

32nd Symposium

Braga, Portugal
Universidade do Minho

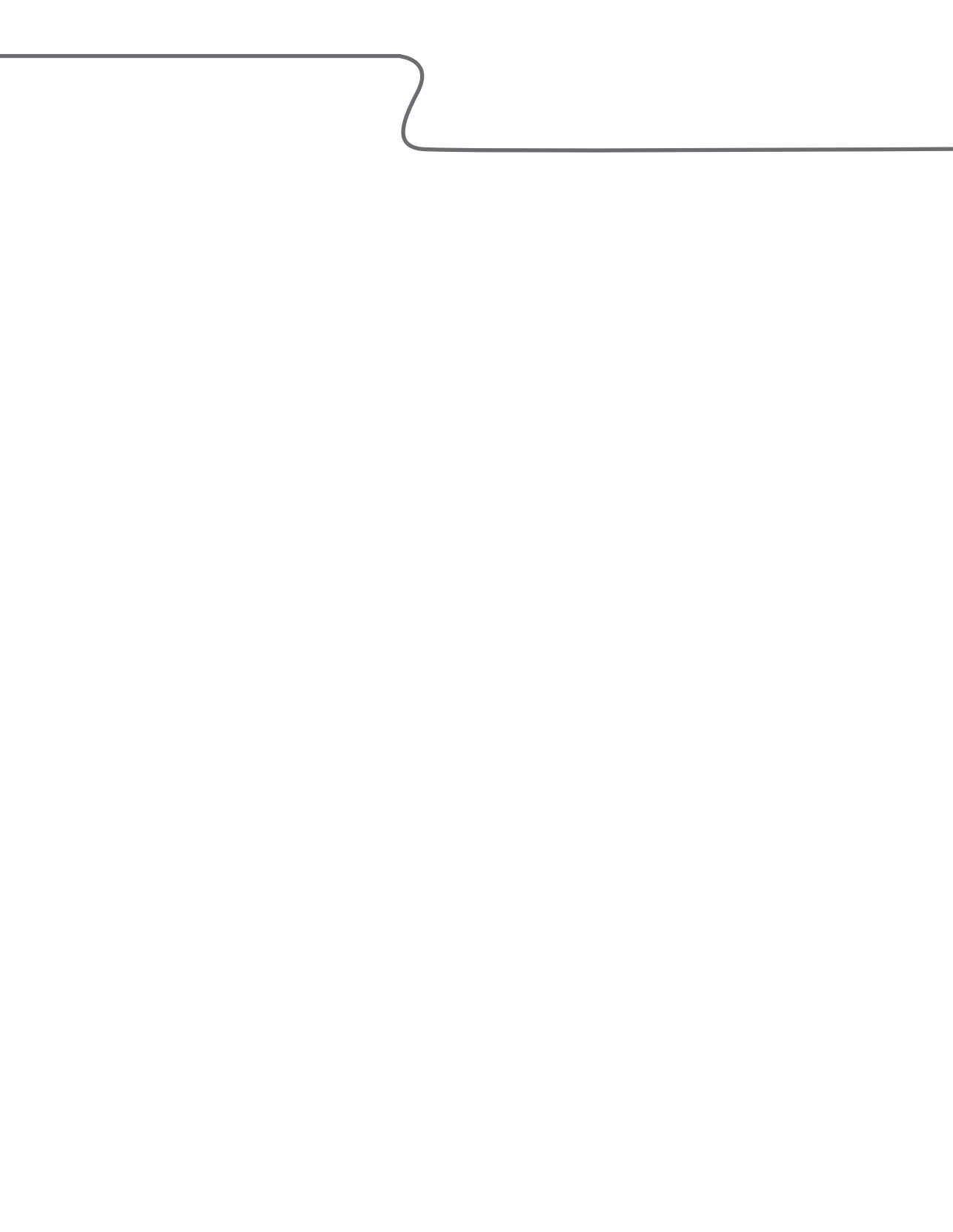
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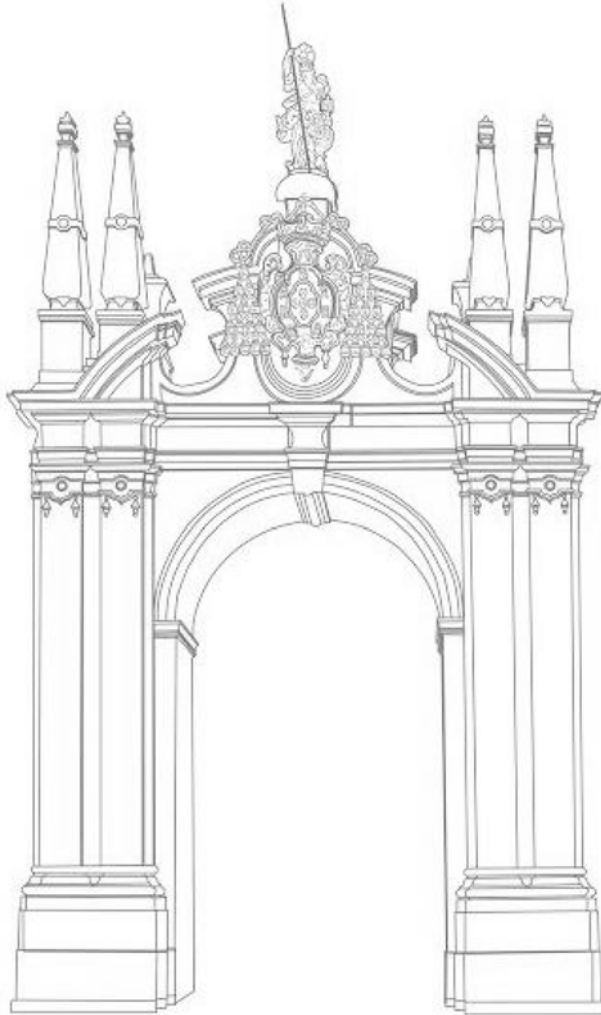
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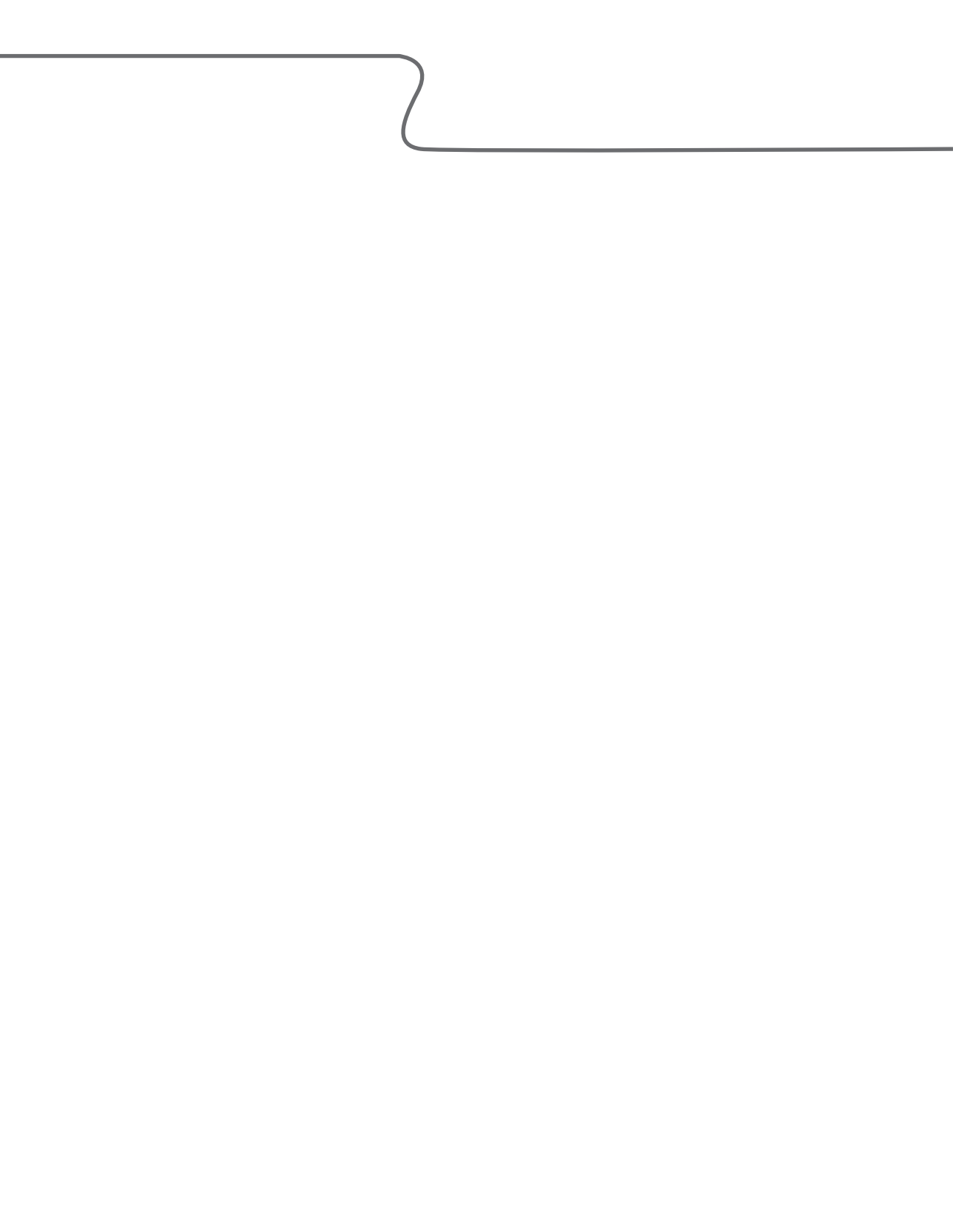
Welcome Message	7
Committees	9
Scientific Programme	11
Plenary lectures - Abstracts	17
Oral Abstracts	27
Poster Abstracts	181
Author Index	375
List of Participants	405





