable for at least two years when stored at room temperature or at 4C. In greenhouse trials pasteurized sandy loam was amended with various concentrations of *D. oviparasitica* peat formulation and seeded with Swiss chard. After seedling emergence, the pots were infested with 750 J2/100 cm^3 of *Heterodera schachtii*. Fungal peat amendment at 1% (DW/DW) reduced the number of recovered nematode cysts 5 weeks after infestation by up to 70% compared to the infested control with autoclaved amendment. As little as 0.05% *D. oviparasitica* peat amendment reduced the number of cysts by up to 50%. These results suggest that BioAPT peat may serve as a useful media and carrier for the introduction of *D. oviparasitica* into soil for population suppression of the sugar beet cyst nematode.

NEMATOLOGY EDUCATION IN EUROPE: CLIPPING OR SPREADING WINGS. **Smol[1], N. and W. Decraemer[1,2].** [1]Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium; [2]Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium.

The aim of university level education in Nematology at Ghent University is to train specialists equipped to take up job positions in all subjects related to nematodes, as well as in broader areas. The programme therefore has a multidisciplinary approach and for this cooperation with other universities and institutes within Europe and beyond is important. However, cooperation and mobility in and out of Europe involves a cascade of administrative and logistic problems. An overview will be given of the different options for collaborative education in Europe at MSc and PhD level, dealing with important topics such as grants, enrolment fees, curriculum and visa issues. The masters course has been in existence at Ghent University for 21 years; the intake of students and the jobs taken up by alumni will be presented and discussed. In addition, the following points will be discussed: How effectively has the availability of this MSc in Nematology been disseminated? Is there a need for Nematology training? Who is willing to cooperate to provide this training? How do we attract students to this field? How do we attract industrial partners? Who can, and is willing to, sponsor education?

NEMATOLOGICAL RESEARCH AND DEVELOPMENT IN PAKISTAN: HISTORICAL PERSPECTIVE AND FUTURE PROSPECTS. **Soomro, M.H. and F. Shahina.** National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan.

Nematodes have been affecting man since times immemorial as parasites of human, animals and agricultural pests. Plant parasitic nematodes are considered hidden enemies of crops which represent unique challenges to agricultural research. Nematological research in Pakistan has commenced in 1974 and up to now extensive surveys have been conducted throughout Pakistan which resulted in the identification of 760 nematode species and 2 new genera of which 229 were new to science. The main institution involved in Nematological research is the National Nematological Research Centre (NNRC) at the University of Karachi, which has been contributing significantly on the biodiversity and biosystematics of nematode fauna especially the description and identification of new genera/species. During recent years, significant progress has been made in the morphological, biochemical, molecular biology, genomics, biocontrol and mass production of beneficial nematodes. A genomic library has been developed at NNRC on the basis of ITS, 28S/D2D3, 18S rDNA regions and 12S rDNA genes of the indigenous root-knot nematodes, entomopathogenic nematodes and their symbiotic bacterial species/stains, which adds to the worldwide database. Research on biological agents based on the ecological knowledge provides sustainable alternative control methods and replaces highly toxic chemical insecticides. The research efforts of our group and optimization of nematode production methods at NNRC have provided an impetus and advancement in the biopesticides industry in Pakistan. The team feels that the research endeavours of the last 12 years have enabled us to embark upon commercialization of the native strains of EPNs from Pakistan. However, we have yet to learn from colleagues and professionals from around the globe and are searching for international collaboration.

CARBOHYDRATES IN THE GLIA-SECRETED MATRIX OF *CAENORHABDITIS ELEGANS* SENSE ORGANS. **Spiegel[1,2], Y., Y. Lu[2] and S. Shaham[2].** [1]Department of Entomology and Nematology, Agricultural Research Organization, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel; [2]Laboratory of Developmental Genetics, The Rockefeller University, 1230 York Avenue, New York, New York 10065, USA.

Amphids are the largest, most important sensory organ of nematodes. These bilateral organs consist of 12 sensory neurons and two glial cells, referred to as sheath and socket cells. At the tip of the nematode head these two come together to form a tube – the amphid channel, which contains a material, the matrix, of which some components are secreted by the sheath glia. These 12 neurons have been studied mainly by attraction assays and genetic tools. There is, however, little knowledge about the matrix composition and origin. By labeling wild-type and various mutants (cilia and glia-ablated) of the nematode *Caenorhabditis elegans* with fluorescent and gold-conjugated lectins (carbohydrate-binding proteins), we were able to gain preliminary information about the nature and localization of carbohydrates in the amphids and phasmids glia matrix. N-acetyl-glucosamine (GlucNac) residues are located, in wild type species, in different glia cells around the sensory cilia extended from the neuron dendrites within the amphid channel, and also protracted anteriorly and posteriorly to the amphid channels. Mannose residues are located mainly anteriorly and within the glia cells within the amphid channel and in the inner/outer labial and cephalic neurons in the tip of the nematode 'nose'. Phasmids reveal the presence of both carbohydrate moieties. In several mutants (e.g. *che-2* or *osm-6*), carbohydrate were presented only in the anterior part of the glia cells, and slim label observed in the phasmid. Amphid neuron dye filling defected species (*fig-1* mutant) did not exhibit neither GlucNac, nor mannose residues in both amphids and phasmids.

DISPLACEMENT OF *MELOIDOGYNE INCOGNITA* BY *ROTYLENCHULUS RENIFORMIS* DURING A 10 YEAR COTTON MONOCULTURE AND IMPLICATIONS FOR SITE-SPECIFIC MANAGEMENT. **Spurlock[1], T.N., T.L. Kirkpatrick[2], C.S. Rothrock[3] and S.W. Monfort[4].** [1]University of Arkansas Division of Agriculture, Southeast Research and Extension Center, Monticello, AR, USA; [2]University of Arkansas Division of Agriculture, Southwest Research and

Extension Center, Hope, AR, USA; [3]University of Arkansas Division of Agriculture, Fayetteville, AR; [4]Clemson University, Edisto Research and Extension Center, Blackville, SC, USA.

Meloidogyne incognita and *Rotylenchulus reniformis* are economically important nematode species in cotton production. Evidence from 2001-2003, in a texturally diverse cotton field near Portland, Arkansas, USA, suggested that populations of *M. incognita* had the greatest effect on yield in soils with greater sand content. Additionally, exhaustive nematode assays indicated *R. reniformis* was increasing in population and distribution in the field. Since that work, the field has continued to be planted in cotton until being planted in soybean in 2013. From the original 512 plots, nematode population densities were measured in the fall immediately after the crop was harvested in 2011 and 2013. Over the 10 year period, populations of *M. incognita* declined sharply while *R. reniformis* increased. Univariate *Moran's I* indicated significantly aggregated distributions for both pathogens in all years except 2011 and trend surface models indicated *Range* values decreased for *M. incognita* but increased for *R. reniformis* prior to 2013. Bivariate exploratory spatial analysis and spatial regression indicated a significantly dispersed distribution and inverse relationship in 3 of 4 years and 2 of 4 years respectively confirming competition and displacement of *M. incognita* by *R. reniformis*. Neither *R. reniformis* nor *M. incognita* populations were directly related to yield loss across soil textures. The highest yield impact of nematode populations occurred in the sandier areas of the field indicating site specific management in these textural zones should have the greatest economic return.

NEW REPORTS AND MOLECULAR DIAGNOSTICS OF ROOT-KNOT NEMATODES FROM GOLF COURSE GREENS IN THE WESTERN UNITED STATES. **Skantar[1], A.M., C. Nischwitz[2], Z.A. Handoo[1], S. Subbotin[3], M.N. Hult[1], M.E. Schmitt[4] and M.A. McClure[4].** [1]USDA-ARS Nematology Laboratory, Beltsville, Maryland, 20705; [2]Dept. of Biology, Utah State University, Logan, Utah, 84322; [3]California Department of Food and Agriculture, Sacramento, California 95832; [4]School of Plant Sciences, University of Arizona, Tucson, Arizona 85721.

Several species of root-knot nematodes (*Meloidogyne* spp.) are known to occur on turfgrass. A survey of 238 golf courses in the Western United States foundroot-knot nematodes in 60% of putting greens sampled. Morphology and phylogenetic trees inferred from multiple DNA markers were used to identify specimens from 110 golf courses. *Meloidogyne naasi* was the most common species, distributed from coastal Southern California to Washington; *Meloidogyne graminis* and *Meloidogyne marylandi* were found mostly in the warmer regions of the Southwest. PCR-RFLP of a mitochondrial DNA region distinguished these two species, providing a simple method for future diagnosis of these nematodes from turf. Specimens from two golf courses in Washington were identified as *Meloidogyne minor* based on analysis of the 28S, ITS1&2, and IGS2 ribosomal markers and the nuclear protein-coding gene Hsp90 sequences. Sequence-characterized amplified region (SCAR) primers previously reported as specific for *Meloidogyne fallax* were found to cross-react with *M. minor*. A population from California was determined to be *M. fallax* based on juvenile morphology and analysis of the ribosomal markers and Hsp90. The phylogenetic relationships of these populations and known root-knot nematode species reconstructed using Hsp90 gene sequences were congruent with those obtained from ribosomal RNA genes. Resolution of *M. fallax* and *Meloidogyne chitwoodi* using Hsp90 was equivalent to species separation obtained with 28S rDNA alignments. The strengths and weaknesses of ribosomal and Hsp90 markers, and the use of SCAR PCR as diagnostic tools will also be discussed.

MOLECULAR IDENTIFICATION OF PLANT-PARASITIC NEMATODES OF QUARANTINE CONCERN AT THE UNITED STATES DEPARTMENT OF AGRICULTURE (USDA). **Skantar, A.M., Z.A. Handoo, L.K. Carta and D.J. Chitwood.** USDA-ARS Nematology Laboratory, Beltsville, Maryland, 20705.

One mission of the USDA Agricultural Research Service Nematology Laboratory in Beltsville, Maryland is to provide nematode identifications that are urgently required by regulatory agencies, federal and state authorities, or other ARS scientists for research, regulatory actions, and control purposes. In addition to traditional morphology-based taxonomic approaches, molecular methods are often required to confirm diagnoses or to provide conclusive identification in those instances when species morphology is ambiguous or when adult specimens are unavailable for microscopic examination. Current approaches, challenges, and new developments in nematode molecular identification will be discussed within the context of some recent diagnostic scenarios involving *Globodera, Meloidogyne*, and *Anguina* spp.

AVICTA® COMPLETE CORN – A PREVENTATIVE SOLUTION AGAINST THE HIDDEN PEST. **Slaats[3], B., J. Dreyer[1] and C. Watrin[2].** [1]Syngenta Crop Protection AG, Thornhill Office Park Block 14, Halfway House 1685, Johannesburg, South Africa; [2]Syngenta Crop Protection AG, 410 Swing Road, Greensboro, North Carolina 27409, USA; [3]Syngenta Crop Protection AG, Schaffhauserstr. 101, 4332 Stein AG, Switzerland.

Maize (*Zea mays* L.) nematode damage is difficult to identify and is often misdiagnosed by the grower. Due to a growing world population and limited production areas, a demand for higher yields in existing production areas has arisen coupled with increased intensification which is very favourable for nematode multiplication. Avicta® Complete Corn is a Syngenta SeedCare solution offering triple protection from the day the seed is planted against nematodes, insects and major soil and seed borne diseases under all environmental conditions, as a convenient ''in the bag'' solution. This first-of-its-kind seed treatment is immediate and effective ensuring maize seedlings are protected at emergence and early growth stages. With

limited maize nematode control options on the market, Avicta Complete Corn offers protection in the convenience of a seed-delivered treatment – helping to increase plant health and yield potential. Syngenta SeedCare initiated an extensive testing of the yield benefit of Avicta Complete Corn in South Africa. The nematicidal component of Avicta® Complete Corn is abamectin which is highly active against a broad range of plant-parasitic nematodes. In one study across 12 trials, Avicta Complete Corn outyielded the standard fungicide/insecticide seed treatment in 80% of the trials delivering a yield increase of 5% on average. In another study across 22 trials under high nematode pressure Avicta® Complete Corn led to higher yields than the soil-applied nematicides Terbuphos or Carbofuran in 60% of the time. Overall applying Avicta® Complete Corn provides reliable protection and allows growers to maximize yield and economic return.

PHYLOGENETIC RELATIONSHIPS OF RHIGONEMATOMORPHA– NEMATODES FROM THE HIND GUT OF DIPLOPODS. **Spiridonov, S.E. and S.V. Malysheva.** A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr., 33, Moscow,119071, Russia.

The posterior part of the diplopod intestine is inhabited with the nematodes of the superfamilies Thelastomatoidea, (Oxyuridomorha), Rhigonematoidea (Rhigonematomorpha) and Ransomnematodiea (Rhigonematomorpha). Thelastomatoidea (pinworms) are similar to the thelastomatids of other arthropods, whereas Rhigonematoidea and Ransomnematoidea are morphologically different from pinworms. The representatives of two families for each of these superfamilies were studied under light and scanning electron microscope. The partial sequences of 18S and 28S rDNA were also obtained. The phylogenetic analysis of each separate ribosomal sequence did not resolve the phylogeny of the nematodes of diplopods with the representatives of separate families forming independent clades. The monophyly of superfamilies was not evident. When partial 18S and 28S rDNA sequences were concatenated forming a 2297 bp long alignment, the phylogeny of each superfamily was securely proven using Bayesian Inference (posterior probabilities 99% and 100% for Rhigonematoidea and Ransomnematodiea, correspondingly). However, the monophyly of Rhigonematomorpha was not supported, with Rhigonematoidea, Ransomnematoidea and Ascaridoidea forming three independent clades with Oxyuridomorpha linking to the basal node of this tripartite evolutionary line. Besides the molecular apomorphies, morphological ones were found for each separate superfamily clade. The following features are considered as secure markers of taxonomic independence of these groups: structure of excretory-secretory system, presence of somatic sensillae, structure of the egg-shell. Phylogenetic analysis based on more representative and characteristic sequences is needed to resolve the phylogeny of these nematodes.

EVOLUTION OF NOVELTY: FROM PREDATORY FEEDING TO NOVEL CHEMISTRY, NEW GENES AND EPIGENETICS. **Sommer, R.J.** Max-Planck Institute for Developmental Biology, Tübingen, Germany.

Developmental plasticity has been suggested to facilitate the evolution of novelty and phenotypic diversity, but molecular mechanisms underlying this relationship are little understood. The nematode*Pristionchus pacificus* is a model system for integrative evolutionary biology, combining laboratory studies with field work in ecology and population genetics. *Pristionchus pacificus*shows phenotypic plasticity in its feeding structures and is able to feed on fungi and other nematodes. *Pristionchus* executes one of two alternative mouth-forms (Eurystomatous vs. Stenostomatous) after an irreversible developmental decision that requires small molecule pheromones consisting of a blend of ascarosides and paratosides. Interestingly, dauer formation and mouth-form regulation require novel and specific compounds for their regulation. Downstream of small molecule pheromones and a conserved endocrine signaling module involving the nuclear hormone receptor DAF-12, we identified a novel regulator of plasticity *eud-1*. *eud-1* acts in a developmental switch; *eud-1* mutations eliminate one mouth form, whereas over-expression of *eud-1* fixes it. EUD-1 is a sulfatase that acts dosage-dependently, is necessary and sufficient to control the sexual dimorphism of feeding forms, and has a conserved function in *Pristionchus* evolution. *eud-1* represents a novel gene that is much younger than the feeding dimorphism and has been added to a complex genetic network at a terminal position. It is epigenetically regulated in part by genes that are not conserved. These studies show that novel structures are determined by an unpredictable mix of conserved (co-opted) and novel determinants and that candidate gene approaches are of limited use for studying novel biological phenomena.