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WELCOME MESSAGE



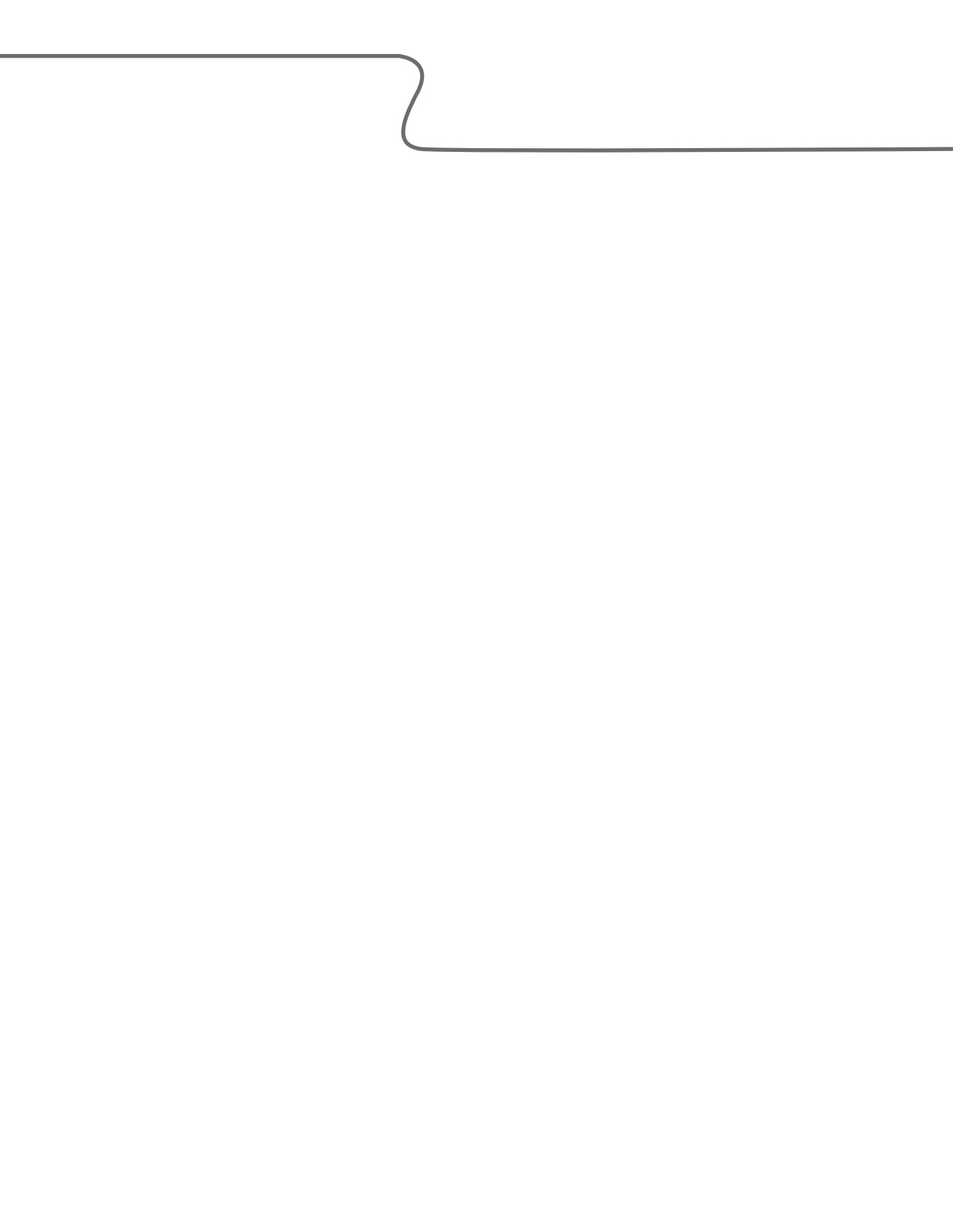
The Local Organizing Committee would like to welcome all of you and express a sincere appreciation to the European Society of Nematologists (ESN) for the choice of Braga “city of Baroque”, Portugal, as the place for the 32nd ESN Symposium. This meeting will be an international forum to gather researchers from around the world, to share and discuss their findings regarding the broad and interdisciplinary field of Nematology. Therefore, scientific contributions will cover a variety of topics including biocontrol, biodiversity, biology, ecology, epidemiology, genetics, management, phylogeny, quarantine, systematics and taxonomy. Throughout the meeting, we hope to create an atmosphere where everyone, students and professionals, can exchange ideas and establish collaborations.

We look forward to welcoming and hosting you in Braga, one of the most attractive cities in Portugal, and hope this meeting becomes an unforgettable moment.

On behalf of the Local Organizing Committee,

Maria Teresa M. Almeida

Chair of the Local Organizing Committee



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Centre for Functional Ecology, Department of Life Sciences,
University of Coimbra, Portugal

Maria Teresa Martins de Almeida

Centre of Molecular and Environmental Biology,
Department of Biology, University of Minho, Portugal

James Baldwin

Department of Nematology, University of California, Riverside, USA

Philippe Castagnone-Sereno

Institut Sophia Agrobiotech, INRA, Sophia Antipolis, France

Loes den Nijs

NPPO, National Reference Centre, Geertjesweg, Wageningen, The Netherlands

Ralf-Udo Ehlers

Department for Biotechnology and Biological Control.
Institute for Phytopathology. Christian-Albrechts-University Kiel, Kiel, Germany

Eric Grenier

Biology of Organisms and Populations applied to Plant Protection,
INRA, Domaine de la Motte, Le Rheu, France

Johannes Helder

Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

John Jones


The James Hutton Institute, Invergowrie, Dundee, UK

Chemical communication in nematodes via a modular glycolipid library <u>Stephan von Reuss</u>	19
Aging research in <i>Caenorhabditis elegans</i> : how to double a worm's lifespan? <u>Bart Braeckman</u>	20
Do soil microbial communities diversity modified plant-bioagressor interactions? <u>Christophe Mougél</u> , Anne-Marie Cortesero, Sylvain Fournet, Kevin Gazengel, Anne-Yvonne Guillerm-Erckelboudt, Tom Lachaise, Lionel Lebreton, Juliette Linglin, Maria Manzanares-Dauleux, Josselin Montarry, Denis Poinso, Catherine Porte	21
Pathogenic mechanisms of entomopathogenic nematodes <u>Nelson Simões</u>	22
Natural products from entomopathogenic bacteria and their role in symbiosis and pathogenicity <u>Helge Bode</u>	23
Utilising the potato genome for rapid mapping of disease resistances in wild <i>Solanum</i> species <u>Ingo Hein</u>	24
Immunomodulatory properties and therapeutic potential of the the secreted <i>Acanthocheilonema viteae</i> product, ES-62 <u>William Harnett</u> , Miguel A. Pineda, Felicity E. Lumb, David Rodgers, James Doonan, Colin J. Suckling and Margaret M. Harnett	25
Beyond the nematode genomes <u>Makedonka Mitreva</u>	26


- 01. PWN biology and epidemiology**
Convenors: Luis Fonseca, Alfonso Navas 29
- 02. Biocontrol of plant-parasitic nematodes**
Convenors: Yitzhak Spiegel, Aurelio Ciancio 34
- 03. Sensory perception and communication**
Convenor: Rolo Perry 39
- 04. Plant hormone/metabolite manipulations by nematodes**
Convenors: Shahid Siddiqui 42
- 05. Quarantine aspects and management of PWN in the EU**
Convenors: Edmundo Sousa, M. Clara A. Serra 45
- 06. Plant-mediated interactions between nematodes and other organisms**
Convenor: Matthias Erb 49
- 07. A. Plant response to nematode infection (compatible)**
Convenors: Godelieve Gheysen, Carolina Escobar 54
- B. Plant response to nematode infection (compatible)**
Convenors: Godelieve Gheysen, Carolina Escobar 59
- 08. Phylogeny and parasitism evolution in nematodes**
Convenors: James Baldwin, Mark Blaxter 64
- 09. Biogeography and impact of climate change**
Convenor: Johannes Helder 67
- 10. Synthetic and natural compounds for nematode control**
Convenor: Brigitte Slaats 69


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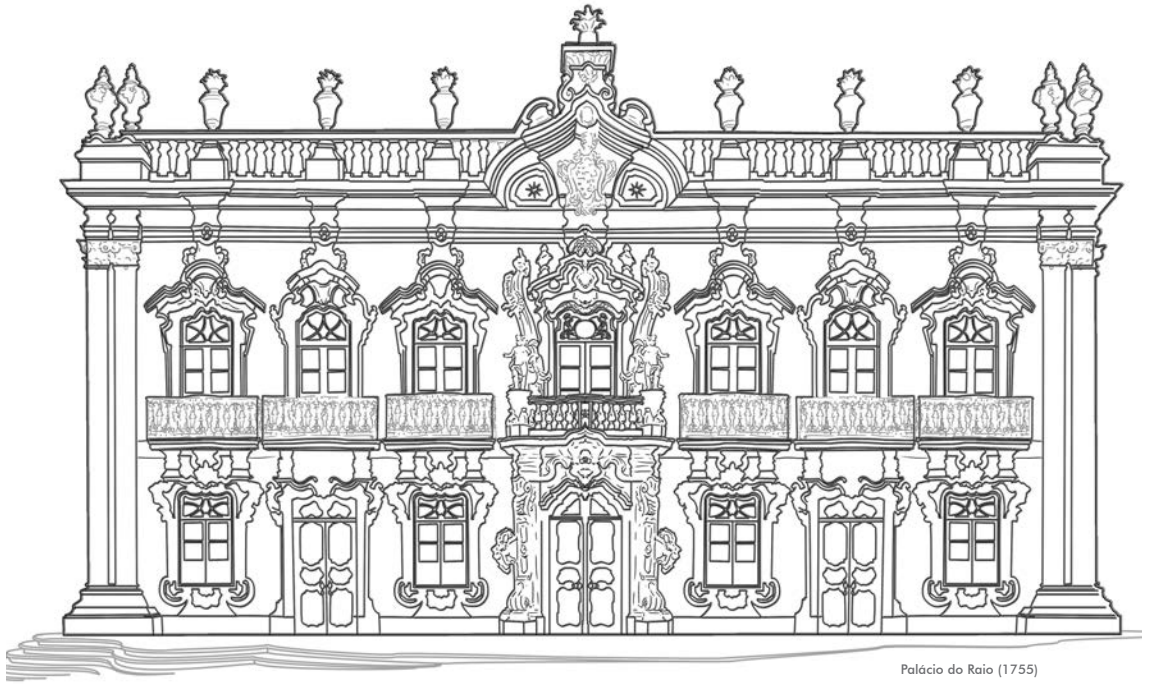
- | | | |
|---|---|-----|
| 11. EPN biology and diversity | Convenors: Vladimir Puza, Bart Brackman | 74 |
| 12. Plant-parasitic nematode management in tropical and arid crops | Convenor: Richard Sikora | 79 |
| 13. Intraspecific diversification and genetic structure of nematode populations | Convenors: Eric Grenier, Vivian Blok | 84 |
| 14. Applications of EPN in agriculture | Convenors: Ralf-Udo Ehlers, Javad Karimi | 89 |
| |  <p>sponsored by
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BIOLOGICAL SYSTEMS</p> | |
| 15. A. Plant-parasitic nematodes management in temperate crops - integrated control strategies | Convenors: Leendert Molendijk, Wim Wesemael | 94 |
| B. Plant-parasitic nematodes management in temperate crops - methodology | Convenor: Thomas Been, Loes den Nijs | 99 |
| C. Plant-parasitic nematodes management in temperate crops - nematode control methods | Convenors: Michaela Schlatthölter, Matt Back | 104 |
| 16. A. Nematodes as bio-indicator in terrestrial, fresh water and marine habitats | Convenors: Maria Viketoff, Thomaé Kakouli-Duarte | 110 |
| B. Nematodes as bio-indicator in terrestrial, fresh water and marine habitats | Convenors: Maria Viketoff, Thomaé Kakouli-Duarte | 115 |

- 17. A. Nematode effectors including suppressors of host plant resistance**
Convenors: Sophie Mantelin, Jose Lozano-Torres 120
- B. Nematode effectors including suppressors of host plant resistance**
Convenors: Sophie Mantelin, Jose Lozano-Torres 125
- 18. A. Ecology and genetics of nematode communities**
Convenors: Pablo Castillo, Thierry Mateille 130
- B. Ecology and genetics of nematode communities**
Convenors: Pablo Castillo, Thierry Mateille 135
- 19. A. Nematode genomics and transcriptomics**
Convenors: Etienne Danchin, Sebastian Eves-van den Akker 140
- B. Nematode genomics and transcriptomics**
Convenors: Etienne Danchin, Sebastian Eves-van den Akker 145
- 20. Quarantine aspects in nematology**
Convenors: Björn Niere, Nicole Viaene 151
- 21. Sustainable management of plant resistance and nematode adaptation**
Convenors: Sofia Costa, Soledad Verdejo 156
- 22. A. Nematode systematics and taxonomy**
Convenors: Wilfrieda Decraemer, Reyes Peña-Santiago 161
- B. Nematode systematics and taxonomy**
Convenor: Wim Bert 166
- 23. Molecular basis of nematode resistance and avirulence**
Convenors: Aska Goverse, Caroline Djian-Caporalino 171
- 24. Species delineation, molecular barcodes and diagnostics**
Convenors: Geraldine Anthoine, Sebastian Kiewnick 176

- | | | |
|--|--|-----|
| 01. PWN biology and epidemiology | | 183 |
| 02. Biocontrol of plant-parasitic nematodes | sponsored by
 | 190 |
| 03. Sensory perception and communication | | 209 |
| 04. Plant hormone/metabolite manipulations by nematodes | | 216 |
| 05. Quarantine aspects and management of PWN in the EU | | 219 |
| 06. Plant-mediated interactions between nematodes and other organisms | | 224 |
| 07. Plant response to nematode infection (compatible) | | 229 |
| 08. Phylogeny and parasitism evolution in nematodes | | 241 |
| 09. Biogeography and impact of climate change | | 242 |
| 10. Synthetic and natural compounds for nematode control | sponsored by

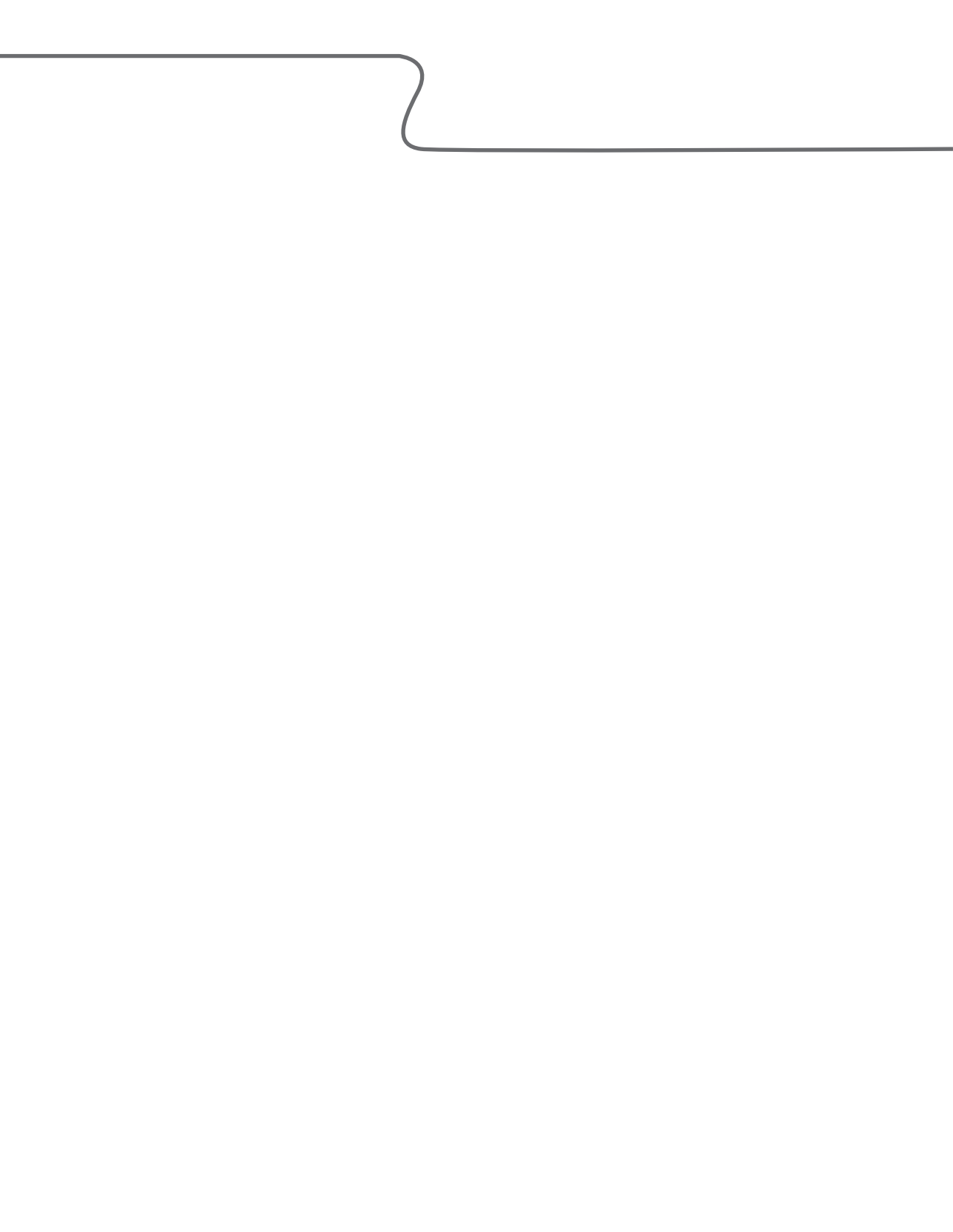
<i>The miracles of science™</i> | 246 |
| 11. EPN biology and diversity | | 259 |
| 12. Plant-parasitic nematode management in tropical and arid crops | | 267 |
| 13. Intraspecific diversification and genetic structure of nematode populations | | 274 |
| 14. Applications of EPN in agriculture | sponsored by
 | 277 |