INTERACTIONS OF DROUGHT STRESS, *MELOIDOGYNE INCOGNITA* AND ARBUSCULAR MYCORRHIZAL FUNGI ON SUGARCANE. **Souza[1], C.C.M., E.M.R. Pedrosa[1], and U.M.T. Cavalcante[2].** [1]Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Recife, 52171-900, Pernambuco, Brazil; [2]Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife/PE, 50670-901, Brazil.

The objective of this study was evaluating effects of the interaction of drought stress [50 and 100% of pot water capacity (PWC)] and *Meloidogyne incognita* (0 and 12.000 eggs per pot)] and arbuscular mycorrhizal fungi – AMFs (*Glomus etunicatum*, *Gigasporos albida*, *Acaulosporo longula* and *Scutellospora heterogama* at 200 spores per pot in same proportion) on early morphological development and physiological activity of sugarcane (variety RB863129), as well as the effects on nematode and fungi multiplication. Sugarcane was inoculated with AMFs 20 days after seedlings acclimation in greenhouse and, with *M. incognita*, 30 days after the AMFs inoculation. Evaluations were carried out 45 days after nematode inoculation. Drought stress and *M. incognita* limit the initial vegetative development and fresh and dry biomass of sugarcane.

Drought stress also increased AMFs density in soil and roots. The AMFs increased sugarcane fresh stalk and root biomass but decreased gall and nematode numbers in roots. Drought stress as well *M. incognita* decreased ascorbate peroxidase contents in sugarcane, but the nematode infestation increased polyphenoloxidase and total soluble protein in the sugarcane plants. Drought stress decreased phosphorus content in the leaves and AMFs increased phosphorus content in stalk, however, the increase in phosphorus promoted by the AMFs did not occur under drought stress. Catalase, peroxidase and proline levels were not affected by drought stress, *M. incognita* and/or AMFs.

NEMATODES AS BIOINDICATORS OF SOIL SUPPRESSIVENESS. **Steel[1,2], H. and H. Ferris[1].** [1]Department of Entomology and Nematology, University of California Davis, One Shields Avenue, Davis, California 95616-8751, USA; [2]Department of Biology, Nematology Research Unit, Ghent University, K.L. Ledeganckstraat 31, 9000 Ghent.

We tested the hypothesis that the numerical, biomass or functional abundance of predator nematodes, either specialists or generalists, are useful indicators of soil suppressiveness to opportunistic plant-feeding species. In concept, regulation or suppression of target pest species should be enhanced when an abundance of predator species is supported by ample availability of bacterial- fungal- and non-damaging plant-feeding prey species. Besides their direct effects on the prey, the predator nematodes are bioindicators of other soil organisms performing the same ecosystem services. We selected soils from natural and managed environments that represented different levels of resources and disturbance and introduced test prey not already resident in the soils (*Meloidogyne incognita* and *Steinernema feltiae*) into microcosm chambers of each soil either in its natural state or after heat defaunation. Survival of the test prey was determined after a 5 day bioassay exposure. Across the soils tested, predator abundance and biomass were greater in undisturbed soils with plentiful resources and lower in soils from agricultural sites. Suppressiveness to the two introduced species increased with both numerical abundance and metabolic footprint of the predator assemblages. The relationship between suppressiveness and metabolic footprint of the predators was best described by a logarithmic function which increased rapidly at first and then dampened to an asymptotic level possibly determined by temporal and spatial aspects of the bioassay system. The applied implications of this study are that soil suppressiveness to pest species may be enhanced by increasing resources, removing chemical and physical constraints to the survival and increase of predators, and altering management practices so that predators and target prey are co-located in time and space.

HOST PLANT RESISTANCE IN GROUNDNUT TO *DITYLENCHUS AFRICANUS*. **Steenkamp[1], S., A.H. Mc Donald[2] and D. de Waele[3].** [1]Agricultural Research Council-Grain Crops Institute, Private Bag X1252, Potchefstroom, 2520 South Africa; [2]Unit for Environmental Sciences and Management, North west University, Private Bag X 6001, Potchefstroom 2520, South Africa; [3]Laboratory of Tropical Crop Improvement, Department of Biosystems, KU Leuven, Willem de Croylaan 42 – box 2455, 3001 Leuven, Belgium.

Groundnut is an important cash crop for both commercial and small-scale farmers in South Africa. The main effect of *Ditylenchus africanus* on groundnut is qualitative, causing downgrading of groundnut consignments with significant financial losses for producers. This nematode is difficult to control with currently available management tools because of its high reproduction and damage potential. The objective of this study was to investigate the potential of host-plant resistance as an effective and economically-feasible alternative for the control of *D. africanus* on groundnut. Selected groundnut genotypes were evaluated in microplot and field trials over two consecutive growing seasons. PC254K1 and CG7 were identified as resistant to *D. africanus* under microplot and field conditions with resistance that was sustainable even under high infestation levels. These lines consistently produced high quality kernels throughout all of the trials and can therefore be used as major sources of resistance to *D. africanus* in groundnut breeding programmes for the development of *D. africanus*-resistant groundnut cultivars. PC287K5 also showed a lesser degree of resistance and can be used in fields with a lower infestation rate.

THE EFFICACY OF MULTIGUARD PROTECT® (A.I. FURFURAL) AS AN IN-SEASON TREATMENT FOR THE CONTROL OF CERTAIN NEMATODES ON TOMATOES, PEPPERS, CUCURBITS AND TURF UNDER FIELD CONDITIONS IN FLORIDA, USA. **Steyn[1], A., B. Booker[2] and E. Buntting[1].** [1]Illovo Sugar Ltd, South Africa, PO Box 31003, Merebank, 4059, Durban, South Africa; [2]Florida Ag Research, 3001, North Kingsway Road, Thonotosassa, 33592, Florida, USA.

With the phasing out or banning of certain toxic nematicides in the agricultural sector of many countries, the need for production of safer nematicides developed. Illovo Sugar South Africa has developed a number of new non systemic agricultural products derived from sustainable sugar cane resources. The active ingredient of these products is furfural, an aromatic aldehyde, which is naturally found in food crops and is also used in food flavouring. Furfural is a non systemic nematicide and breaks down fairly quick, making it ideal for use in all kinds of farming. The nematicide, Crop Guard®, is registered on multiple crops in South Africa while the nematicide, Multiguard Protect®, is currently registered on turf, ornamentals and non-fruit bearing orchard crops in the USA. A number of efficacy trials on pre-plant application for tomatoes and peppers were executed in the United States of America to determine efficacy of Multiguard Protect® on the control and

suppression of certain plant parasitic nematodes. Results from field trials at Florida Ag Research station in Florida and from Pacific Ag in California, USA, have indicated that a single pre-plant treatment of 9 gal/A can control most nematodes during the early season. More trials are currently being executed on Multiguard Protect® in the USA while registration on food crops is pending.

THE EFFICACY OF MULTIGUARD PROTECT® (A.I. FURFURAL) AS A PRE-PLANT SOIL TREATMENT FOR THE CONTROL OF CERTAIN NEMATODES ON TOMATOES AND PEPPERS UNDER FIELD CONDITIONS IN FLORIDA, USA. **Steyn[1], A., B. Booker[2], O. Cuevas[3] and E. Buntting[1].** [1]Illovo Sugar Ltd, South Africa, PO Box 31003, Merebank, 4059, Durban, South Africa; [2]Florida Ag Research, 3001, North Kingsway Road, Thonotosassa, 33592, Florida, USA; [3]Pacific Ag Research, 1840 Biddle Ranch Road, San Luis Obispo, 93401, California, USA.

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VERIFICATION OF *MELOIDOGYNE INCOGNITA* RACE 2 RESISTANCE IN *AMARANTHUS* AND *CAPSICUM* GENOTYPES IN SOUTH AFRICA. **Steyn[1], W.P., M.S. Daneel[1] and M.M. Slabbert[2].** [1]Agricultural Research Council – Institute for Tropical and Sub Tropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa; [2]Department of Horticulture, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa.

Root-knot nematode resistance identified in crop genotypes in greenhouse studies should be verified for sustainability under natural environmental conditions. Therefore, the *Amaranthus* genotype 'Local 33' and *Capsicum* genotype 'Tobasco', identified in greenhouse studies as resistant to *Meloidogyne. incognita* race 2 together with susceptible *Amaranthus* and *Capsicum* genotypes 'Bosbok Thepe' and 'Paprika' respectively, were subjected to a range of initial nematode inoculum densities (Pi), namely 0 (control), 5 000, 20 000, 30 000 and 50 000 eggs and J2 per seedling at planting in a micro plot study. Reproduction factor values (Rf) were used as main criterion to evaluate for resistance. Results indicated that *Amaranthus* genotype, 'Local 33' displayed Rf-values of ≤ 1 at all inoculation levels. This confirms the resistance of this genotype against *M. incognita* race 2 as seen in the greenhouse trials. *Capsicum* genotype 'Tobasco' did however, not show resistance at all Pi levels used in this study. At inoculation levels of 30 000 and higher, 'Tobasco' exhibited Rf-values >1, indicating susceptibility to this root-knot nematode species. Strong relationships were obtained between the Pi and reproduction factor values for both *Amaranthus* and *Capsicum* genotypes and were described using the Logistic Dose Response Models (LDR) y=a+b/(1+(x/c)^d for both *Amaranthus* genotypes and y=a/(1+(x/b)^c for both *Capsicum* genotypes. Significant R^2 values of 99% and 98% were obtained for *Amaranthus* genotypes 'Bosbok Thepe' and 'Local 33', respectively and 98% and 95% for *Capsicum* genotypes ''Paprika' and 'Tobasco', respectively. The need exists for more frequent and extensive screenings of these vegetable genotypes in order to provide resource-poor producers with better options for improved and sustainable yields.

A NEW SUGARCANE FARMING SYSTEM IMPROVES SOIL HEALTH AND ENHANCES SUPPRESSIVENESS TO PLANT-PARASITIC NEMATODES. **Stirling, G.R.** Biological Crop Protection Pty. Ltd., 3601 Moggill Road, Moggill, Queensland, 4070, Australia.

A multi-disciplinary team of scientists in Australia has shown that a sugarcane farming system based on permanent raised beds, residue retention, a leguminous rotation crop and controlled traffic using GPS guidance improves soil health and consistently increases yield. Soil physical properties improve markedly when beds are widened to accommodate the heavy machinery used to plant, harvest and transport the crop. The introduction of a non-grass rotation crop reduces populations of fungal and nematode pathogens that constrain productivity. A reduction in tillage increases earthworm populations, with consequential effects on macroporosity and water infiltration rates. Improved water capture in periods of low rainfall is another reason for increased yields, while improved percolation through macropores and retention of surface cover protects soils from erosion during intense tropical storms. From a biological perspective, suppressiveness to *Pratylenchus zeae* and *Meloidogyne javanica*, the main nematode pests of sugarcane, is enhanced by components of the new farming system. Populations of plant-parasitic nematodes do not resurge as strongly when tillage is eliminated, while a blanket of crop residue

on the soil surface creates a layer of soil that is amenable to root growth and highly suppressive to *Pratylenchus* (and possibly other root pathogens). Initial results suggest that numerous organisms (including nematophagous fungi and predatory nematodes) are contributing to the suppressiveness observed. Future research aims to determine whether predatory micro-arthropods also contribute regulatory services when compaction is removed through traffic control; and whether *Pasteuria penetrans* and *Pasteuria thornei* play a greater role in suppressing their hosts when soil is no longer tilled.

POTENTIAL OF ENTOMOPATHOGENIC NEMATODES TO CONTROL WOOLLY APPLE APHID (*ERIOSOMA LANIGERUM*). **Stokwe[1,2], N.F. and A.P. Malan[2].** [1]Stellenbosch University, Department of Conservation Ecology and Entomology, Private Bag X1, Matieland, Stellenbosch 7602, South Africa; [2]Agricultural Research Council, ARC Infruitec-Nietvoorbij, Private Bag X 5026, Stellenbosch 7600, South Africa.

Woolly apple aphid, *Eriosoma lanigerum*, is an important pest of apples, occurring throughout the apple-growing regions of the world. It feeds above ground, and on the roots of apple trees, causing hypertrophic gall formation, which affects the transport of water and nutrients. Entomopathogenic nematodes (EPN) of the two families, Steinernematidae and Heterorhabditidae, and their symbiotic bacteria have generated extensive interest as inundative biological control agents of insects. With the development of resistance of woolly apple aphid to certain chemicals, export restrictions, and the inability of parasitoids to control the aphid successfully early in the season, it is important to consider EPN as an alternative biocontrol agent. Apple roots from an orchard, infested with woolly apple aphids, were kept in large plastic containers to maintain a steady supply of insects. Seven EPN species were tested for their pathogenicity against *E. lanigerum,* including*Steinernema khoisanae*, *Steinernema yirgalemense, Steinernema citrae, Steinernema feltiae, Heterorhabditis zealandica, Heterorhabditis bacteriophora*, and *Heterorhabditis safricana*. Laboratory bioassays identified *S. yirgalemense* and *H. zealandica* as being the most virulent against the subterranean stage of the woolly apple aphid, with a mortality rate of 48% and 38%, respectively. Studies on the effect of wooly apple aphid size showed that the last instar is most susceptible to infection by *S. yirgalemense* and *H. zealandica,* whereas smaller instars appear to be too small for nematode penetration and infection. The haemolymph of woolly aphid apple showed an inhibitory effect on the development of the symbiotic bacteria *Xenorhabdus* sp. and *Photorhabdus* sp., which are associated with *S. yirgalemense* and *H. zealandica*, respectively.

THE IMPORTANCE OF *PRATYLENCHUS* SPP. ON THE FIELD TO FORK PRODUCTION CHAIN OF APPLE TREES IN THE WESTERN CAPE, SOUTH AFRICA. **Storey, S.G. and L. van der Walt.** Nemlab, cnr. of R44 and Anyswortelrug Rd, Klapmuts, 7625, South Africa.

Agriculture forms a key component of the Western Cape's economy. The export of fresh fruit is vital to this economy as it brings in much needed foreign currency and ensures job security. The production of pome fruit (apples and pears) is the largest sector of the agricultural fruit production sector. The export of fruit to Europe, China, Russia and Japan provides important foreign exchange. Of the total apple production during the 2011/2012 growing season exports amounted to 45.34%. The field-to-fork chain in apple production must be maximised to ensure the sustainability of the economic well-being of this sector. Nematodes impact heavily on various facets of this chain. *Pratylenchus* spp. play a key role in the demise of nursery trees, apple replant disease and the size of the fruit exported. The impact is already measurable at nursery level, as affected trees have to be held back as the stems are not yet strong enough to sell to producers. When the trees are planted they are more susceptible to apple replant disease. Trees with this disease bear fruit 2-3 years later than uninfected trees and also have lower yields throughout their shortened life span. Once bearing, potassium-uptake is affected by *Pratylenchus,* which is directly linked to fruit size. Export prices are determined by size, thus direct impact on income is expected. GlobalGAP and European retailers have strict regulations regarding minimum residue levels making control strategies complex.