15. Plant-parasitic nematodes management in temperate crops	282
16. Nematodes as bio-indicator in terrestrial, fresh water and marine habitats	301
17. Nematode effectors including suppressors of host plant resistance	312
18. Ecology and genetics of nematode communities	321
19. Nematode genomics and transcriptomics	327
20. Quarantine aspects in nematology	331
21. Sustainable management of plant resistance and nematode adaptation	334
22. Nematode systematics and taxonomy	340
23. Molecular basis of nematode resistance and avirulence	362
24. Species delineation, molecular barcodes and diagnostics	366



Palácio do Raio (1755)

PLENARY LECTURE ABSTRACTS



Chemical communication in nematodes via a modular glycolipid library

Stephan von Reuss

University of Neuchâtel, Laboratory of Bioanalytical Chemistry, Institute of Chemistry, Avenue de Belleveaux 51, CH-2000 Neuchâtel, Switzerland

corresponding author: svonreuss@ice.mpg.de

Nematode communication via chemical signals has been known since the 1960s but the identity of the corresponding effectors has remained elusive for many decades. In recent years advances in analytical chemistry and the development of highly sensitive and selective mass spectrometric screens uncovered a widely conserved modular library of nematode-derived glycolipids based on the 3,6-dideoxysugar ascarylose. The ascarosides are involved in a large diversity of biological processes, the extent of which has just started to emerge, including the regulation of nematode development and behaviour as well as cross-kingdom interactions with plants and fungi. While ascaroside signalling appears to be highly conserved, more detailed analysis of just a few species demonstrates that the ascaroside core structures also serve as a scaffold for the combinatorial assembly of additional units derived from various primary metabolic pathways (such as carbohydrate, fatty acid, amino acid, and nucleoside metabolism) to generate a fascinating array of species-specific signalling components that are considered to provide highly dedicated 'private' channels for intraspecies communication in sympatric nematodes. Comparative analysis of mutant metabolomes along with stable isotope labelling techniques revealed how biosynthetic precursors from the environment are integrated into ascaroside signals and enabled the identification of several genes and primary biosynthetic pathways that have been co-opted for ascaroside biosynthesis.

Keywords: Biosynthesis; chemical analysis; chemical communication; glycolipids; modular library.

Aging research in *Caenorhabditis elegans*: how to double a worm's lifespan?Bart Braeckman

Biology Department, Ghent University, B-9000 Ghent, Belgium

corresponding author: Bart.Braeckman@ugent.be

Ageing research in *Caenorhabditis elegans* started four decades ago, but only gained major attention in the late 1990s when the first genetic pathway that modulates lifespan was characterised in detail: the insulin/IGF-like signalling pathway (IIS). Genetic interactions, tissue-specificity and downstream targets of this pathway were scrutinised because many genes involved have human homologues. Yet, we should realise that longevity of IIS mutants is likely the result of the activation of a dauer-like program that allows a switch to a specialised physiological state which is non-existent in humans. A key component of this physiology is the shift from protein synthesis to enhanced fat and glycogen accumulation. These accumulations can be used for the synthesis of the protectant trehalose which stabilises proteins and membranes. The twofold lifespan extension in IIS mutants is spectacular and, understandably, it is widely covered in the literature with currently about one new publication every three days. However, the neglected axenic dietary restriction treatment also results in a twofold lifespan extension and acts via independent genetic pathways. We are now exploring its genetic components and recently found that it acts via activation of the CREB-binding protein in the GABAergic neurons. Also, the mitochondrial unfolded protein response is activated under axenic dietary restriction but it is not linked to the longevity phenotype.

Keywords: Aging; axenic dietary restriction; *Caenorhabditis elegans*; IIS pathway.

Do soil microbial communities diversity modified plant-bioagressor interactions?

Christophe Mougel, Anne-Marie Cortesero, Sylvain Fournet, Kevin Gazengel, Anne-Yvonne Guillerm-Erckelboudt, Tom Lachaise, Lionel Lebreton, Juliette Linglin, Maria Manzanares-Dauleux, Josselin Montarry, Denis Poinot and Catherine Porte

INRA, Université Rennes 1, AGROCAMPUS OUEST, Institut de Génétique, Environnement et Protection des Plantes, UMR1349, Domaine de la Motte, F-35653 Le Rheu, France

corresponding author: Christophe.Mougel@rennes.inra.fr

The consequences of decline in biodiversity for ecosystem processes and functioning have long been of considerable interest. The significance of biodiversity loss in microbial communities is challenged by the concept of functional redundancy. Since different species can have the same function in ecosystems, functional redundancy predicts that the loss of species does not necessarily alter ecosystem functioning because of their replacement by other species for maintaining processes. Therefore, it is classically assumed that ‘the more, the better’ in terms of biodiversity levels; however, species-rich microbial communities potentially led to high competition and antagonistic interactions that may reduce community functioning, and may have consequences on plant growth and health or soil biological barrier effect against pathogens. We investigate the consequence of *Brassica napus* - microbial communities diversity interaction on different bioagressors fitness following a life-history traits approach. Microbial diversity was manipulated through a removal-recolonisation experiment. The impact of the removal experiment on the microbial diversity was evaluated using high-throughput sequencing. *Brassica napus* were cultivated in these soils and confronted to three different bioagressors: the protist *Plasmodiophora brassicae*, the nematode *Heterodera schachtii* and the cabbage root fly *Delia radicum*. The effects of soil microbial communities on each bioagressor were evaluated by measuring different life history traits, which are a reliable estimate of bioagressor feeding success and expected fitness of the system. The result obtained showed a reduction of disease index or bioagressors fitness in the less diverse communities. These results are discussed in regard of plant metabolites and community ecology.

Keywords: Bioagressors interactions; *Brassica napus*; microbial communities.

Pathogenic mechanisms of entomopathogenic nematodes

Nelson Simões

Centro de Biotecnologia dos Açores, Universidade dos Açores, Rua da Mãe de Deus, 9500-801
Ponta Delgada, Portugal

corresponding author: Nelson.JO.Simoes@azores.gov.pt

Entomopathogenic nematodes are a helminth-bacteria association able to parasitise and to kill a relatively large number of insects, and therefore are used in biological control. Despite not fully known, the mechanisms underlying the parasitic process and the associated disease show that both organisms are able to induce mortality in the insect. Host invasion is accomplished by the nematode that carries the bacteria into insect haemocoel, where the bacteria is released initiating development. Invasion seems to be supported by released enzymes. The analysis of transcripts and secreted – excreted products (ESP) of *Steinernema carpocapsae* during the initial phase of parasitism reveals a great amount of serine proteases. The most abundant serine protease (Sc-SP-1) released during this phase was shown to be able to bind to insect midgut epithelium and to cause cell detachment forming holes in basal lamina. Another serine protease caused histolysis in the insect mid-gut and induced apoptosis in Sf9 insect cells, thus suggesting its involvement in invasion. In ESP protease inhibitors were also found that interact with insect defences. That is the case of a kunitz that targeted recognition proteins of insect immune system such as masquerade-like and serine protease-like homologues in a protein-protein assay. Another protease inhibitor found in ESP forms a complex with three proteins in the haemolymph of lepidopteran required for clotting, apolipoporphin, hexamerin and trypsin-like, thus impairing immune clot reaction of parasitised insects. The associated bacteria *Xenorhabdus nematophila* also expresses and releases a few proteases, which knockout of the encoding gene significantly reduces the virulence. These findings highlight the mechanisms of pathogenicity the entomopathogenic nematodes and are opening new avenues in the improvement of these organisms as biological control agents.

Keywords: Proteases; protease inhibitors; secreted - excreted products; transcripts; virulence factors.

Natural products from entomopathogenic bacteria and their role in symbiosis and pathogenicity

Helge Bode

Department of Biosciences and Buchmann Institute for Molecular Life Sciences (BMLS), Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

corresponding author: h.bode@bio.uni-frankfurt.de

Heterorhabditis and *Steinernema* nematodes live in symbiosis with *Photorhabdus* and *Xenorhabdus* bacteria, respectively. During the last years we and others have studied the natural product from these bacteria and could identify several antibiotic or cytotoxic compounds. However, our main goal is to find the natural function of these natural products in the natural system including nematodes, insect larvae and additional food competitors. Thus, we have developed molecular biology and chemical tools to identify these compounds and to identify their targets. We have also generated several mutants in the bacteria that show the importance of natural products for nematode development. Upon manipulation of global regulators we could show that all natural product biosynthesis are influenced, leading to severe loss in nematode development. We have also identified transcriptional regulators that we regard as 'insect' sensors since they are widespread in insect-associated bacteria and their deletion results in a decrease of insect virulence.