THE EFFECT OF CONSERVATION AGRICULTURE ON NEMATODE POPULATIONS AND SOIL MICROBIAL COMMUNITY DYNAMICS. **Swart[1], A., M. Marais[1], and J. Habig[2].** [1]National Collection of Nematodes, Biosystematics Programme, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa; [2]Soil Microbiology, Soil Microbiology and Plant Pathology Programme, ARC-Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa.

The ARC Zeekoegat experimental farm at Roodeplaat, Gauteng, was selected for a conservation agriculture trial in which several conservation agriculture practices were tested. A multidisciplinary approach was followed and the focus area included soil, and other biological parameters. The key plots examined included six treatments consisting of maize monoculture, maize and soybean rotation, and maize and cowpea intercropping. All these treatments were combined with either low of high fertiliser levels, and either reduced or conventional tillage. Nematodes and soil microbial communities play important roles in nutrient cycling and have been used as indicators of soil health. Population numbers of different nematode trophic groups were compared with each other during the 2010-2013 seasons. In most treatments a decline in the incidence and population numbers of free-living nematodes were noticed from 201-2013. Overall, the reduced tillage treatments show a slightly higher percentage of fungivores than bacterivores, while the conventional tillage plots slightly favoured bacterivores over fungivores. The population numbers of the free-living nematodes in conventional tilled plots were generally higher than

those in reduced tillage plots. Of concern is the very high population numbers of herbivores compared to free-living nematodes in all the treatments. As mentioned previously, soil microbial community diversity and activity play an integral part in maintaining ecosystem function. Carbon source utilisation profiles indicated differences between different rotation systems and tillage practices. Overall enzyme activity was higher under reduced tillage practices, compares to conventional tillage practices. This presentation will combine all findings on nematodes as well as soil microbial data.

FORMULATING A NEW VISION AND DIRECTING CHANGE IN NEMATOLOGY EDUCATION IN SUB-SAHARAN AFRICA TO REFLECT A SCIENCE OF THE 21ST CENTURY. **Talwana, H.** Department of Agricultural Production College of Agricultural and Environmental Sciences Makerere University. P.O. Box 7062 Kampala Uganda.

Nematology is a fascinating cross-disciplinary science. However, in sub-Saharan Africa, Nematology is at crossroads or lost completely due to lack of a critical mass of nematologists; nematologists rarely working full time on the nematode problems; trained nematologists rarely remaining in the discipline, and most importantly, higher learning institutions in Africa are not teaching Nematology at all and/or where offered, it is taught as part of Plant Pathology or Entomology. Consequently, there is a general lack of awareness of even the existence of nematodes by the public – policy makers, farmers, formative education teachers, etc. On the other hand, there is growing apathy in today's students of Biological Sciences about Nematology, which in most cases is a result of the content and style of delivery by trainers. With little practice in laboratories and in the field due to lack of appropriate equipment, limited local contemporary examples due to low research output, and limited inference to modern day biosciences trends and achievements, there is a negative perception of the applicability and transferability of Nematology to 'real-world' situations and employment opportunities. Thus, even the most interested students are turned away. This calls for change in the way the content of Nematology is delivered and the onus is on the trainers to inspire and entice a new generation into Nematology as an ultimate career path. The opportunity that the convergence of advances in Nematology and emerging (bio)-technologies provides should particularly be harnessed to deliver Nematology as a model biological science.

TOWARDS A REFERENCE TRANSCRIPTOME OF *ANGIOSTRONGYLUS CANTONENSIS*. **Tang[1,2], P., T-W. Chen[2], C-C. Lee[2], K-Y. Chen[1], S-H. Chang[1], J.-W. Shin[3] and L-C. Wang[1]**. [1]Department of Parasitology, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan; [2]Bioinformatics Center, Chang Gung University, Taoyuan 333, Taiwan; [3]Department of Parasitology, National Cheng Kung University, Tainan 701, Taiwan.

Although *Angiostrongylus cantonensis* is the most common cause of eosinophilic meningitis in Southeast Asia and the Pacific Basin, relatively little is known about this neglected parasite at molecular and genomic level. In order to understand the regulation of development, adaptations to different microenvironments, and the interaction between the host, the Chang Gung University initiated an *A. cantonensis* systems biology project that aimed to study the genome, transcriptome and proteome of this parasite. For the past two years, we have performed pilot studies on gene, miRNA and protein expression profiling. In the present investigation, we focus on the generation of a reference transcriptome of *A. cantonensis*. More than 300 million paired-end reads (28Gbp) were generated from the Illumina Hiseq sequencing platform from different developing stages. Assembling of these reads resulted in more than 44,000 contigs with a N50 of 1,023bp. BLAST search revealed that 31.5% of these putative transcripts showed significant sequence homology to known protein or protein motifs/domains. Functional classifications indicated that most of these putative transcripts are involved in embryo development or egg hatching, larval development, regulation of growth rate and locomotion. The protein expression levels of these transcripts were verified by using two-dimensional electrophoresis and LC/MS/MS. The reference transcriptome established in the present study will provide a foundation for the ongoing whole genome sequencing project and comparative transcriptomic/proteomic analyses.

COMPARISON OF EXTRACTION METHODS FOR EXTRACTION OF MIGRATORY ENDOPARASITIC NEMATODES FROM ROOTS. **Tangchitsomkid[1], N., T. Chanmalee[2] and M. Hodda[3].** [1]Biotechnology Research and Development Office, Department of Agriculture, Chatuchak 10900 Bangkok, Thailand; [2]Centre for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand; [3]CSIRO Ecosystem Sciences, GPO Box 1700 Canberra, ACT 2601, Australia.

This poster reports tests of the efficiency of ultrasonic methods in extracting the nematode, *Radopholus similis*, from the roots of the aquatic plant, *Anubias* sp. The first test compared different extraction methods. In water, with a duration of 10 minutes and frequency of 50/60 KHz, ultrasonic extraction recovered a little more than three times the number of nematodes as a mist chamber running for 48 hours (a mean of 26.4 nematodes versus 8.2 nematodes). The second test compared the extraction rate and damage to both plant roots and extracted nematodes at different durations of sonication. Duration times of 5, 10, 20, 40 and 60 minutes were investigated. Minimal root damage and nearly maximal extraction rates were obtained after sonication for 20 minutes, allowing for the plants to be replanted and the nematodes to be identified easily. The ultrasonic technique can require less time and effort for root preparation than most other techniques for migratory endoparasites, yet has a similar or superior extraction efficiency per sample, thus allowing more samples to be processed more cheaply.

A NEW NEMATODE EXTRACTION KIT FOR FIELD WORK USING ULTRASONIC TECHNOLOGY. **Tangchitsomkid[1], N., T. Chanmalee[2] and M. Hodda[3].** [1]Biotechnology Research and Development Office, Department of Agriculture, Chatuchak 10900 Bangkok, Thailand; [2]Centre for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140 Thailand; [3]CSIRO Ecosystem Sciences, GPO Box 1700 Canberra, ACT 2601 Australia.

An efficient, easy-to-use extraction kit was developed for extracting root migratory endoparasites—such as *Hirshmanniella*, *Radopholus* and *Pratylenchus*—in the field. Ultrasonic waves in a water medium at 40 KHz causes alternate compression and decompression inducing nematodes to leave the roots and enter the water within 20 minutes. Consolidated into a single unit for fieldwork, the kit is a cube of about 20 cm size, made of stainless steel for durability. It has a water chamber of 9 cm wide by 15 cm long by 6 cm deep, with a single-frequency ultrasonic wave generator connected to the bottom. It has an automatic timer and operates at 220 volts. It is also equipped with a mini microscope fixed at 50 x magnification 10 cm long. The kit comes with a carry bag into which it fits for convenient transportation. The kit can be used by farmers to detect nematode outbreaks in cultivated fields, especially in the roots of plants for export (such as aquatic plants, *Philodendron* species, orchids, *Anthurium* species, *Zamioculcas zamiifolia* and other ornamental plants). The kit is also suitable for quarantine inspection and certification to enable rapid examination and detection of nematodes, thus saving both time and expense. The kit was tested and compared with other methods available, with extraction efficiencies equal to, or better than, the sucrose-centrifugation and mist-chamber techniques. However, we consider it more practical and convenient than either. The kit and its basic technology was invented in Thailand and is now patent pending (Application no.1101000659).

ULTRASONIC EXTRACTION OF PLANT-PARASITIC NEMATODES FROM PLANT ROOTS. **Tangchitsomkid[1], N., T. Chanmalee[2] and M. Hodda[3].** [1]Plant Protection Research and Development Office Department of Agriculture, 50 Phaholyothin Road, Chatuchak, Bangkok, 10900 Thailand; [2]Kasetsart University, Kamphaeng Saen Campus, 1 Malaiman Rd, Kamphaeng Saen District, Nakhon Pathom 73140, Thailand; [3]CSIRO Ecosystem Sciences, GPO Box 1700, Canberra ACT 2601 Australia.

Extraction of nematodes from plant roots is a very time- and labour-intensive process, so new methods offering advantages for either speeding or lessening workloads are highly desired, but must be tested before adoption. A novel method using ultrasonic waves is fast, simple and non-destructive to roots. The method was tested and optimized for extraction of burrowing nematodes (*Radopholus similis*) from the roots of the aquatic plant *Anubias* sp. in water. Different durations of treatment and ultrasonic frequencies were tested, and the number of nematodes and plant damage recorded. The highest extraction efficiencies per sample were obtained after 40 minutes or longer sonication at a frequency of 40 KHz, but the condition of the extracted nematodes was poor and not suitable for identification.. Considering efficiency as number of nematodes extracted per unit time, optimal extraction was obtained with a duration of 20 minutes. With this duration there was little observable damage to the nematodes (allowing use in experiments or inoculation trials), or the plants (so that successful replanting is possible). The ultrasonic method was compared with other extraction techniques, and was much more efficient than root submersion, shaking or mist chamber techniques, but a little less efficient per sample than the sucrose-centrifuge method. Ultrasonic extraction requires less time and effort for root preparation than other methods, so it can be a lower cost method for routine use. Furthermore, it can be incorporated into a simple, cheap, mass-manufactured, portable and automated kit, which can be made widely available.

DIFFERENT CITRUS JUICES AND OILS AS SOIL AMENDMENTS USED IN CONTROL OF *MELOIDOGYNE INCOGNITA* ON TOMATO. **Tefu, G., M.S. Daneel, W.P Steyn, C. Arries and T. Selabela.** Agricultural Research Council-Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa.

Glasshouse experiments were conducted to evaluate the effect of soil amendments with extracted juices and oils of different citrus plants (grapefruit, lemon, lime, naartjie and sweet orange) for the control of *Meloidogyne incognita*. Soil was inoculated with 3000 root-knot nematode eggs after which the treatments were applied and 3 days later tomato seedlings were transplanted in each bag. The height of the tomato plants was measured bi-weekly and root weight, plant mass, root system, gall index and nematode numbers were determined at 56 days after transplanting. The organic amendment consisting of orange juice gave persistently the best improvement in growth as well as best reduction of nematodes. Citrus oils consistently performed weaker than the juice and a clear difference was observed between the different juices with orange followed by naartjie, grapefruit, lime and lemon. Lime and lemon were similar to the control. Further studies are required to confirm the potential of these organic amendments in the control of *Meloidogyne incognita* in the field.

THE EFFECT OF TEMPERATURE AND TIME ON THE POPULATION DYNAMICS OF *MELOIDOGYNE. CHITWOODI* IN POTATO TUBERS DURING STORAGE. **Teklu, M.G., T.H. Been, C.H. Schomaker and J.E. Beniers.** Plant Research International, Wageningen University and Research Centre, Plant Sciences Group, 6700 AA, Wageningen, The Netherlands.

This experiment was carried out to assess the population dynamics of *Meloidogyne chitwoodi* in tubers under different storage conditions used in The Netherlands. Infected tubers were produced by growing the susceptible cv. Désirée in 100, 10 kg pots at a density (*Pi*) of = 12 J2 g dry soil^{-1}. Infected tubers of all pots were combined and sorted into classes based on their tuber-knot index. Thirteen batches of infected tubers were composed of equal amounts of tubers from each index. The infected tubers were stored at 98% relative humidity at temperatures of 4, 8 and 12 °C and nematode numbers were assessed after 0, 2, 4, 6 and 8 months storage time. After hardening, peeling and mixing of the skins, replications were placed in the spray-mist chamber for 7 weeks to extract the juveniles. The logistic model was fitted to the cumulative hatch to follow the hatching process of *M. chitwoodi* from the peel and the estimated maximum cumulative hatch was used to evaluate the treatments. Both storage temperature and time influenced the population dynamics of *M. chitwoodi* in potato tubers. Storage temperatures of 4 and 8° C reduced populations of *M. chitwoodi* up to 96% and 89% after 8 months of storage. However, at 12°C the population density remained at the same level over time. Recovered juveniles of *M. chitwoodi* from tubers after 6 and 8 months of storage, at all three temperatures, proved to be still infective on the susceptible cv. Désirée.

TESTING FOR TUBER RESISTANCE AGAINST *M. CHITWOODI* IN PARTIALLY RESISTANT POTATO GENOTYPES WITH *AVENA STRIGOSA*. **Teklu[1], M.G., T.H. Been[1], C.H. Schomaker[1], L.P.G. Molendijk[2], T.G. van Beers[2] and J. E. Beniers[1].** [1]Plant Research International, Wageningen University and Research Centre, Plant Sciences Group, 6700 AA, Wageningen, The Netherlands; [2]Applied Plant Research International, 8219 PH Lelystad, The Netherlands.

A pilot experiment was carried out using two partially resistant genotypes AR04-4096, 2011M1 and the control cv. Désirée to test whether these genotypes possess both tuber and root resistance. Four plants of *Avena strigosa*, and one potato plant, consisting of a single sprout, were sown in 10 kg pots. The resistant genotypes were tested with and without *A. strigosa* and cv. Désirée was used as a susceptible control. Pots were inoculated with a moderate nematode density: 16 J2 g dry soil^{-1}. Final population densities were estimated from the soil, roots and tuber peel. The roots and peel were incubated in a spray-mist chamber for 4 and 7 weeks, respectively. The numbers of nematodes extracted from soil, roots and tubers in the pots with *A. strigosa* and the resistant genotypes increased compared to those without *A. strigosa*. The relative susceptibilities (%RS) of the genotypes AR04-4096 and 2011M1, without *A. strigosa,* were 0.18 and 0.52, respectively, which is consistent with previous results. The presence of *A. strigosa* increased the relative combined host status (RH) for AR04-4096 compared to Désirée till 25%. *Avena strigosa* slightly increased the estimated relative susceptibility of tubers of the tested genotypes: $\%RS_T$ of AR04-4096 increased from 0 to 0.04%; that of 2011M1 from 0.56 to 2.96%. The negative effect of *A. strigosa* on the percentage of nematode-free tubers of AR04-4096 was negligible, but nematode-free tubers decreased from 84% to 21% in 2011M1. It can be concluded that the tested partially resistant genotypes possessed partial resistance in both roots and tubers.

SURVEY FOR THE SOYBEAN CYST NEMATODE IN MANITOBA, CANADA. **Tenuta[1], M., M. Madani[1] and D. Lange[2].** [1]Department of Soil Science, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada; [2]Manitoba Agriculture, Food and Rural Development, Altona, MB, R0B 0B0, Canada.

We report the findings of a survey conducted in 2012/2013 of soybean fields for the soybean cyst nematode (*Heterodera glycines*) in Manitoba. In total, 48 fields were sampled with fields prone to infestation selected. Fields were sectioned into areas that could be responsible for pest introduction. In total, 282 composite soil samples were analyzed from 48 fields. Overall, 37 of the samples from 22 fields had nematode cysts. Sixty cysts were recovered. Most cysts were heavily damaged but 26 were sufficient for examination. Of these, 23 had circumfenestrate vulval cone structures. Three cysts had bifenestrate vulval cone structures characteristic of the genus *Heterodera*. Not all the cysts contained nematodes either as juveniles or eggs. Most were empty except for 15 circumfenestrate and one bifenestrate cysts. These 16 cysts were then followed up with molecular analyses by ITS sequencing, three SCN species-specific PCR approaches (Ou et al. 1993, Subbotin et al. 2001, Madani et al. 2013), and in one case, by 28s rDNA gene sequencing. ITS sequencing was possible for 14 of the circumfenestrate cysts. These best matched the sequence database for the cyst nematode, *Cactodera*. Only one of the bifenestrate cysts yielded DNA for analysis and its identification was ambiguous between *H, glycines* and *Cactodera*. *Cactodera* is not a pest of soybean or other crop plants in Manitoba. These cyst nematodes are likely naturally present, living on weeds and grasses in the sampled fields. The findings of the current study mean *H. glycines* has still not been detected in Manitoba.

DETERMINING THE SPECIES OF STEM NEMATODE ON GRAIN PEA AND CREEPING THISTLE IN THE CANADIAN PRAIRIE PROVINCES. **Tenuta[1], M., M. Madani[1], S. Briar[1], and S.A. Subbotin[2].** [1]Department of Soil Science, University of Manitoba, Winnipeg, MB, R3T 2N2 Canada; [2]Plant Pest Diagnostic Center, California Department of Food and Agriculture, Sacramento, CA, 95832-1448, USA.

The stem nematode (*Ditylenchus dipsaci*) was first described as a parasite of creeping thistle (*Cirsium arvense*) in the Canadian Prairied in 1979. Recently, *Ditylenchus* on creeping thistle in Russia was shown to be *Ditylenchus weischeri*, which is not an agricultural pest. This study examined if *D. weischeri* and not *D. dipsaci* is present in yellow pea (*Pisum sativum*) grain harvest samples in 2009 and 2010 and from creeping thistle in Saskatchewan, Alberta and Manitoba. Samples from 538

fields (mainly yellow pea) were provided by 151 pea growers. Two percent of the samples were *Ditylenchus* positive. Numbers ranged from 4-1500 nematodes kg^{-1} pea and were related to presence of creeping thistle seeds. Positive samples occurred in 2009 but not 2010 and were from different geographical locations. The nematode was morphologically dissimilar to *D. dipsaci*. Creeping thistle plants were then collected from yellow pea fields in the Provinces. All plants from Saskatchewan and Manitoba were infested with *Ditylenchus*. The ITS-rRNA regions ITS1, 5.8S and ITS2 was amplified using the TW 81 and AB 28 primer set. For some samples the ITS1 and ITS2 were separately amplified using TW81 and Dit58SR or Dit58SF and AB28 primers, respectively. A fragment of *hsp90* was also obtained with U831 and L1110 primers. PCR-ITS products were subjected to RFLP using *Bsh*1236I, *Hinf*I, *Msp*I, *Rsa*I and *Taq*I. The results match those published for *D. weischeri*. The results indicate the stem nematode found in yellow pea grain is *D. weischeri* and that creeping thistle seeds in grain its source.

A TRANSCRIPTOMIC STUDY OF COLD TOLERANCE MECHANISMS IN THE ANTARCTIC NEMATODE *PANAGROLAIMUS DAVIDI*. **Thorne[1], M., M. Clark[1], C. Marshall[2] and D. Wharton[3].** [1]British Antarctic Survey, Cambridge, UK; [2]Department of Biochemistry, University of Otago; [3]Department of Zoology, University of Otago, PO Box 56, Dunedin, NZ.

Panagrolaimus davidi is an Antarctic nematode from coastal terrestrial habitats of the McMurdo Sound region, where it survives both desiccation and freezing. It can survive complete water loss (anhydrobiosis) and is the only organism known to survive intracellular ice formation. It has several cold tolerance strategies, including; freeze avoidance, cryoprotective dehydration, freezing tolerance and anhydrobiosis. We have conducted a study to determine gene expression during cold acclimation (+5°C), freezing at -1°C (which induces cryoprotective dehydration), freezing at -10°C (which produces intracellular freezing) and during recovery from freezing. After exposure to these conditions the total RNA was extracted and sequenced on on an Illumina HiSeq 2000 resulting in 286,447,212 paired-end reads of >100bp, after quality control, and a transcriptome constructed from the data from all the treatments. To determine upregulation in specific treatments, expression levels were compared by fold difference and a ratio test of mapping counts. I will present here the data on acclimation and cryoprotective dehydration. Most prominent amongst genes upregulated during acclimation are those associated with cuticle synthesis and embryonic development. This is surprising since there is no population growth in cultures below +6.8°C. Maintenance of cuticle structure may be important for preventing inoculative freezing during cryoprotective dehydration. Genes upregulated during freezing at -1°C, and recovery from freezing, include those associated with enzymes and transport, signalling and control, and lipid metabolism. Genes associated with stress responses are present in the transcriptome, suggesting that the main survival mechanisms are expressed constitutively.

SEASONAL VARIATIONS OF NEMATODE ASSEMBLAGES AND DIVERSITY IN VERTISOLS, CAMBISOLS AND ARENOSOLS SOIL GROUPS IN KENYA. **Thuo[1], A.K., J.W. Kimenju[1], G.M. Kariuki[2], G.N. Karuku[1], P.K. Wendot[2] and H. Melakeberhan[3].** [1]Department of Plant Science and Crop Protection, University of Nairobi, P.O Box 30197, G.P.O, Kenya; [2]Kenyatta University, Department of Agricultural Science and Technology, P.O. Box 43844-00100 Nairobi, Kenya; [3]Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University (MSU), East Lansing, Michigan 48824, USA.

Soil health assessment has been based on narrow disciplinary approaches that overlook the multiple and interacting biological processes that are the basis of sustainable crop productivity. This study was carried out to determine the influence of seasonal variations in nematode assemblages in different soil groups, sites and disturbance levels as an indicator of soil health. Sampling was done in areas characterized by small scale subsistence agriculture in Kenyan northern and southern sites over three distinct seasons. The sampling points included disturbed (tilled) and the adjoining undisturbed (untilled) soils within three soil groups, namely vertisols, cambisols and arenosols. Nematodes were extracted using the centrifugal-floatation technique, enumerated and assigned to their respective trophic groups. Total nematode abundance in the three seasons varied significantly with a mean of 68.4, 93.1 and 51.6 nematodes in 200 cm^3 of soil in season 1, season 2 and season 3, respectively. Nematodes abundance in the undisturbed soils was higher (97.74) compared to the disturbed soils (62.08 nematodes per 200 cm^3. Mean abundance of nematodes was highest in Ccmbisols. In addition, nematode abundances, in all trophic levels across the three seasons, were significantly higher in the northern compared to the southern sites. Bacterivores (28%) had the highest incidence followed by herbivores (27%) and fungivores (21%) while omnivores (11%) had the least. The study demonstrated that nematode communities do vary in the different soil groups and between seasons, suggesting that they can be utilized as viable bio-indicators of soil health.

EFFECT OF TILLAGE AND FUMIGATION ON *PASTEURIA PENETRANS*. **Timper, P.** USDA ARS, P.O. Box 748, Tifton, Georgia 31794, USA.

The endospore-forming bacterium *Pasteuria penetrans* is a parasite of *Meloidogyne* spp. In this study, the effect of tillage and the fumigant 1,3-dichloropropene (1,3-D) on numbers of *P. penetrans* and suppression of *M. incognita* was evaluated from 2011-2013. A split-plot experiment was established in a field infested with both the nematode and bacterium. The main

plots were tillage (strip vs conventional) with five fumigation treatments in 4-year sequences within each tillage treatment (C=no fumigant; F=fumigant): C-C-C-C, F-F-F-F, F-C-F-C, F-F-C-F, and C-F-F-C. Abundance of *P. penetrans* endospores was determined in the spring after tillage/fumigation and average root-gall indices on cotton plants were determined at the end of the season. Additionally, a greenhouse bioassay was conducted to determine reproductive potential of *M. incognita* in soil collected from each plot at planting time. Abundance of *P. penetrans* was greater in C-C-C-C than in most of the fumigated treatments and greater in strip tillage than in conventional tillage in 2012 and 2013. Nevertheless, the reproductive potential of *M. incognita* in the greenhouse was not affected by the fumigation or tillage treatments. Endospore abundance was greater in 2012 than in the other two years and was negatively correlated with reproductive potential only in that year. In the no-fumigation treatment, endospore abundance was negatively correlated with gall index indicating that *P. penetrans* was contributing to nematode suppression. Tillage mixes and redistributes soil and may move endospores away from the planting furrow. Although1,3-D had no direct effect on *P. penetrans*, it reduced the number of available hosts leading to lower endospore numbers.

DISTINCTION OF '*TRYPOPHLOEI*' GROUP - A NEW TYPOLOGICAL GROUP AMONG WOOD NEMATODES OF THE GENUS *BURSAPHELENCHUS* FUCHS, 1937. **Tomalak, M., A. Filipak, T. Malewski and J. Pomorski.** Department of Biological Pest Control and Quarantine, Institute of Plant Protection - National Research Institute, Władysława Wegorka 20, 60-318 Poznan, Poland.

The nearly 90 known species of the genus *Bursaphelenchus* Fuchs, 1937 have been divided into 13 typological groups. *Bursaphelenchus xylophilus* is considered the most dangerous pest of pine trees worldwide and the *xylophilus* group seems to be the most extensively studied. At present it contains 15 species characterized by a relatively uniform set of distinctive morphological characters, such as the morphology of male spicules, extended anterior vulval lip, lateral fields with four incisures, and number and arrangement of male caudal papillae. Recent inclusion of new species gradually widened the morphological variation within this group. Additional data on the nematode genetics and ecology clearly indicate that species presently assigned to the *xylophilus* group may represent two closely related phylogenetic groups which also differ in their ecological relations with vector insects. We therefore suggest the establishment of a new typological group designated as *trypophloei*. This group would comprise species located in a separate but adjacent to main *xylophilus* group clade, and at the present it would include *Bursaphelenchus trypophloei*, *Bursaphelenchus masseyi*, *Bursaphelenchus tokyoensis*, *Bursaphelenchus fagi*, and two other species, presently under description, which are located in the same phylogenetic clade. These nematodes are morphologically most similar to the *xylophilus* group with some variation in spicule morphology. They all are associated with bark beetles (Curculionidae: Scolytinae). In contrast, all species of the classical *xylophilus* group with known insect association use long horned beetles (Cerambycidae) as vectors.

MOLECULAR CHARACTERIZATION OF *HETERODERA* SPP AND *PRATYLENCHUS* SPP. FROM WINTER WHEAT IN THE EAST ANATOLIAN REGION OF TURKEY. **Toktay[1], H., M. Imren[2], A. Ocal[3], E.B. Kasapoglu[4], A.A. Dababad[5] and H. Elekcioglu[4].** [1]University of Nigde, Faculty of Agricultural Sciences and Technologies, Nigde, Turkey; [2] Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection Bolu, Turkey; [3]West Mediterranean Agricultural Research İnstitute. Antalya, Turkey; [4]University of Çukurova, Faculty of Agriculture, Department of Plant Protection, 01360, Adana Turkey, Adana, Turkey; [5]CIMMYT (International Maize and Wheat Improvement Centre), Wheat Improvement Program, Ankara, Turkey.

Turkey is one of the top ten wheat producing countries in the world, with the East Anatoloian region being one of the most important winter wheat areas in the country. Plant parasitic nematodes are one of most important biotic agents that cause economical yield loss in wheat growing regions. Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are some of the most important pest of wheat worldwide. Limited knowledge of the nematode pests of wheat exists in Turkey. This study aims to characterize *Heterodera* and *Pratylenchus* species in this region by using morphological and molecular tools and to establish phylogenetic relationships between the populations. Forty-three *Heterodera* and thirty *Pratylenchus* populations were identified using sequence analysis of the Internal Transcribed Spacer region of the ribosomal DNA (ITS-rDNA), phylogenetic analysis using ITS-rDNA sequences, and morphological methods. Forty poulations were identified as *Heterodera filipjevi* and five populations as *H. Latipons*. *Pratylenchus* species identified were *P. thornei, P.penetrans and P. neglectus*.

DEVELOPMENT OF QPCR ASSAYS FOR QUANTITATIVE DETECTION OF *HETERODERA AVENAE, H. FILIPJEVI* AND *H. LATIPONS*. **Toumi[1,2], F., L. Waeyenberge[1,2], N. Viaene[1,3], A. Dababat[4], J.M. Nicol[4], F.C. Ogbonnaya[5] and M. Moens[1,2].** [1]Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium; [2] Faculty of Bio engineering, Ghent University, Coupure links 653, 9000 Ghent, Belgium; [3]Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium; [4]International Maize and Wheat Improvement Centre (CIMMYT), Ankara, Turkey; [5]International Center for Agricultural Research in the Dry Areas, PO Box 5466, Aleppo, Syria.

Heterodera avenae, *H. filipjevi* and *H. latipons* are considered of major economic significance in cereals. Precise identification and quantification of these nematodes are necessary to develop effective integrated pest control. We report the results of a qPCR assay that we developed for the quick detection and quantification of the three species. Three qPCR primer sets comprising two primers and a probe, were designed and optimized. All developed assays were able to detect a single second-stage juvenile (J2). Their specificity was confirmed by the lack of amplification of 13 other *Heterodera* species. A qPCR using DNA extracted from 106, 114 and 114 J2 + eggs of *H. avenae*, *H. filipjevi* and *H. latipons* resulted in steady Ct-values (Ct = 22.33 ± 0.1, Ct = 21.83 ± 0.05 and Ct= 18.6 ± 0.12, respectively). Dilution series of DNA extracted from known numbers of J2 + eggs of the three species were made. The assays resulted in a standard curve showing a highly significant linearity between the Ct-values and the dilution rates (R^2 = 0.99; slope = -3.05, R^2 = 0.99; slope = -3.4 and R^2 = 0.99; slope = -3.5 for *H. avenae*, *H. filipjevi* and *H. latipons* respectively). The three qPCR assays provide a sensitive and valid tool for the rapid detection and quantification of the three species whether they occur alone or in mixtures with other species. Unfortunately, the assay for the detection and quantification of *H. filipjevi* was not successful for all *H. filipjevi* populations, which could be contributed to DNA polymorphism.