Keywords: *Heterorhabditis*; natural product; *Photorhabdus*; *Steinernema*; *Xenorhabdus*.

Utilising the potato genome for rapid mapping of disease resistances in wild *Solanum* species

Ingo Hein

The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

corresponding author: ingo.hein@hutton.ac.uk

Potato is the most important non-cereal food crop worldwide and consumed by more than a billion people. Pests and pathogens represent a serious and continuing threat to potato production. Some of the most widespread and economically significant diseases are late blight, nematodes and viruses. The ability to withstand multiple biotic and abiotic stresses is critical for wild potatoes. With the availability of significant germplasm resources including the Commonwealth Potato Collection (CPC) at the James Hutton Institute and improved genomics methods, the potential to exploit natural diversity is considerable to secure potatoes as a major food source in the future. Sequencing the potato genome has provided unprecedented insight into the distribution and organisation of genes that underpin resistances. We have developed gene-specific target enrichment and sequencing platforms to more rapidly map and clone functional pattern-recognising receptors as well as NB-LRR genes. Using target enrichment in combination with bulked-segregant analysis and long sequencing technology, resistances to *Globodera pallida*, *Phytophthora infestans* and Potato Virus Y have been mapped and candidate genes identified.

Keywords: Disease resistance; genome; potato; *Solanum*.

Immunomodulatory properties and therapeutic potential of the secreted *Acanthocheilonema viteae* product, ES-62

William Harnett¹, Miguel A. Pineda², Felicity E. Lumb¹, David Rodgers², James Doonan¹, Colin J. Suckling³ and Margaret M. Harnett²

¹ Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G4 0RE, UK

² Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow G12 8TA, UK

³ Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, UK

corresponding author: w.harnett@strath.ac.uk

ES-62, the major secreted product of the adult stage of the rodent filarial nematode, *Acanthocheilonema viteae*, is immunomodulatory by virtue of covalently attached phosphorycholine (PC) moieties. The molecule has a number of effects on the immune system, including reducing IL-17 responses, polarising dendritic cell maturation, inhibiting mast cell degranulation and inducing generation of regulatory B cells. ES-62 acts by subverting immune system cell signal transduction pathways, for example, in macrophages its activity is dependent on the expression of TLR4 and the nematode product causes autophagolysosomal degradation of the TLR adaptor, MyD88. The ability of a range of parasitic worm species to protect against disease development in mouse models of allergy and autoimmunity has contributed to the belief that anti-inflammatory drugs can be developed from these organisms. This is supported by ES-62's immunomodulatory activity allowing it to protect against development of pathology in mouse models of rheumatoid arthritis, systemic lupus erythematosus, skin inflammation and asthma. Such results have encouraged us to generate novel drug-like ES-62 Small Molecule Analogues (SMA) that are based around the active anti-inflammatory PC moiety. Investigation of the effects of these SMAs in the mouse models of inflammatory diseases has revealed that they broadly mirror ES-62 in protective activity and generally share the same mechanisms of action. Finally, the SMA have registered favourable ADMET analysis, can inhibit inflammatory responses of human immune system cells *in vitro*, and have recently been found to be protective in a mouse model (collagen-induced arthritis) when administered as dendritic cell therapy. Overall therefore, subject to satisfactory necessary further pre-clinical analysis, the SMA may be suitable for phase I clinical trials.

Keywords: Immunomodulation; secreted product; therapy.

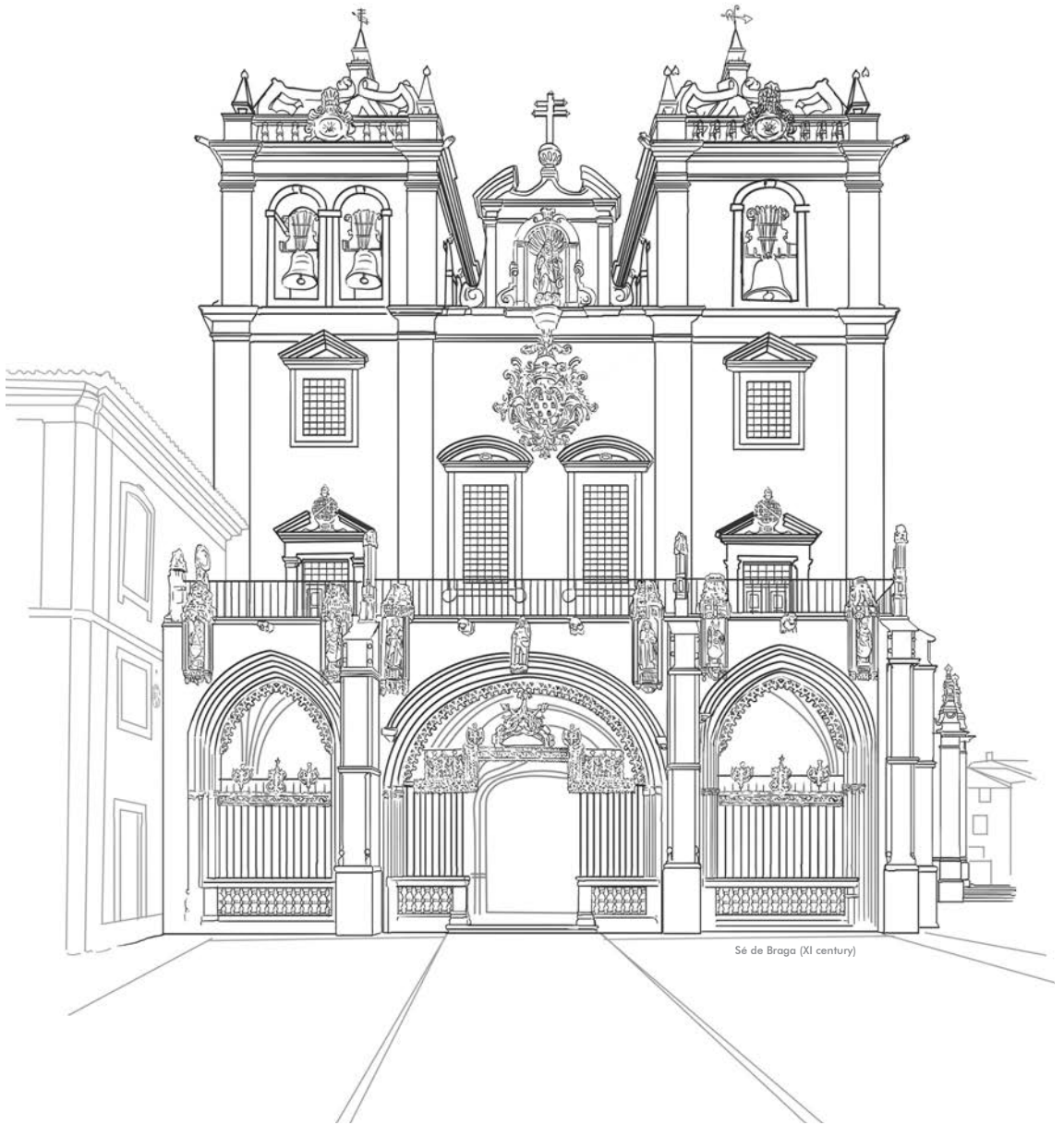
Beyond the nematode genomesMakedonka Mitreva

McDonnell Genome Institute and Department of Medicine, Washington University School of Medicine, 4444 Forest Park Boulevard, St. Louis, MO 63108, USA