DEVELOPMENT OF REAL-TIME PCR PRIMERS FOR MAJOR PLANT PARASITIC NEMATODES IN RICE FIELDS IN MYANMAR. **Toyota[1], K. and Y.Y. Min[2].** [1]Tokyo University of Agriculture and Technology, Japan; [2]Yezin Agricultural University, Japan.

Myanmar has 100% self-sufficiency rate of cereals and is the seventh largest rice producing country. Rice yield increased from 1997 to 2007 in major rice producing countries, while decreasing in Myanmar, suggesting the presence of obstacles against rice production. The objectives of this study were to identify major plant-parasitic nematodes in rice fields in Myanmar and to estimate possible damage to yield. Soil was collected from 26 paddy fields in different regions in Myanmar and nematodes were extracted with the Baermann-funnel method. The D2/D3 regions of 28S rDNA was amplified from individual plant-parasitic nematodes and sequenced. The rice root nematode *Hirschmanniella oryzae* and the root-knot nematode *Meloidogyne graminicola* were identified. In addition, the rice stem nematode (ufra) *Ditylenchus angustus* was also detected in some fields. Then, real-time PCR primers were designed to quantify the species in soil. *H. oryzae* and *M. graminocola* were detected in 23 and 3 fields, respectively. Nematicide was applied into two paddy fields infested with *H. oryzae* and *D. angustus* to estimate potential damage by nematodes. The results showed 20% to 70% increase in the grain yields by nematicide application. This study enabled the quantification of the major plant parasitic nematodes in Myanmar, and suggested that nematodes cause a significant reduction in rice yields in certain regions of Myanmar.

TRANSGENIC NEMATODE RESISTANCE FOR AFRICAN FOOD SECURITY: NEMATODE RESISTANT BANANAS AS A CASE STUDY. **Tripathi[1], L., H. Roderick[2], A. Babirye[1] and H.J. Atkinson[2].** [1]International Institute of Tropical Agriculture, PO Box 7878, Kampala, Uganda; [2]Centre for Plant Sciences, University of Leeds, Leeds LS2 9JT, UK.

Transgenic crops can support future food security when they overcome agronomic challenges refractory to other approaches. Examples include nematode resistant plantains and cooking bananas for Africa that prevent current losses of up to 70%. The crop's sterility enhances both the biosafety of this biotechnological intervention and its rate of progress relative to that of classical plant breeding. Our efficient genetic transformation system for plantain produced over 200 transgenic lines expressing a maize cystatin that limits nematode feeding success and/or a synthetic peptide that suppresses root invasion. Nematode challenge of plantlets in the glasshouse identified lines with promising levels of resistance. Twelve of these lines were grown for an authorised, contained field trial in Uganda. Nematodes from infected banana roots were added to the pot soil of selected lines before transplanting to the field in a randomised block design. Subsequent plant growth was measured non-destructively using digital hemispherical photography to calculate the leaf area index (LAI). *Radopholus similis* dominated the nematode population at 7 months post-planting and caused severe necrosis to control plant roots. Several transgenic lines had significantly larger LAI values and less root necrosis than the control non-transgenic plants. A significant reduction in recovered nematodes for three lines corresponded to 89-98% resistance. The experiment is continuing for a further 2-3 harvests. Plantains and cooking bananas are a vital food for about 100 million Africans. The future challenges for this not-for-profit public research are to ensure biosafe uptake for plantain and then application to other subsistence crops that suffer appreciable nematode damage.

INTEGRATED MANAGEMENT OF ROOT-KNOT NEMATODES ON TOMATO WITH ARBUSCULAR MYCORRHIZAL FUNGI, *PAECILOMYCES LILACINUS* AND *MUCUNA* SPP. SOIL AMENDMENTS. **Udo[1], I.A., M.I. Uguru[2] and R.O. Ogbuji[2].** [1]Department of Crop Science, University of Calabar, PMB 1115, Calabar, Nigeria; [2]Department of Crop Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

A greenhouse trial was conducted to evaluate the efficacy of five arbuscular mycorrhizal fungi (AMF), five *Mucuna* spp. soil amendments and bioformulated *Paecilomyces lilacinus* singly and combined in the management of *Meloidogyne incognita* race 1 on tomato. The treatments involved the combination of five AMF (*Glomus etunicatum*, *Glomus mosseae, Glomus clarum, Glomus deserticola and Gigaspora gigantea*), five *Mucuna spp* (*Mucuna pruriens utilis, Mucuna ghana,*

Mucuna cochichinensis, *Mucuna jaspaeda* and *Mucuna pruriens* IR2) and inoculation with bioformulated *P. lilacinus*. For the respective factors, pots without *Mucuna* amendment, not inoculated with *P. lilacinus* or arbuscular mycorrhizal fungi served as the controls. The tomato seedlings were inoculated with 5000 eggs of *M. incognita* per plant. The plants were grown to full maturity. The *Mucuna* spp. differed in their mineral contents. *M. ghana* and *M. jaspaeda* had the highest nitrogen (N) content (>4%) and the lowestt C : N ratio (7-9). The combination of the three control agents significantly ($P<0.05$) suppressed root galling and egg production more than single applications. *G. mosseae* when combined with all the *Mucuna spp* and with bionematicide application had plants with the lowest gall index (GI=2.0) and the lowest egg masses. Shoot growth, root and shoot dry matter accumulation and fresh fruit yield were enhanced with the combination of the three factors compared with the control. *M. jaspaeda* and *M. ghana* when combined with most of the AMF species and the bionematicides were the most efficient *Mucuna* spp. in gall inhibition, growth and yield enhancement.

IDENTIFYING NOVEL ROOT-KNOT NEMATODE GENES AND CHARACTERIZING THEIR POTENTIAL ROLES IN PLANT SUSCEPTIBILITY. **Utermark, J. and C.A. Gleason.** Georg-August-Universität Göttingen, Schwann-Schleiden-Forschungszentrum, D-37077 Göttingen, Germany.

Some of the most economically damaging plant-parasitic nematodes belong to the group of root-knot nematodes (*Meloidogyne* spp). These nematodes have an extremely broad host range and can be serious pests on major food crops. In order to establish successful plant infections, the nematode presumably secretes a large repertoire of effectors that can suppress plant defences and/or alter host cell physiology to form the successful feeding cells. Using the known root-knot nematode secretome and genome(s), we identified several novel proteins from the nematode and are currently undertaking a multi-step approach that will help us prioritize these candidates for further mechanistic investigations. In one approach, we are utilizing a novel effector screen in which nematode genes are heterologously expressed in the bacterial pathogen *Pseudomonas syringae*. In addition, we are following the gene expression profiles in various nematode life-stages to further narrow our candidate list. Following this pipeline for new effector discovery, we are now in the early stages of characterizing a select list of root-knot nematode genes and their possible roles in compatible interaction with *Arabidopsis thaliana*. One of our current protein candidates interacts with a plant protein involved in vesicular transport, highlighting the possibility that the nematode may be manipulating intercellular trafficking, a process linked to plant immunity. Thus, by using the genetic and genomic resources available for *Arabidopsis*, we hope to further our understanding of the nematode's relationship with the host plant.

ENVIRONMENTAL FATE OF 1, 3-DICHLOROPROPENE AND EVALUATION OF GROUNDWATER PROTECTION. **van Wesenbeeck[1], I., S. Knowles[2], and K. Racke[1]**. [1]Dow AgroSciences, 9330 Zionsville Road, Indianapolis, Indiana, USA; [2]Dow AgroSciences, 3 Milton Park, Abingdon, UK.

Most nematicides exhibit some degree of mobility in the soil environment, and it is important to carefully evaluate the potential for leaching and ground water contamination under field use conditions. The soil fumigant 1,3-dichloropropene (1,3-D) is rapidly degraded in soil, but high application rates, high water solubility, and low soil sorption indicate leaching mobility should be considered for both parent and degradates. Although laboratory and lysimeter studies provide information on transformation and partitioning behaviour, it is important to evaluate mobility on a landscape scale to account for the complexity and heterogeneity of soil water flow and aquifer recharge. Retrospective surveys of ground water for 1,3-D and degradates were conducted in major 1,3-D use areas. An initial survey of wells at sites in 5 vulnerable regions in the US yielded only a single, transient detection of 1,3-D during 3 years of monitoring. A national survey of more than 500 drinking water wells in vulnerable soils in high 1, 3-D use areas in the US revealed only 4 trace level detections of 1,3-D in nearly 2000 samples and a small number of degradate detections. In Europe, monitoring of wells in 5 regions with high historical 1, 3-D use reported just two low-level detections of a degradate in more than 5000 samples. The overall conclusion drawn from the large amount of 1,3-D ground water monitoring data is that, due to its rapid soil degradation and high vapor pressure, 1,3-D and its metabolites are not expected to be found in drinking water sources when applied according to good agricultural practices.

PATHOLOGICAL REACTIONS TO HOST PLANTS OF NINE *HETERODERA SCHACHTII* (SCHMIDT 1871) POPULATIONS IN THE WESTERN CAPE. **van Zyl, J.** Department of Agriculture, Western Cape, Private Bag X1, Elsenburg 7607, South Africa.

Population variability and genetic diversity exists within nematode species. The aim of this study was to determine whether pathological differences as to their effect on host plants within the nine populations of *Heterodera schachtii* found in the greater Cape Flats area did exist in order to effectively manage and control *H. schachtii* in the Western Cape. Soil samples were obtained from nine localities in the greater Cape Flats, these localities were as follows Durbanville, Guguletu, Khayelitsha, Kraaifontein, Kuilsriver, Lynedoch, Mitchell's Plain, Philippi and Stellenbosch. The top and root weight of beetroot and cabbage infected with the Lynedoch and Philippi populations of *H. schachtii* were significantly ($P=0.05$) lower than those of the other populations. The Philippi population showed significantly ($P=0.05$) the highest Pf/Pi ratio of 27 and

the highest number of eggs per cyst. Significantly (P=0.05) more *H. schachtii* juveniles from the Lynedoch and Philippi populations penetrated the seedling roots of beetroot and cabbage. The Kraaifontein population had the lowest penetration level on beetroot and the Mitchell's Plain population the lowest penetration on cabbage roots. The number of juveniles emerging from cysts from Lynedoch and Philippi were higher (P=0.05), over a shorter period of time, especially during the first seven days. During the first seven days 67.6% of the total number of juveniles emerged from the Lynedoch population and 65.5% from the Philippi population. The results demonstrated physiological variation in *H. schachtii* populations as far as virulence, root penetration and larval emergence from cysts to hosts were concerned.

EFFECTIVENESS OF RESISTANT TOMATO CULTIVARS FOR MANAGEMENT OF ROOT KNOT NEMATODES IN FLORIDA. **Vau, S. and D.W. Dickson.** Department of Entomology and Nematology, PO. Box 110620, University of Florida, Gainesville, Florida 32611-0620, USA.

In Florida, fresh market stake tomato (*Solanum esculentum* L.) is grown on 15,000 ha each year with a value exceeding US $460 million. With the suspension of methyl bromide and few suitable alternatives growers will be challenged to manage soilborne pathogens, especially root-knot nematodes (*Meloidogyne* spp.). Our objective was to determine the effectiveness of resistance in tomato for root-knot nematode management. This trial, conducted during the spring of 2013, was arranged in a randomized split design consisting of five replicates with six treatments, each treatment consisting of susceptible and resistant tomato cultivars, BHN 602 and Amelia, respectively. The treatments included: non-treated control; metam potassium (561 l/ha); chloropicrin (263 kg/ha); methyl bromide 50-50 (386 kg/ha); 1,3-D (140 l/ha); and 1,3-D + chloropicrin 35% (327 l/ha). There was no interaction among cultivars and treatments for average total marketable yield and total marketable yield per plant ($P \leq 0.05$). Total marketable yield and total marketable yield per plant for Amelia (59.9 kg and 0.30 kg/plant) was consistently lower compared with BHN 602 (66.4 kg and 0.43 kg/plant), independent of the treatments ($P \leq 0.05$). Plots treated with methyl bromide and chloropicrin produced the highest yield and were different from non-treated and 1,3-D treated plots ($P \leq 0.05$). The percentage of galling on Amelia was less than 10% and didn't show any interaction among treatments; whereas galling on BHN 602 averaged 51, 49, 26, 14, 12 and 0% for non-treated, metam potassium, chloropicrin, 1,3-D, 1,3-D + chloropicrin, and methyl bromide, respectively. Results from this study show the difficulty in root-knot nematode management using resistant cultivars in Florida sandy soils.

GENETIC RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN SOUTH AFRICAN SOYBEAN CULTIVARS AND VALIDATION OF THE TRAIT. **Venter, C., H. Fourie, J. Berner and A. Jordaan.** North-West University, Unit for Environmental Sciences and Management, Private Bag X 6001, Potchefstroom 2520, South Africa.

Soybean production in South Africa is progressively adversely affected as a result of *Meloidogyne incognita* parasitism. However, no synthetically-derived and/or biological agents are registered locally as nematicides on soybean. The use of resistant cultivars thus represents one of the most viable and environmentally-friendly strategies to protect soybean against nematode pests. Twenty-three locally adapted together with seven pre-released cultivars and the resistant standard LS5995 were evaluated for their host status to *M. incognita* in greenhouse trials. Resistance identified was then verified using enzymatic activity and cellular changes induced by *M. incognita* in resistant cultivars were ultimately compared to those formed in its susceptible counterparts. Reproduction factor values <1, indicating resistance to *M. incognita*, were recorded only for cultivar LS5995 and the seven pre-released cultivars. In terms of enzyme activity, significant ($P \leq 0.05$) increases in guaiacol peroxidase activity in leaf and root samples of the *M. incognita*-resistant cultivars GCI7 and LS5995 that were inoculated with J2 were recorded 24 hours after onset of the experiment. For lipoxygenase activity recorded, substantial variation existed between the *M. incognita*-resistant and susceptible cultivars tested, while no useful data were recorded for catalase activity. Light microscope observations revealed distinct differences in the appearance and development of giant cells in roots of the *M. incognita*-resistant cultivars LS5995 and GCI7 compared to those in roots of the susceptible cultivars Dundee and LS6248R. Resistance identified in the GCI7 pre-released cultivar was verified against that of the resistant standard LS5995 during this study and accentuate the importance of proper screening protocols to identify root-knot nematode resistance.

TAXONOMIC STUDY OF DORYLAIMOIDEA (NEMATODA: DORYLAIMIDA) FROM THE SEEKOEIVLEI NATURE RESERVE, SOUTH AFRICA. **Vermeulen, A. and C. Jansen van Rensburg.** Department of Zoology and Entomology, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa.