corresponding author: mmitreva@genome.wustl.edu

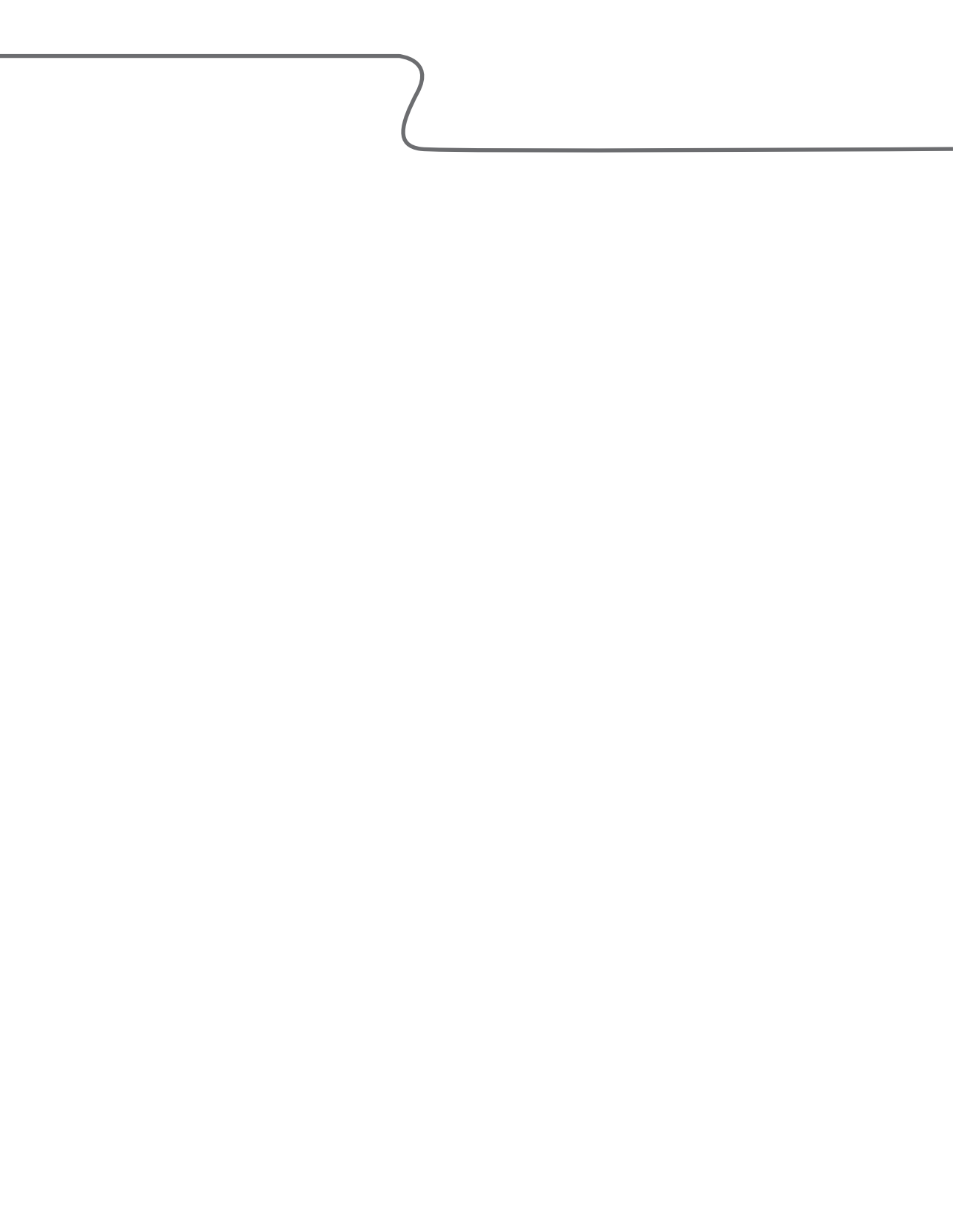
There has been constant increase in the number of nematode genomes published (and in progress), which is a trend that correlates to ever-improving molecular techniques for obtaining biological material, performing DNA extraction and library construction, and predominantly to ever-evolving sequencing chemistry and instrumentation, as well as to decreased sequencing costs. These nematode genomes provide an invaluable resource to enable development of postgenomic tools to investigate the immunobiology of parasitic nematode diseases and accelerate discovery for the development of vaccines and diagnostics. Such post-genomics tools (functional genomics, proteomics, interactomics *etc.*) provide a basis for a multi-omics approach to obtain omics-based discovery of information essential for developing of novel diagnostics, vaccines and anthelmintics. It is essential to follow-up to the genome project by depicting the complexity of the nematode proteome (structure and function), evolutionary insights gained from comparative analysis, and a subsequent laboratory studies to confirm the predictions. Specific post-genomic application we are working on will be presented, including i) bioinformatics and cheminformatics approaches, along with laboratory screening of nematodes spanning the phylum Nematoda, that have led to identify and characterise targets with a broad-control potential, ii) systems biology approach including immunoaffinity chromatography and mass spectrometry to efficiently identify candidate diagnostic antigens, and iii) deep-sequencing approaches to routinely characterise clinical isolates and through variant studies explain varied pathogenicity and responsiveness to treatment.

Keywords: Comparative omits; genomics; next generation sequencing; post-genomic applications.



Sé de Braga (XI century)

ORAL ABSTRACTS



An unique set of biomarkers for differentiating populations of the pinewood nematode *Bursaphelenchus xylophilus* as revealed by quantitative proteomics and Character Compatibility Analysis

Sergio Ciordia¹, Lee Robertson², Susana C. Arcos², María Rosa González², María del Carmen Mena¹, Paula Zamora³, Paulo Vieira⁴, Isabel Abrantes⁵, Manuel Mota⁴, Philippe Castagnone-Serenó⁶ and Alfonso Navas²

¹ Centro Nacional de Biotecnología, CSIC, calle Darwin 3, Campus de Cantoblanco, Madrid 28049, Spain

² Museo Nacional de Ciencias Naturales, CSIC, calle José Gutiérrez Abascal 2, Madrid 28006, Spain

³ Centro de Sanidad Forestal de Calabazanos, Junta de Castilla y León, Villamuriel de Cerrato, s/n. 34190 Palencia, Spain

⁴ NemaLab/ ICAAM (Instituto de Ciências Agrárias e Ambientais Mediterrânicas), Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal

⁵ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

⁶ INRA UMR1355/Université de Nice-Sophia Antipolis/CNRS UMR7254, Institut Sophia Agrobiotech, 400 route des Chappes, F-06603, Sophia Antipolis, France

corresponding author: anavas@mncn.csic.es

Bursaphelenchus xylophilus (the pinewood nematode; PWN) is a severe forest pathogen in countries where it has been introduced and is considered a worldwide quarantine organism. Protein markers for differentiating populations of this nematode were identified by studying differences among four selected Iberian and one American population. The Iberian populations were selected because they have different phylogenetic background. These populations were compared by quantitative proteomics (iTRAQ) approaches. Two biological replicates were performed for each population and three technical replicates for each biological replicate. A total of 2860 expressed proteins were identified in the populations proteomes using the public database from the *B. xylophilus* genome project, but only 216 proteins were unambiguous and significantly differentially regulated in the studied populations. Comparisons of their pairwise ratio were statistically treated and supported in order to convert them into discrete character states, suggesting that 141 proteins were not informative as population specific markers. Application of the Character Compatibility Analysis (Perfect Phylogeny criterion) on the remaining 75 proteins (belonging to families with different biological functions) excludes 27 which are incompatible among them. Considering only the compatible proteins, the method selects a subset of 30 specific unique protein markers which allowed the compared classification of the Iberian isolates. This approach makes it easier search for diagnostic tools and phylogenetic inference within species and populations of a pathogen exhibiting a high level of genetic diversity.

Keywords: Biomarkers; iTRAQ.

Comparative proteomic analysis of *Bursaphelenchus xylophilus* and *B. mucronatus* secretomes

Joana M.S. Cardoso¹, Sandra I. Anjo^{2,3}, Luís Fonseca¹, Bruno Manadas³,
Conceição Egas³ and Isabel Abrantes¹

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² Faculty of Sciences and Technology, University of Coimbra, Rua Silvio Lima, Pólo II, 3030-790 Coimbra, Portugal

³ CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3004-504 Coimbra, Portugal

corresponding author: joanasa_cardoso@hotmail.com

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is an invasive species responsible for the development of the pine wilt disease. The PWN has been recognised worldwide as a major forest pest, causing several economic and ecological problems. On the other hand, *B. mucronatus*, a close related species with similar morphological and ecological characteristics, clearly differs from *B. xylophilus* on its pathogenicity, making this nematode a good candidate for comparative analysis studies. In the present study, secretome profiles of *B. xylophilus* and *B. mucronatus* were obtained and proteomics differences between these two secretomes were evaluated using a quantitative SWATH-MS approach. From the 609 proteins initially identified, 422 were quantified and compared between *B. xylophilus* and *B. mucronatus* secretomes and from these, 243 proteins were found differentially regulated: 158 and 85 proteins were increased in *B. xylophilus* and *B. mucronatus* secretomes, respectively. While *B. xylophilus* secretome revealed a strong enrichment in proteins with peptidase activity, the oversecreted proteins in *B. mucronatus* were mainly related to oxidative stress responses. The changes in protein levels were validated at transcript level by reverse transcription quantitative real time PCR. The comparative analysis here presented expands our knowledge about the molecular basis of *B. xylophilus* and *B. mucronatus* interaction with their hosts and supports the hypothesis of a key role of secreted peptidases in *B. xylophilus* pathogenicity.

Keywords: Pathogenicity; peptidases; pinewood nematode; secreted proteins.