Nematodes are the most abundant and diverse metazoans in freshwater habitats. Classical nematode taxonomy is a complex study area and identification of species in genera containing a large number of species can be especially challenging to biologistsThe order Dorylaimida is a major taxonomic group of free-living nematodes having a universal distribution existing in almost every ecological niche. Dorylaimoidea are especially interesting nematodes due to their intricate taxonomy and their relatively wide morphological variability. Material for this study was obtained from existing material in the nematology study group collection of the Department of Zoology and Entomology, University of the Free State. Additional soil samples were collected at the Seekoeivlei Nature Reserve in May 2013. This reserve is known as one of the largest

wetland systems in the grassland biome of South Africa and is a Ramsar site. Nematodes were extracted from soil samples using the sugar-sieving-centrifugation-flotation method and fixed using standard techniques in preparation for light and scanning electron microscopy. Currently, nematodes are being identified and compared to the Juan Heyns reference material from the national Collection of Nematodes, Biosystematics Programme, Agricultural Research Council, , Pretoria. Results obtained to date include members from the genera *Dorylaimus*, *Mesodorylaimus, Prodorylaimus, Eudorylaimus* and *Allodorylaimus*. The aim of this study is to review all existing literature on the above-mentioned genera and to broaden the understanding of the taxonomy of this group of nematodesin South Africa.

VIRULENCE AND OXIDATIVE STRESS RESPONSE OF THE PINE WOOD NEMATODE *BURSAPHELENCHUS XYLOPHILUS*. **Vicente[1,2], C.S.L., Y. Ikuyo[1], R. Shinya[1,4], M. Mota[2,3], K. Hasegawa[1].** [1]Department of Environmental Biology, College of Bioscience & Biotechnology, Chubu University, 1200 Matsumoto, Kasugai, Aichi 487-8501, Japan; [2]ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Departamento de Biologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal; [3]INIAV/Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal Av. da República, Quinta do Marquês 2784-159 Oeiras, Portugal; [4]HHMI and Division of Biology and Biological Engineering, California Institute of Technology, 1200 East California Blvd, Pasadena, California 91125, USA.

Bursaphelenchus xylophilus is the causal agent of pine wilt disease and the most devastating plant parasitic nematode attacking coniferous trees (mostly *Pinus* species) in the world. In the early stages of invasion, this nematode has to manage host defence mechanisms, such as strong oxidative stress. Only successful virulent nematodes are able to resist the basal immune plant counterattack, and further migrate and proliferate in numbers inside of the host tree. Our main objective was to study the oxidative stress tolerance of the virulent/avirulent *B. xylophilus* isolates and avirulent *Bursaphelenchus mucronatus*, and understand in which degree this feature is related with their virulence level. For this purpose, we used the most prominent reactive oxygen species H_2O_2 (hydrogen peroxide) as oxidative stress agent to evaluate the tolerance of the virulent/avirulent isolates of *B. xylophilus* and *B. mucronatus*, assessing catalase enzymatic activity, H_2O_2 neutralization and relative gene expression of different antioxidant enzymes. In addition, transgenic of *Caenorhabditis elegans* overexpressing *B. xylophilus* catalase were constructed and evaluated for survival under oxidative stress conditions. Here we show the correlation between *B. xylophilus* virulence and oxidative stress resistance; virulent *B. xylophilus* expressed more antioxidant enzymes and could have more tolerance against oxidative stress than avirulent *B. xylophilus* and *B. mucronatus*. Moreover, transgenic *C. elegans* overexpressing *B. xylophilus* catalase were able to resist better than wild type *C. elegans*. Our study suggests that oxidative stress tolerance of *B. xylophilus* is important to withstand against host plant counter-attack and can be considered a pathogenicity factor.

ECTOPIC EXPRESSION OF CELL CYCLE INHIBITOR GENES EFFECTIVELY INTERFERES WITH ROOT-KNOT NEMATODE FEEDING SITE DEVELOPMENT. **Vieira[1], P., N. Rodiuc[1], L. De Veylder[2], G. Engler[1,3], P. Abad[1], J. de Almeida Engler[1,4].** [1]Institut National de la Recherche Agronomique, UMR 1355 ISA/Centre National de la Recherche Scientifique, UMR 7254 ISA/Université de Nice-Sophia Antipolis, UMR ISA, 400 route des Chappes, Sophia-Antipolis, France; [2]Department of Plant Systems Biology, Flanders Institute for Biotechnology, Technologiepark 927, 9052 Gent, Belgium; [3]PVE-UCB/CAPES, Brasilia, Brazil; [4]PVE-UnB/CAPES, Brasilia, Brazil.

Root-knot nematodes (*Meloidogyne* spp.) modify plant root cells by inducing specialized feeding structures. Selected root vascular cells are changed to form complex feeding cells that supply nutrients for the nematodes to develop and reproduce. Giant-feeding cells are characterised by a dense cytoplasm filled with proliferating organelles The changes that occur during feeding cell morphogenesis are accompanied by a drastic rise in ploidy levels, metabolic activity and cell size. We have shown that activation of the cell cycle plays a key role in feeding site development but how precisely nematodes manipulate this process in their favour remains to be better comprehended. A systematic comparison of the temporal and spatial expression pattern of core cell cycle genes between galls and uninfected control roots of *Arabidopsis thaliana* resulted in the identification of a collection of genes up- or down regulated in nematode feeding sites. Their functional analyses resulted in the identification of a subset of genes strongly impairing gall development. The disruption of many *Arabidopsis* cell cycle regulators has been shown to affect both mitotic and endoreduplication cycles. Among them, negative regulators such as the ICK2/KRP2 gene are highly expressed during gall development and are candidates to control the cell cycle in NFC. ICK2/KRP2, a member of the cyclin-dependent kinase/kip-related proteins (ICK/KRP), regulates mitosis-to-endocycle transition in plant cells and is expressed in endoreduplicating cells. Herein we present data related with four KRP genes exerting different functions during feeding site development.

SYNERGISTIC INTERACTION BETWEEN PLANT-FEEDING NEMATODES AND THE FUNGUS *RHIZOCTONIA SOLANI* IN POTATO. **Viketoft[1], M. and E. Edin[2].** [1]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Department of Forest Mycology and Plant Pathology, Uppsala, Sweden.

Stem canker caused by the pathogenic fungus *Rhizoctonia solani* is a major problem for potato cultivation throughout Sweden, especially as it leads to great economic losses. Advisors and growers have developed a theory that there is a correlation between the severity of stem canker and plant-feeding nematodes, as high occurrences of stubby-root and root-lesion nematodes were found in potato fields with high incidence of stem canker. The aim of this project was to investigate any interactions between these nematodes and the fungus under controlled conditions. In the first experiment, pre-germinated minitubers (cv King Edward) were planted in pots with sterilised sand and placed in a climate chamber at conditions corresponding to a Swedish spring. Fungal mycelium and/or nematodes (*Pratylenchus penetrans*) were added to the pots in various combinations. The pots were harvested ten weeks after inoculation and the parameters measured were fresh and dry weight of stems, stolons, tubers and roots, as well as the gradation of the damage caused by the nematodes and the fungus of the different plant parts. The nematodes produced feeding symptoms on stems, tubers, stolons and roots, but the roots also became brownish to different degrees. The fungus caused stem canker of different severity on the stems, burned off stolons and produced sclerotia on the roots and on the majority of the tubers. We found a synergistic effect between *R. solani* and *P. penetrans*, as the tuber yield decreased significantly when both nematodes and fungus were present in the pots.

GENE TRANSCRIPTION CHANGES DURING COMPATIBLE AND INCOMPATIBLE INFECTIONS OF POTATO BY *GLOBODERA ROSTOCHIENSIS*. **Walter, A., E. Alexandersson and E. Andreasson.** Swedish University of Agriculture, Department of Plant Protection Biology, Sweden.

The golden potato cyst nematode, *Globodera rostochiensis*, is one of the most important pests of potato in Sweden. Several pathotypes of the nematode exist, and they may be controlled by one of several sources of single-gene resistance in potato. Here we report that SW93-1015, a breeding line from the potato breeding program at SLU Alnarp, has phenotypic resistance to the Ro1/4 pathotype of *G. rostochiensis*. Reproduction of *G. rostochiensis* pathotype Ro1/4 on SW93-1015 is approximately 5% of reproduction on the susceptible cultivar Desiree. To further investigate the interaction between potato cyst nematodes and susceptible and resistant host plants, changes in gene transcription were monitored just after root penetration and during syncytium formation in Desiree and SW93-1015 potato plants infected with Ro1/4 *G. rostochiensis*. RNA expression in root tissue was analysed on a microarray at 0, 8, and 48 h post-infection, corresponding to pre-infection, root penetration, and early syncytium formation. Changes in gene transcription over the process of infection in susceptible and resistant genotypes are discussed. The resistance will be further investigated using a crossing population if Desiree and SW93-1015.

COMPARATIVE TRANSCRIPTOME ANALYSIS OF TWO RACES OF *HETERODERA GLYCINES* AT DIFFERENT DEVELOPMENTAL STAGES. **Wang[1,2], G., D. Peng[1], B. Gao[3], W. Huang[1], L. Kong[1], H. Long[1,4], H. Peng[1], H. Jian[2].** [1]The key Laboratory for Biology of Insect Pests and Plant Disease, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; [2]Department of Plant Pathology, China Agricultural University, Beijing 100193, China; [3]Huzhou Modem Agricultural Biotechnology Innovation Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Huzhou 313000, Zhejiang, China; [4]Key Laboratory of Pests Comprehensive Governance for Tropical Crops, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Science, Danzhou, China.

The soybean cyst nematode, *Heterodera glycines*, is one of the important pests of soybeans, but little is known about its genetic diversity. In order to elucidate the differences, we investigated the transcriptional diversities within race 3 and race 4 inbred lines during their compatible interactions with soybean host Zhonghuang 13. Six different race-enriched cDNA libraries were constructed with limited nematode samples collected from the three typical sedentary stages, parasitic J2, J3 and J4 female, respectively. Among 689 putative race-enriched genes isolated from the six libraries with functional annotations, 92 ones were validated by quantitative RT-PCR (qRT-PCR), including eight putative effector coding genes. More race-enriched genes validated were identified within race 3 and race 4 with their development in soybean roots. Gene Ontology analysis of all the race-enriched genes at J3 and J4 female stages showed that most of them functioned in metabolic processes. Relative transcript level analysis of 13 selected race-enriched genes at four developmental stages showed that the differences in their expression abundance took place at either one or more developmental stages. Gene developmental expression analysis also indicated that inconsistent gene expression tendencies of six selected genes existed between race 3 and race 4, which was a new observation in soybean cyst nematodes. It was the first investigation into the transcription diversities between two *H. glycines* races throughout their sedentary stages, increasing the recognition of the genetic diversities of *H. glycines*.

TRANSGRESSIVE SEGREGATION OF ROOT-KNOT NEMATODE RESISTANCE IN COTTON DETERMINED BY QTL ANALYSIS. **Wang[1,2], C., M. Ulloa[3], and P.A. Roberts[2].** [1]Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, 150081, China; [2]University of California, Riverside, CA 92521, USA; [3]USDA-ARS, Lubbock, TX 79415, USA.

Transgressive resistance to root-knot nematode, *Meloidogyne incognita*, was found in intraspecific (*Gossypium hirsutum*; resistant Acala NemX x susceptible Acala SJ2) and interspecific (*G. barbadense* susceptible Pima S-7 x *G. hirsutum* Acala NemX) cotton recombinant inbred line (RIL) populations. Similar contributions to both root-galling and egg-production resistance for SSR marker CIR316 linked to resistance gene *rkn1* in NemX on chr11 were identified in each population. Transgressive factor *RKN2* from Pima S-7 clustered with *rkn1* in NemX and produced higher resistance. QTL analysis of test-cross NemX x F_1(Pima S-7 x SJ2) showed Pima S-7 CIR069-271bp allele marker linked to CIR316 contributed up to 63% of resistance to galling in the presence of *rkn1*. Similarly, in RIL Acala NemX x Acala SJ-2, markers closely linked to CIR316 contributed up to 82% of resistance to root-galling, indicating that interaction of factors from both susceptible and resistant parents occurred in the *rkn1* resistance region. These results were confirmed in BC_1F_1 population Acala SJ-2 x F_1(NemX x SJ-2) and F_2 population NemX x SJ2, in which up to 44% and 37% increase in resistance to galling was found, respectively. These results indicated transgressive factor(s) from susceptible Acala SJ-2 exist in the region of *rkn1*, similar to transgressive factor *RKN2* from susceptible Pima S-7, and stronger transgressive resistance occurred in later than in early generations in the intraspecific cross NemX x SJ-2. The complex recombination and interactions in the *rkn1* resistance region in the Acala NemX background provides a model to study transgressive resistance in plants.

IDENTIFICATION OF *PRATYLENCHUS* SPECIES FROM THE RHIZOSPHERE OF PEANUT IN CHINA. **Wang[1,2], H., K. Zhuo[1,2], J. Liao[1,2].** [1]Laboratory of Plant Nematology, South China Agricultural University, Guangzhou 510642, China; [2]Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou 510642, China.

Pratylenchus species are regarded as important pests of peanut, causing economic losses worldwide. During the past three years, surveys for the presence of *Pratylenchus* species from the rhizosphere of peanut in China were conducted. Eighty-seven samples were collected from nine provinces in China. *Pratylenchus* spp. were detected in 22 of these samples. All the populations were purified and cultured on carrot disc, then identified by using comparative morphological, morphometric and molecular studies. Finally, seven populations were identified as *Pratylenchus coffeae*, four populations as *Pratylenchus brachyurus*, two populations as *Pratylenchus zeae* and one population as *Pratylenchus neglectus*. The remaining eight populations were not identified to species as yet. These results indicate that *Pratylenchus* spp. are abundant as parasites of peanut throughout China.

BENEFITS OF MIX COVER CROPPING ON SOIL HEALTH. **Wang[1], K.H. and C.R.R. Hooks[2].** [1]University of Hawaii Manoa, Department of Plant and Environmental Protection Sciences, Honolulu, Hawaii, USA; [2]Department of Entomology, University of Maryland, College Park, Maryland, USA.

One of the criteria of enhancing soil health is to increase soil microbial diversity. Several field trials were conducted in Hawaii and Maryland to examine if mix cover crops could provide more biodiversity below ground than individual cover crop. Mixed cover cropping of sunn hemp (*Crotalaria juncea*) and oat (*Avena sativa*) supported higher abundance of bacterivorous and predatory nematodes in a no-till eggplant (*Solanum melongena*)-cover crop system in a lower elevation of Hawaii. However, mixed planting of sunn hemp and oats or planting of sunn hemp alone increased numbers of nematode-trapping fungi in the rhizosphere of eggplants to a similar level. A second mixed cover cropping trial was conducted in a higher elevation of Hawaii. Among the cover crops examined included common vetch (*Vicia sativa*), bell bean (*Vicia faba*), BioMaster winter peas (*Pisum arvense*), rye (*Secale cereale*), vetch plusrye, and a commercial cover crop mix composed of bell bean, BioMaster peas, Arvika peas (*Pisum sativum*), purple vetch (*Vicia Americana*), hairy vetch (*Vicia villosa*), common vetch and Cayuse oats (*A. sativa*). In Maryland, barley (*Hordeum vulgare*), crimson clover (*Trifolium incarnatum*), and their combination were compared to bare ground treatments followed by no-till planting of corn (Zea mays). In a second trial, soil health conditions were examined under a forage radish (*Raphanus sativus*)-crimson clover (*Trifolium incarnatum*) mixture in strip-till, no-till, conventional till with plastic mulch cover vs a bare ground treatment. In general, cover crop mix enhanced more soil health parameters than their individual counterpart, and provided more benefits for cover cropping.