Stage-specific transcriptomic analysis of the pinewood nematode, *Bursaphelenchus xylophilus*

Suguru E. Tanaka¹, Ryusei Tanaka², Yuko Takeuchi-Kaneko³, Kenji Fukuda¹, Natsumi Kanzaki⁴ and Taisei Kikuchi²

¹ Laboratory of Forest Botany, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8687, Japan

² Division of Parasitology, University of Miyazaki, 5200 Kihara, Kiyotake-cho, Miyazaki-city, Miyazaki, 889-1692, Japan

³ Laboratory of Terrestrial Microbial Ecology, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan

⁴ Forest Pathology Laboratory, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba-city, Ibaraki, 305-8687, Japan

corresponding author: sgr.tnk.021101@fr.a.u-tokyo.ac.jp

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease, which has been severely damaging pine forests in Asia and Europe. Although previous studies have revealed some aspects of the disease, details of the mechanism remain unclear. In this study, we obtained stage-specific RNA-seq data of PWN to understand gene expression patterns along the development of the nematode. RNA samples of each developmental stage of the nematode (eggs, second-, third- and fourth-stage juveniles, and adult male and adult female) were prepared from synchronously-cultured PWN. Dispersal third- and fourth- (DIII and DIV) stage juveniles were produced using a long-incubation method and co-culture with *Monochamus* beetles, respectively. RNA-seq was obtained using an Illumina HiSeq sequencer, mapped to the PWN reference genome (v2.0) and FPKM values of each gene were calculated. To compare gene expressions among developmental stages, first, we excluded 'Oscillation' genes, which seemed oscillating in each stage because of their involvement in the moulting process, using sequence similarity to *Caenorhabditis elegans* 'Oscillation' genes. PWN genes except "Oscillation" were categorised into three types, 'Flat', 'Rising' and 'Others' ('Others' are the remaining genes not included in the other three categories). 'Flat' contained many house-keeping and growth-related genes; 'Rising' included many reproduction-related genes; and 'Others' consisted of some DNA-packaging and PWN-specific genes. When we compared gene expressions of dispersal stages (DIII and DIV) with those of propagative stages, genes with some GO terms, such as 'lipid catabolic process' and 'neuropeptide signalling pathway' were found to be up-regulated in the dispersal stages, which are exposed stressful conditions, e.g., dried and starving condition in dead trees or in trachea of the beetle. Gene expression patterns of PWN provided in this study will be an important resource to fully understand the mechanism of pine wilt disease.

Keywords: Dispersal juveniles; gene expression category; *Monochamus* beetles; propagative juveniles; RNA-seq.

Pinewood nematode effectors: different approaches to predict new genes involved in parasitism

Margarida Espada^{1,2}, Sebastian Eves-van den Akker³, Ana Cláudia Silva⁴, Peter J.A. Cock², Manuel Mota^{1,5} and John T. Jones^{2,6}

¹ NemaLab/ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal

² Cell and Molecular Sciences Group / Information and Computer Sciences group (PJAC), The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

³ Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

⁴ Área de Fisiología Vegetal, Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla La Mancha, 45071 Toledo, Spain

⁵ Departamento de Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal

⁶ School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9TZ, UK

corresponding author: mespada@uevora.pt

The migratory plant-parasitic nematode *Bursaphelenchus xylophilus* is unique in having phytoparasitic and mycetophagous phases throughout its life cycle. During its phytoparasitic stage, the nematode migrates within pine trees feeding on the contents of cortex and xylem parenchymal cells. Interactions of the nematode with the plant host are mediated by effectors - secreted proteins originating from the pharyngeal gland cells. We have been using next-generation sequencing and bioinformatics in a non-biased approach to identify novel effectors from this nematode. We have sequenced the transcriptome of *B. xylophilus* and compared gene expression in the mycetophagous and plant-parasitic stages to identify genes likely involved in plant parasitism. Currently, we are analysing the upstream regions of these novel effectors in order to identify putative promoter elements associated with effectors, which can subsequently be used to predict further effector sequences. The transcriptome analysis approach has allowed us to identify a panel of candidate effectors. We have used *in situ* hybridisation to demonstrate that some of these are expressed specifically in the pharyngeal gland cells. The data obtained to date suggest that effectors are one key part of a multi-layered detoxification strategy deployed by *B. xylophilus*. In order to protect itself from host defence responses, it appears that *B. xylophilus* secretes detoxification enzymes into the host, while simultaneously upregulating other detoxification enzymes within its digestive system. We showed that one of these enzymes – a sigma class glutathione S transferase - has a protective activity against defence compounds produced by the host. These data represent the most comprehensive analysis of novel effectors from this nematode to date.

Keywords: *Bursaphelenchus xylophilus*; detoxification metabolism; parasitism genes; plant-nematode interaction; transcriptome.

Perspectives on the endophytic microbiome of pine trees affected by pine wilt disease: from cultivation to Next-Generation Sequencing

Diogo Neves Proença¹, Romeu Francisco¹, Marisa Carvalho², Susanne Kublik³, Anne Schöler³, Gisle Vestergaard³, Michael Schloter³ and Paula V. Morais^{1,2}

¹ CEMUC, University of Coimbra, 3030-788 Coimbra, Portugal

² Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

³ Research Unit Environmental Genomics, Helmholtz Zentrum München, 85764 Munich, Germany

corresponding author: diogo.proenca@gmail.com

Pine wilt disease (PWD) is one of the most devastating forest diseases in the world with tremendous economic, social and environmental impact. This study aimed to assess the endophytic microbiome (Bacterial and Archaeal) of PWD-susceptible *Pinus pinaster* and the function of bacterial endophytes. The pine tree endophytic microbiome was diverse and rich, composed of 55 different bacterial classes detected in total (37 assigned and 18 not assigned), using Illumina MiSeq. The main components of the community, shared by cultivation and molecular methods, belonging to Proteobacteria, class Gammaproteobacteria that, according to the sampling area, could include also Firmicutes. Six families were the most abundant found in all symptomatic classes from both sampling areas – Bacillaceae, Burkholderiaceae, Enterobacteriaceae, Comamonadaceae, Sphingomonadaceae and Xanthomonadaceae. Archaea were found as part of the endophytic microbiome, most with low similarity to previously described archaea. Based on the endophytic microbiomes, trees grouped according to the sampling area. Symptomatic declining trees showed higher diversity indexes; that might result from the tree's loss of selective ability or control of the endophytes. The core microbiome composed of the genera *Methyloversatilis*, *Ochrobactrum*, *Bacillus*, *Burkholderia*, *Dyella* and *Terriglobus* showed proteolytic, cellolytic and lipolytic activity, produced siderophores, but they were present in different numbers at the different symptomatic stages of the tree. These functions might have a role inside the pine trees and within interactions with pinewood nematode. The modifications observed in endophytic microbiome diversity at different symptomatic stages could include the contribution of the nematode-carried organisms. This study showed the endophytic microbiome of healthy and PWD trees and intends to contribute to the understanding of its role in pine disease.

Keywords: Bacteria; Illumina; microbiome; nematode; pine wilt disease.

Biological control of nematodes: from soil suppression and the search for a silver bullet to synthetic biology

Keith Davies

Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

corresponding author: keith.davies@kgdavies.com; k.davies@herts.ac.uk

Nematode suppressive soils are a well-documented phenomenon and a whole host of microbes have been implicated. Commercial agrochemical companies are looking for a single silver bullet that will replace nematicides; these must be environmentally benign and also compatible with other crop protection methods. Baker and Cook's schematic biological interaction model will be applied to crop, nematodes and their natural enemies to contextualise the issues. To date there are no successful biological control agents that can routinely replace nematicides for use as a silver bullet in the field situation. However, molecular biology has revolutionised our understanding of host-parasite interactions from nematode trapping fungi to bacterial wart disease. Various platforms by which bacteria can be engineered are now available and novel biological functions can be added to both Gram-positive and Gram-negative bacteria; some of these platforms will be reviewed. Finally, the question around whether or not these new approaches may offer a way forward for biological control will be presented.

Keywords: *Bacillus*; genetic engineering; *Pasteuria*; trapping fungi.

Nematode cysts – a promising source for biological control

Samad Ashrafi^{1,2}, Maria R. Finckh², Abdelfattah A. Dababat³ and Wolfgang Maier¹

¹ Julius Kühn-Institut, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

² University of Kassel, Faculty of Organic Agricultural Sciences, Ecological Plant Protection, Witzenhausen, Germany

³ International Maize and Wheat Improvement Centre (CIMMYT), Ankara, Turkey

corresponding author: samad.ashrafi@julius-kuehn.de

Nematode-infested trial fields of CIMMYT-Turkey displaying soil suppressiveness against the cereal cyst nematode *Heterodera fillipjevi* were screened to find fungi and bacteria that could potentially explain the origin of this suppressiveness. Here, we focus only on the fungi isolated during the study. Two different screening approaches were adopted to obtain a potentially wider variety of fungi associated with nematodes. In the first approach nematode cysts and wheat root samples were randomly selected and processed for fungal isolation and identification. This approach yielded several well-known nematode-associated fungi, belonging to the genera *Pochonia*, *Paecilomyces*, as well as several *Fusarium* and *Acremonium* species. Using these fungi in glasshouse studies, a significant reduction of nematode populations on wheat could be observed. In the second approach only nematode eggs showing symptoms of fungal infections were scrutinised. The fungal species obtained from these eggs were identified using both culture-dependent and culture-independent methods. By doing so we can test the identity of isolated fungi with the fungi living in individual eggs and correlate this with the displayed symptoms. This approach resulted in isolation of several so-far undescribed ascomycetous species belonging to genera of *Monocillium*, *Pleospora*, *Ijuhya* and *Ophiosphaerella*. Using light microscopy interesting parasitic interactions of the fungi with the eggs and juveniles could be detected. Also Koch's postulate could be fulfilled for all of these isolates. Because of their novelty to science, their parasitic interaction and because all of these fungi are slow-growing fungi they seemed promising targets to study their natural compounds. So far the compounds of two of these were fully elucidated yielding one known compound (Chaetoglobosin A) with nematocidal activities, and one new compound belonging to the cyclodepsipeptides, a group of compounds known for their anthelmintic effects.