PASTEURIA SPECIES AS A BIO-CONTROL AGENT FOR CONTROL OF PLANT-PARASITIC NEMATODES. **Watrin[1], C. and N. Sekora[2].** [1]Syngenta Crop Protection, Greensboro, North Carolina, USA; [2]Syngenta Crop Protection, Pasteuria Bioscience Laboratory, Alachua, Florida, USA.

Pasteuria species are gram-positive, endospore-forming, obligate bacterial parasite of nematodes with a unique mode of action. The *Pasteuria* spores (active ingredient of CLARIVA™) are highly effective and lethal to nematodes without harming other soil organisms, plants, or the environment. Selected strains of *Pasteuria* can be specific to a single nematode species or genus and will not target other nematode species that may be present. *Pasteuria* spores attach, penetrate, and infect nematodes, ultimately killing them while producing more *Pasteuria* spores. The technology starts to work immediately and may reduce the reproductive rate of infected nematodes before killing them. *Pasteuria* spp. endospores are long-lived in the soil; are resistant to desiccation, and are compatible with most crop protection products. Recent breakthroughs in fermentation technology have allowed for *in vitro* production of *Pasteuria* spores in large scale, a cost effective use of this organism

to control plant-parasitic nematodes. When delivered as a seed or soil treatment, *Pasteuria* spp. provides enhanced convenience and plant-targeted effectiveness for growers. *Pasteuria* technology has the potential to be used across a broad range of crops in which nematodes cause damage and subsequent yield loss. Current development activities include developing *Pasteuria* spp. for control of several plant-parasitic nematode targets including multiple species of *Heterodera*, *Pratylenchus*, and *Meloidogyne*.

PASTEURIA AS A BIO-CONTROL AGENT TO MANAGE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*. **Watrin[1], C. and N. Sekora[2].** [1]Syngenta Crop Protection, Greensboro, North Carolina, USA; [2]Syngenta Crop Protection, Pasteuria Bioscience Laboratory, Alachua, Florida, USA.

Soybean cyst nematode (*Heterodera glycines*) is the most destructive pathogen of soybean (*Glycines max* L.) throughout the world. Annual losses to producers in the United States can reach more than $2 billion a year. Today, *H. glycines* is found in most soybean producing areas of the world. Several management practices have been investigated to control this pest, such as the use of resistant cultivars, chemical control (nematicides), and rotation with non-host crops. Using integrated solution strategies including *H. glycines*-resistant cultivars with CLARIVA™, a *Pasteuria* species seed-applied nematicide, is an effective and consistent strategy to control this pest, and can provide yield advantages in most situations. The *Pasteuria* spores (active ingredient of CLARIVA™) are obligate parasites of nematodes and are lethal without harming other soil organisms, plants, or the environment. *Pasteuria* spores attach, penetrate, and infect nematodes, ultimately killing them while producing more *Pasteuria* spores. The technology starts to work immediately and may reduce the reproductive rate of infected *H. glycines* before killing them. *Pasteuria* endospores are long-lived in the soil, are resistant to desiccation, and are compatible with most crop protection products, allowing CLARIVA™-treated seeds to be an excellent platform for integrated management of *H. glycines* on soybean.

FROM SIDE KICK TO LEAD ROLE - EXPERIENCES WITH SEAWEED-BASED BIONEMATICIDES. **Wesemael[1,2], W.M.L., B.M. Ngala[2,3], Y.V. Vazquez[2], N. Viaene[1,2] and R.N. Perry[2,4].** [1]Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium; [2]Faculty of Science, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium; [3]Crop and Environment Science Department, Harper Adams University, Newport, Shropshire, TF10 8NB, UK; [4]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts AL10 9AB, UK.

Due to decreasing availability of classical nematicides new products are needed and, thus, there is increasing interest in bionematicides. Scientific research focused on biocontrol agents *Pasteuria* spp., *Pochonia chlamydosporium* and *Purpureocillium lilacinus*, all available as commercial products targeting plant-parasitic nematodes. Many plants contain or release compounds with nematicidal activity. Knowledge on their mode of action, stability and practical application is limited. Several products containing bacteria and fungi are commercialised as plant-growth promoters and soil conditioners. Effects on plant-parasitic nematodes have been reported after their application. Seaweed-based products also have an effect on different plant-parasitic nematodes. Seaweed extracts from *Ascophyllum nodosum* used as a soil drench in a pot test improved the growth of *Meloidogyne chitwoodi* and *Meloidogyne hapla* infected tomato plants and reduced hatch and infectivity of second-stage juveniles (J2) *in vitro*. However, the level of control achieved with these applications alone may be insufficient under normal agricultural conditions. Extracts from *Ecklonia maxima* gave an increased movement of J2 towards host plant roots but hampered movement in the presence of a repellent (acetic acid). Further studies on how biological compounds affect the behaviour and life cycle development of plant-parasitic nematodes are needed to facilitate the development of successful bionematicides. As market uptake increases, the need for independent efficacy data is high. Rather than a secondary effect on plant growth, the nematicidal potential of natural products should be a main topic for scientists and companies.

INFLUENCE OF CYST NEMATODES ON THE FOOD CHAIN. **Westphal[1], A. and M. Daub[2].** [1]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Dürener Str. 71, 50189 Elsdorf, Germany.

Cyst nematodes pose a number of management challenges in the food chain. For example *Heterodera schachtii* is widely spread throughout German production regions, and management must bring about a continued suppression of the pest. Along the production chain, different steps can be taken. At the field level, long crop sequences and cover cropping with resistant plants for nematode suppression are used. In Germany, the restructuring of the handling and processing of the plant product has fortuitously reduced the potential spread of the nematode with beet-adhering soil. Farmer operated hauling from the field to the processing plant has been mostly replaced by truck transport of temporarily field-stored and roughly cleaned sugar beets. Also, the transportation within the processing plant has been modified, reducing the loads of potentially nematode-infested wastes. On the contrary, the multi-farm use of equipment carries the risk of spreading nematode infestation with contaminated harvesters. Other examples are potato cyst nematodes, *Globodera pallida* and *Globodera rostochiensis* that are severe problems of potato production. European regulations and national versions of these rules specify how to deal with plant products and the concomitant soil. Seed potatoes must only be produced on fields found to be free of *Globodera* spp. If

a field is found to be infested, management options are prescribed to suppress the nematode. Primary options are resistant cultivars. In Germany, these are registered by the Julius Kühn-Institut in collaboration with the Federal Plant Variety Office. Changes in the production chain need careful attention to manage the risk with these nematodes.

IMPROVED GROWTH OF *BETA VULGARIS* AFTER DIGESTATE FERTILIZATION IN *HETERODERA SCHACHTII*-INFESTED SOIL. **Westphal[1], A., M. Kücke[2] and H. Heuer[3].** [1]Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Institute for Crop and Soil Science, Julius Kühn-Institut, Bundesallee 50, 38116 Braunschweig, Germany; [3]Institute of Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany.

Heterodera schachtii is one of the most important pests of sugar beet. It occurs in many sugar beet producing areas. Management of *H. schachtii* relies on crop rotation with sugar beet planted not more than every three years, and the planting of resistant cover crops.. Recent policy changes have resulted in increased amounts of organic waste materials from biogenic energy production. Digestate is one such product from bio-energy fermenters. The aim of this study was to determine if digestate improved growth of sugar beet (*Beta vulgaris saccharifera*) and mangold (*Beta vulgaris vulgaris*). In sugar beet production, *H. schachtii*-infested microplots were fertilized with equivalents of 60, 120, or 240 kg nitrogen (N)/ha contained in digestate or with calcium ammonium nitrate fertilizer. Phosphate (P) and potassium (K) were adjusted correspondingly with mineral fertilizers. In mangold, an additional treatment applied only 30 kg N/ha. Controls were unfertilized plots and those that received the 120 kg N/ha rate plus the nematicide Fosthiazate. In both crops, digestate amendments were followed by lower nematode penetration rates and improved early growth; yield data were less distinct between the two fertilizer forms but there were increased yields with increasing fertilizer rates. In sugar beet, increased digestate amounts resulted in reduced nematode penetration and improved early growth, but yields were highest in the nematicide-treated plots. In both crops, unfertilized plots had high penetration rates, low initial growth, and poor yields. This data indicate that fertilizing sugar beet and mangold with digestate was beneficial in nematode suppression and concomitant nutrient supply.

DISTRIBUTION AND GENETIC DIVERSITY OF *BAKERNEMA INAEQUALE* (CRICONEMATIDAE) IN NORTH AMERICA. **Whitlock[1], K.J., E.C. Bernard[1] and T.O. Powers[2].** [1]Entomology & Plant Pathology Department, The University of Tennessee, 2505 E. J. Chapman Drive, 370 Plant Biotechnology Building, Knoxville TN 37996-4560 USA; [2]Plant Pathology Department, The University of Nebraska, 406 Plant Science Hall, Lincoln, NE 68583-0722 USA.

Bakernema inaequale is a widespread criconematid nematode species endemic to eastern North America. Because this nematode is unique in appearance, easily recognizable under a stereo microscope, and known only from forest sites, it has potential as an indicator species for environmental health and climate change. Many criconematid species actually are species complexes; therefore, extensive sampling for *B. inaequale* was carried out and specimens were characterized morphologically and molecularly (COI). We hypothesized that northward migration of *B. inaequale* following the last ice age caused the population to split into western and eastern populations separated by the Appalachian Mountains. Soil samples were collected from diverse habitats of non-agricultural land from northern Pennsylvania to the Florida Panhandle and Alabama, and added to samples already obtained from more northern U.S. states. After extraction with a sugar flotation method, up to five live specimens from each sample were imaged and measured, then prepared for DNA amplification. Although much more sampling needs to be done, *B. inaequale* appears to be a distinct lineage with little haplotype diversity observed so far. This nematode radiated rapidly from a southern Pleistocene refugium of taiga and northern hardwoods without significant genetic drift or east-west speciation. *Bakernema inaequale* is amphimictic and spread of genes through the mountains via mating probably has maintained genetic stability within the species.

INVOLVEMENT OF PECTATE LYASES IN THE FORMATION OF FEEDING STRUCTURES INDUCED BY CYST AND ROOT-KNOT NEMATODES. **Wieczorek[1], K., A. Elashry[2], M. Quentin[3], F.M.W. Grundler[2], B. Favery[3], G.J. Seifert[4] and H. Bohlmann[1].** [1]Department of Crop Sciences, Division of Plant Protection, University of Natural Resources and Life Sciences, Konrad-Lorenz Straße 24, A-3430 Tulln, Austria; [2]Institute of Crop Science and Resource Conservation, Department Molecular Phytomedicine, University Bonn, Karlrobert-Kreiten-Str. 13, D-53115 Bonn, Germany; [3]Institut Sophia Agrobiotech UMR INRA 1355-CNRS 7254-Université de Nice Sophia Antipolis, F-06903, Sophia Antipolis, France; [4]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria.

Pectin in the primary plant cell wall is thought to be responsible for its porosity, charge density, and microfibril spacing and is the main component of the middle lamella. Plant-parasitic nematodes secrete a cocktail of cell wall degrading enzymes that macerate the plant tissue, facilitating the penetration and migration within the roots. In sedentary nematodes, these enzymes are released only during the migration of infective juveniles through the root. At later stages nematodes manipulate the expression of host plant genes including various cell wall enzymes in order to induce specific feeding sites. In this study we investigated pectin and pectate epitopes together with the expression of two *Arabidopsis thaliana* pectate lyase-like genes, *PLL18* (At3g27400) and *PLL19* (At4g24780), in both syncytia induced by the cyst nematode *Heterodera schachtii* and giant

cells induced by the root-knot nematode *Meloidogyne incognita*. We analyzed their expression in both types of feeding sites and confirmed their upregulation based on our previous GeneChip and microarray results by qRT-PCR and *in situ* RT-PCR. Furthermore, the functional analysis of mutants demonstrated the important role of both *PLL*s in the development and maintenance of syncytia but not giant cells. Our results show that both enzymes play distinct roles in different infected root tissues as well as during parasitism of different nematodes.

NEMATODE TOLERANCE ENHANCED USING SOIL AMENDMENTS ON TSG2-GUAVA TREES IN SOUTH AFRICA. **Willemse, A.S., M.S. Daneel, K. de Jager and W.P. Steyn.** Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa.

In the past the South African guava industry was mainly based on one commercial cultivar namely 'Fan Retief'. With the outbreak of Guava Wilt Disease which was first detected in the Mpumalanga Province in the early 1980s it became critically important for the guava industry to breed a cultivar with an adequate degree of tolerance against Guava Wilt Disease. In 1995, resistant cultivar, 'TS-G2' was released by the ARC-ITSC. While cultivar 'Fan Retief' seemed to be resistant to root-knot nematodes, nematodes were never perceived as a problem until 'TS-G2' was released, as this cultivar was highly susceptible to root- knot nematodes. Normal chemical nematode control practices do not seem to be sufficient and alternatives were investigated where enhanced root growth together with nematode control was evaluated. A greenhouse trial consisted of *Moringa* leaf extracts, Biostart, Gwamis, Nitrospray, mycorrhrizae, two nematicides Vydate and Nemacur and an untreated control. 'TS-G2' seedlings were inoculated with 9000 *Meloidogyne* spp. eggs collected from an infested guava orchard. Plant and root growth was observed with several of the treatments compared to the untreated control with Vydate, guano manure (Gwanis) and mycorrhizae showing the best growth enhancement, however nematode control was less obvious. It is therefore most likely that a combination of chemical nematicides and organic amendments might give sufficient nematode control over an extended period.

GENETIC MAPPING AND PHYSICAL LOCALIZATION OF VIRULENCE TRAITS IN *MELOIDOGYNE HAPLA*. **Williamson[1], V.M., V.P. Thomas[1], J. Jimeno[1], S. Fudali[1], D. Bird[2,3] and D. Nielsen[4].** [1]Department of Plant Pathology, University of California, Davis, CA 95616, USA; [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA; [3]Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695, USA; [4]Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA.

The root-knot nematode *Meloidogyne hapla* can reproduce on a wide range of crop species, but there is variability in host range and virulence both within and between isolates. The genome sequence of several strains of this species has been obtained, identifying an abundance of scorable DNA polymorphisms. Due to the unusual reproductive mechanism, that is, facultative meiotic parthenogenesis, controlled crosses can be carried out to produce F2 lines resembling recombinant inbred lines. These lines can be maintained as cultures and scored for both pathogenicity phenotypes and molecular markers to generate integrated genetic linkage maps. Progeny lines from a cross of two *M. hapla* isolates that reproduce poorly on the wild potato *Solanum bulbocastanum* SB22 differ dramatically in ability to reproduce on this host with some lines able to reproduce to higher levels than either parental strain. Three quantitative trait loci contributing to reproductive ability of the nematode on SB22 were identified and positioned on linkage groups. Similarly, analysis of progeny from a cross between two *M. hapla* strains that differ in their ability to reproduce on the resistant common bean variety NemaSnap identified a single, major genetic locus that mediates variety-specific virulence. The availability of genome sequence scaffolds together with the integrated genetic map have allowed us to physically localize this virulence trait to a region of <100 kb in the *M. hapla* genome providing an inroad for its future identification.

ASSESSMENT OF SOILS FOR THE PRESENCE OF THE NEMATOPHAGOUS FUNGUS *DACTYLELLA OVIPARASITICA* BY A PCR-BASED STRATEGY. **Witte[1], H., J. Yang[2], J. Borneman[3], J. Smith Becker[1] and J.O. Becker[1].** [1]Departments of Nematology, [2]Botany and Plant Sciences, [3]Plant Pathology and Microbiology, University of California, Riverside, California 92521, USA.

The nematophagous fungus *Dactylella oviparasitica* (syn. *Brachyphoris oviparasitica*) is considered the primary causal agent of a long-term *Heterodera schachtii* population suppression in a field of the Agricultural Operations, University of California-Riverside. This strain has been shown to successfully suppress sugarbeet cyst nematode populations after its introduction into various field soils. We hypothesize that parasitism of *Heterodera schachtii* by *D. oviparasitica* might be a major factor in regulating cyst nematode populations in the Imperial Valley, California's main sugarbeet production area. As little is known about the geographical distribution of this fungus, we developed an assay for the detection of *D. oviparasitica* and closely related organisms in soils. The fungus was baited by planting a soil sample with *H. schachtii*-infested Swiss chard. White *H. schachtii* females were picked from the roots and subjected to DNA extraction. A qPCR assay utilizing sequence-selective primers targeting the ITS region of the rRNA gene sequence of *D. oviparasitica* was used to amplify and detect the desired sequence. This procedure was used to screen soil samples from 77 different sugar beet fields in the Imperial Valley. Preliminary results indicate the presence of *D. oviparasitica* or closely related fungi in 15 samples. The obtained

qPCR products from those samples will be sequenced to determine strain identity and further validate the results. This strategy presents a feasible methodology to assess soils for the presence of *D. oviparasitica* and could potentially be adapted for the detection of other nematophagous organisms.

STEM POWDER OF *PAEONIA SUFFRUTICOSA*AS A CONTROL AGENT FOR *MELOIDOGYNE INCOGNITA*. **Yanhua, W., Z. Xiaoqiang and B. Wei.** College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, People's Republic of China.

Paeonia suffruticosa var. papaveracea (Andr.) Kerner is cultivated in China and used for both ornamental and traditional medicine. In 2001, stem extracts of *P. suffruticosa* showed strong nematicidal activity against plant nematodes in a laboratory bioassay.In 2009, a pot trial was conducted in the greenhouse. The stem powder of *P. suffruticosa* was mixed thorough with soil in the pot at concentrations of5ml, 10ml and 15ml powder per litre soil (one pot). Seedlings of cucumber or swamp cabbage plants were transplanted into pots and 1000 juveniles of *Meloidogyne incognita* were inoculated into the pot. The pot trial lasted for 45 days in greenhouse. The experiment showed that the stem powder of *P. suffruticosa* effectively controlled root-knot disease caused by *M. incognita* on cucumber and swamp cabbage plants. The control efficiency was 44%, 66% and 71%, 82% for the 10 ml and 15 ml powder treatments, respectively. Numbers of juvenile nematodes in the soil also decreased sharply compared to the control.

MOLECULAR DIVERSITY OF NEMATODES ASSOCIATED WITH NATIVE SOUTH AFRICAN FIGS. **Wöhr, M.S., J.C. Erasmus and J. M. Greeff.** Department of Genetics, University of Pretoria, Pretoria 0002, South Africa.

Despite three decades of fig nematode observations and descriptions, only four nematodes have been described from the 25 native South African fig species and none on a molecular basis. With expanding data on fig associated nematode description for most parts of the world, there is a rising need for such descriptions in Africa to gain better insight into species diversity and relationships. At least two groups of nematodes have radiated on *Ficus* and sample collection has been ongoing and will aim to obtain representatives of both clades of nematode, as well as other genera. To address this lack of South African fig nematode data, DNA isolated from nematodes harvested from eleven native South African *Ficus* species was amplified using marker gene primers from literature. The sequences obtained were compared to genomic nematode datasets to identify the nematode genus or genera present in the samples. Phylogenetic analysis could place the novel species among their genus and show species that did not closely match the current molecular fig nematode dataset. Additionally, nematodes were permanently mounted for microscopic observation, with which we could confirm the DNA data based on signature morphological features of each genus, but also uncover species that were not represented molecularly. This study has thus far identified 16 nematodes species belonging mainly to the two nematode genera associated with figs, *Parasitodiplogaster* and *Schistonchus*, but also two *Teratodiplogasters*, an unknown Tylenchid species as well as an unknown Diplogasterid.

DRAFT GENOME AND TRANSCRIPTOME SEQUENCES OF *DITYLENCHUS DESTRUCTOR* AND *DITYLENCHUS. DIPSACI*. **Yu[1], Q., H. Peng[2], E. Ponomareva[1], J. Cullis[1], C.T. Lewis[1], A.C. Levesque[1] and D.L. Peng[2].** [1]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Center, Ottawa, Ontario, Canada; [2]Institute of Plant Protection, Chinese Academy of Science, Beijing, China.

Ditylenchus destructor, the potato rot nematode, and *Ditylenchus dipsaci*, the stem and bulb nematode are important nematode pests causing great losses to many crops. They are also both quarantined pests for many countries including Canada. . A *D. destructor* population from the Jiangsu province, China was cultured on *Fusarium oxysporium* on potato dextrose agar, and on pea root cultures on Gamboug B5 medium. The *D. dipsaci* population from Ontario, Canada was also cultured and on pea root cultures on Gamboug B5 medium. The genome and transcriptome of the two species were sequenced. An Illumina HiSeq instrument was used to perform paired-end, mate-pair, and RNA-seq sequencing for both species. Paired-end DNA and RNA sequencing was performed using read lengths of 101bp and an insert size of 300bp. In addition, two runs of mate-pair sequencing were performed on each species using read lengths of 51bp and insert sizes of 3000bp and 8000bp, which generated 53M paired-end reads (6bp trim), 129M 3kbp mate-paired reads (6bp trim), and 116M 8kbp mate-paired reads (2bp trim) for *D. destructor* and 107M 3kbp mate-paired reads (5bp trim), 135M 8kbp mate-paired reads for *D. dipsaci*. *D. destructor* RNA consisting of four samples made up of two replicates, each grown in *F. oxysporum* or plant media and *D. dipsaci* of one sample from plant medium were sequenced and are being analysed. The genome and transcriptome assemble and annotation is our future focus.

ENTOMOPATHOGENIC NEMATODES PREVENT THE TERMITE PEST, *MACROTERMES BELLICOSUS* FROM RECONSTRUCTING ITS NEST. **Zadji[1,4], L., H. Baimey[1], L. Afouda[1], M. Moens[2] and W. Decraemer[3,4].** [1]University of Parakou, P.O. Box 123, Parakou, Benin; [2]Institute for Agricultural and Fisheries Research (ILVO) Crop Protection Department, Burg Van Gansberghelaan 96, 9820 Belgium; [3]Royal Belgian Institute of Natural Sciences, Brussels; [4]Ghent University, Department of Biology, Ledeganckstraat 35, Ghent, Belgium.

Termites cause significant losses to citrus in Benin. A field experiment was conducted to assess the effectiveness of two indigenous entomopathogenic nematode species (EPN), *viz*. *Heterorhabditis sonorensis* and *Heterorhabditis indica*, against an underground nest population of *Macrotermes bellicosus*. We hypothesized that after aboveground nest demolition, *M*. *bellicosus* would reconstruct its nest in three months time. Aboveground nests were first demolished before 50 2-week old EPN-infected *Galleria mellonella* larvae were scattered over the demolished surface. Three treatments were compared: (1) *G*. *mellonella* infected with *H*. *sonorensis*, (2) *G*. *mellonella* infected with *H*. *indica*, and (3) untreated control. To monitor nest reconstruction progress, nest volumes were calculated before demolition and at 10-days interval after demolition. Data indicated that nest reconstruction rates (NRR) differed significantly among treatments but were always lower than in the untreated control. The highest nest reconstruction rates were observed for the control (107%) 70 days after application of the EPN. The nest reconstruction rates for treatments 1 (37.6) and 2 (43%) remained constant at 40 and 50 days after application, respectively, until 70 days after application. Both EPN species persisted well in the nests and were retrieved up to 70 days after application. At that time, the underground populations of 71 (treatment 1) and 60 % (treatment 2) were found dead after excavation; the underground populations of the untreated control nests, however, were all alive. These findings suggest that tested indigenous EPN can provide effective biological control of *M*. *bellicosus* in the field.

SYMBIOTIC SPECIALISATION, STABILITY AND PATHOGENIC RELATIONSHIPS AMONG RHABDITID ENTOMOPATHOGENIC NEMATODES *HETERORHABDITIDOIDES* AND THEIR SYMBIOTIC BACTERIA. **Zhang, K.** Department of Zoology, College of Life Sciences, Nanjing Agricultural University, Nanjing, 210095, China.

Two representive rhabditid entomopathogenic nematodes *Heterorhabditidoides chongmingensis* DZ0503CMFT (DZ) and *H*. *rugaoensis* RG081015 (RG), with their respective native symbiotic bacterial strains *Serratia nematodiphila* DZ0503SBS1 (S1) and *S*. *nematodiphila* DR186 (186), were set up to combine four different monoxenic nematode-bacterium complexes (DZ/S1, DZ/186, RG/S1, RG/186), in order to examine symbiotic specialisation, stability and pathogenic relationships among host/symbiont combinations. DZ showed a strong symbiotic specialisation relationship with its native symbiont S1, whereas RG was not as strongly specialised with its native symbiotic bacterial strain 186. The symbiotic stability of the DZ/S1 complex was stronger than that of DZ/186, as well as both RG/186 and RG/S1 complexes. Developmental rate and sex ratio (85.43% and 2.61, respectively) of the third infective larvae of monoxenic DZ/S1 complex were sharply higher than those of the DZ/186 complex (26.72 % and 0.63, respectively),but these parameters differed little between the RG/186 and RG/S1 complexes. The sizes of infective juveniles (IJs) of the different monoxenic nematode complexes did not differ significantly. Further studies found that after multigenerational passage through *Galleria mellonella* larvae *in vivo*, pathogen ratios of all four monoxenic nematodes appeared to decrease and were positively correlated with their pathogenicity to *G*. *mellonella*. Pathogen ratio and pathogenicity of DZ/186 declined sharply, whereas those of the other complexes declined smoothly. Our results suggested that *H*. *chongmingensis* has a more stable and also more specialized mutualistic symbiotic relationship with its native symbiotic bacterial strain S1, whereas *H*. *rugaoensis* has a stronger, more stable ability to carry non-native strains of *S*. *nematodiphila*.

ANALYSIS OF PRIMARY STRUCTURE LOOPS FROM HAIRPINS 35 AND 48 OF THE NEMATODA SSU RRNA GENE PROVIDES FURTHER EVIDENCE THAT THE GENERA *TRIPYLINA* BRZESKI, 1963, *TRISCHISTOMA* COBB, 1913 AND *RHABDOLAIMUS* DE MAN, 1880 ARE MEMBERS OF ENOPLIDA. **Zhao[1], Z., D. Li[2] and T.R. Buckley[1].** [1]Landcare Research, Private Bag 92170, Auckland, New Zealand; [2]Plant Health & Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland, New Zealand.

The primary structure of the small subunit (SSU) rRNA Hairpin 35 and 48 loops are highly conserved among multicellular animals in general. Rare nucleotide substitutions were reported in the evolutionarily conserved loops of Hairpin 35 and 48 of the SSU rRNA gene in all the marine Enoplida. This study focuses on the nucleotide substitutions in the SSU rRNA Hairpin 35 and 48 regions with the aim of investigating possible molecular synapomorphies among *Trischistoma* Cobb, 1913, *Tripylina* Brzeski, 1963 and *Rhabdolaimus* de Man, 1880 and other genera from Enoplida. A comparison of SSU rDNA sequences has revealed that a rare nucleotide transition A → G and a transversion G→Y have occurred simultaneously in the evolutionarily conserved loops of Hairpins 35 and 48 of the SSU rRNA gene of *Tripylina*, *Trischistoma* and *Rhabdolaimus*. The same pair of substitutions occurred in all Enoplida analysed but not in other nematodes. Analyses of the primary structure of the SSU rRNA gene seem to provide useful tools for nematode systematic studies. The nucleotide transition in the SSU rRNA Hairpin 35 and transversion in the SSU rRNA Hairpin 48 may have potential for use as a universal molecular diagnostic tool and also for design of selective primers and probes.

THE EFFECTS OF ENDOPHYTIC *PAECILOMYCES LILACINUS* AND *CHAETOMIUM GLOBOSUM* ON ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* AND COTTON APHID *APHIS GOSSYPII*. **Zhou[1], W., J. Starr[2] and G. Sword[1].** [1]Department of Entomology, Texas A&M University, College Station, TX, USA; [2]Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX, USA.

Both *Paecilomyces* and *Chaetomium* fungi have been reported to have anti-nematode activities. For *Paecilomyces* several studies have demonstrated their negative effects against insect and nematode herbivores. For *Chaetomium*, several metabolites (i.e., chetomin, chetocin, cochliodinol, etc) have been reported to negatively affect both insects and nematodes. We evaluated two endophytic fungal strains, *Paecilomyces lilacinus* and *Chaetomium globosum*, which were isolated from cotton plants in Texas, for their endophytic effects in cotton against both nematode and insect herbivores in greenhouse trials. Upon introducing either of the strains into cotton as an endophyte, both negatively affected root-knot nematode (*Meloidogyne incognita*) infection and reproduction. Similar anti-insect effects of endophytic *C. globosum* against phloem-feeding aphids (*Aphis gossypii*) were found in insect feeding assays under greenhouse conditions. Plant growth enhancement effects by both endophytes were also examined.

MAJOR SPERM PROTEIN IN THE SPERMATOZOA OF FREE-LIVING MARINE NEMATODE *ENOPLUS BREVIS* (ENOPLIA, ENOPLIDA). **Zograf[1,2], J. and K. Yakovlev[1].** [1]A.C. Zhirmunsky Institute of Marine Biology FEB RAS, 17 Paltchevski Str., 690041 Vladivostok, Russia; [2]Far Eastern Federal University, 8, Sukhanova str., 690950, Vladivostok, Russia.

Nematode spermatozoa are considered to represent an aberrant type of male gametes. In general, nematode spermatozoon is an amoeboid cell subdivided into an anterior pseudopodium and posterior main cell body. The major sperm protein (MSP) is a nematode specific small protein and is able to form filaments in the cytoplasm of spermatozoa and plays a role in the spermatoza motility. The structure of major sperm protein had been extensively studied. The presence of MSPhas not been proven in any of the free-living marine nematodes. Nevertheless, the free-living marine nematodes include taxa that preserve a large number of plesiomorphic features, thereby providing rich material for solving major problems in comparative histology and phylogeny of nematodes. In the present study we show MSP presence, in granular form. in spermatozoa of the enoplid nematode *Enoplus brevis*. The locality of MSP was studied using immunofluorescence and confocal microscopy. The MSP dissociate during sperm activation resulting in the appearance of fibrils. Such a structure of MSP has been shown for other nematodes, representatives of the class Chromadorea. Major sperm protein is thus found in both Enoplea and Chromadorea classes and could be considered as synapomorphy of the type Nematoda.