Keywords: Biological control; compound; fungi.

The use of *Daldinia concentrica*, an endophytic fungus, and its bioactive volatiles against the plant-parasitic nematode *Meloidogyne javanica*

Sigal Braun Miyara, Patricia Bucki, Orna Liarzi, Payal Sanadhya and David Ezra

Department of Entomology and the Nematology and Chemistry units, ARO The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

corresponding author: sigalhor@volcani.agri.gov.il

Endophytic organisms are located during most of their life-cycle inside plant tissues without causing any visible damage. Many endophytes secrete specialised metabolites that possess biological activity. Recently, we demonstrated that the endophytic fungus *Daldinia concentrica* secretes biologically active volatile organic compounds (VOC). In the present study we examined the ability of *D. concentrica* and its VOC to control *Meloidogyne javanica* second-stage juveniles (J2) both *in vitro* and in glasshouse experiments. We exposed, without any direct contact, the nematodes to the fungus culture or to an artificial mixture containing four of its VOC. The J2 exposure to a *D. concentrica* culture showed nematocidic activity and caused 67% J2 mortality. A mixture of VOC: 3-methyl-1-butanol, (\pm)-2-methyl-1-butanol, 4-heptanone and isoamyl acetate, at the ratio of 1:1:2:1, increased J2 mortality by up to 99%. Although each of the four VOC significantly increased the J2 mortality relative to the control, only 4-heptanone exhibited the same nematocidic activity and mortality level as the whole mixture. Interestingly, only the mixture, but not the fungus, affected hatching of *M. javanica*. Finally, application of the mixture to inoculated soil in the presence of *M. javanica* on susceptible tomato seedlings showed a significantly lower galling index and smaller number of eggs per g of soil, with no effect on root weight. On the basis of these results we suggest the use of *D. concentrica* and/or an artificial mixture of its VOC as an alternative and natural approach, to control root-knot nematodes.

Keywords: Biological control; endophyte; fungus; root-knot nematodes; volatiles.

Rhizomodulation by *Pochonia chlamydosporia* and chitosan for nematode biocontrol and crop yield promotion

Luis Lopez-Llorca

Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) Ramon Margalef, University of Alicante, E-03080 Alicante, Spain

corresponding author: lv.lopez@ua.es

The multitrophic interactions in the rhizosphere include communication among roots, beneficial microbes and pathogens, resulting in losses or gains for plants. The nematophagous fungus, *Pochonia chlamydosporia*, is a well-known cause of soil suppression to plant-parasitic nematodes (PPN). The fungus is a root endophyte in economically important crops and in the model plant *Arabidopsis thaliana*, *P. chlamydosporia* induces a moderate plant response to stress. This could explain why the fungus also triggers resistance to root pathogenic fungi. Some isolates reduce flowering time, stimulate plant growth and increase yield. Nutrient solubilisation or enhancing plant hormones by *P. chlamydosporia* can explain these effects. Precocious flowering is associated with expression changes in key flowering-time genes. Jasmonate signalling modulates progression of plant colonization by the fungus. We have identified signals putatively involved in tomato - root-knot nematode - *P. chlamydosporia* interactions. Root exudates act as signals that can be manipulated for improving plant growth and yield and reduce disease. The genome of *P. chlamydosporia* reflects its multitrophic lifestyle. The endophytic habit of the fungus can be explained by the presence of carbohydrate active enzymes which can degrade plant cell wall materials. However the most striking feature is the large expansion of *P. chlamydosporia* genes encoding enzymes for chitin and chitosan degradation. Chitin is a structural component of cuticles and cell walls of invertebrates and fungi. Its deacetylated form, chitosan, kills or inhibits fungal pathogens, but *P. chlamydosporia* is resistant to chitosan. In *P. chlamydosporia*, chitosan activates conidiation and expression of serine proteases involved in nematode eggs penetration. As a consequence, chitosan enhances pathogenicity of the fungus to nematodes. Rhizomodulation using *P. chlamydosporia* and chitosan is our current paradigm to improve crop yield and reduce damage by PPN.

Keywords: Carbohydrate active enzymes; endophytism; plant immunity; plant-nematode communication; rhizosphere multitrophic interactions.

Diversity of nematophagous fungus communities in olive nurseries in Morocco as affected by climate and substrates

Mohamed Aït Hamza¹, Hicham Lakhtar¹, Hafssa Tazi¹, Abdelmajid Moukhli², Odile Fossati³, Lucie Miché⁴, Sebastianos Roussos⁴, Zahra Ferji⁵, Hassan Boubaker², Thierry Mateille⁴ and Abdelhamid El Mousadik¹

¹ Université Ibn Zohr, Faculté des Sciences d'Agadir, Laboratoire Biotechnologie et Valorisation des Ressources Naturelles, BP 8106, 80000 Agadir, Morocco

² INRA, CRRA, BP 513, Menara, Marrakech, Morocco

³ IRD, UMR Centre de Biologie pour la Gestion des Populations, 755 Avenue du Campus Agropolis, CS30016, 34988 Montpellier-sur-Lez Cedex, France

⁴ IRD, UMR Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale, Faculté des Sciences Saint Jérôme, Case 421, 13397 Marseille Cedex 20, France

⁵ Institut Agronomique et Vétérinaire Hassan II, Campus d'Agadir, Département de Protection des Plantes, BP 18/S, 80000 Agadir, Morocco

corresponding author: mohamed.aithamza@edu.uiz.ac.ma

The plant-parasitic nematodes (PPN) are important plant pests in agriculture. Annual losses caused by PPN are estimated from 8.8 to 14.6% of the world crop production (100–157 billion USD per year). In Morocco, nematode parasitism is enhanced in intensive cropping systems (tomato, olive etc.) especially with root-knot nematodes (*Meloidogyne* spp.). Because of crop intensification and environment pollution with chemical pesticides, the search of alternative strategies for nematode control is necessary. Biological control using nematophagous fungi is one option, especially at the nursery state before planting. However, few studies have been conducted with native strains. Thus, the present study aims at evaluating the nematode population levels and isolating, identifying, and assessing the *in vitro* predatory activity of nematophagous fungi that had been isolated in 25 commercial olive nurseries in contrasting soil substrates across five regions in Morocco (Souss-Massa, Haouz, Guerouane and Sefiane). Among the 305 soil samples recovered from these nurseries, conidial traps of nematode destroying fungi were recorded in all the areas surveyed. Seventy-three pure isolates were obtained. Diversity indices showed that the fungal isolates were more numerous in the Souss region (southern Morocco), while the fungal communities were more numerically alike in the Guerouane region (northern Morocco). *In vitro* predatory activity of the fungal strains against *M. javanica* juveniles showed that *Talaromyces assiutensis* has been identified as the most efficient nematophagous fungus. The Orbiliaceae species (*Arthrobotrys* spp., *Drechslerella brochopaga* and *Monacrosporium* spp.) were able to trap 50 to 80% of the nematode juveniles. *Paecilomyces* and *Trichoderma* species were able to kill 30 to 50% of the juveniles. The development of such indigenous nematophagous fungi as biocontrol agents can be considered.

Keywords: Diversity; *in vitro* predation; nematophagous fungi; olive nurseries; root-knot nematodes.

How the FLP do nematodes jump?

Robert Morris, Leonie Wilson, Neil D. Warnock, Deborah Cox, Matthew Sturrock, Aaron G. Maule and Johnathan J. Dalzell

MBC, Queen's University Belfast, BT9 7AE, Belfast, Northern Ireland, UK

corresponding author: rmorris10@qub.ac.uk

Entomopathogenic nematodes (EPN) are a guild of obligate insect parasites that share many physiological and behavioural traits with mammalian strongylid and strongyloidid parasites; including host-finding nictation behaviour. EPN are also interesting from the perspective of insect biocontrol. Like other parasitic nematodes, EPN employ a sophisticated chemosensory apparatus to detect potential hosts and communicate with conspecifics. Understanding the underlying molecular basis of relevant host-finding behaviours could facilitate improved EPN biocontrol approaches, and could lend insight to similar behaviours in economically important animal parasites. FMRFamide-like neuropeptides are enriched and conserved across the Phylum Nematoda, and have been linked with motor and sensory function, including dispersal and aggregating behaviours in the free-living nematode *Caenorhabditis elegans*. RNA interference (RNAi) was used to knockdown the expression of the *flp-21* gene in *Steinernema carpocapsae*. Our results show that knockdown of *flp-21* has a significant impact on dispersal behaviour, nictation and jumping of *S. carpocapsae*, relative to controls. Immunocytochemical localisation of FLP-21 to paired anterior neurons corroborates the RNAi data suggesting a role in sensory modulation. This study represents the first demonstration of a functional neuronally-sensitive RNAi pathway in *S. carpocapsae*, linking the neuropeptide FLP-21 to dispersal, nictation and jumping phenotypes in a parasitic nematode for the first time.

Keywords: Insect; neurobiology; parasitology; sensory; volatile.

TrakBox: A low cost option for real-time tracking and behavioural analysis of microscopic nematodes

Lindy Holden-Dye¹, Vincent O'Connor¹ and Christopher James²

¹ Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK

² School of Engineering, University of Warwick, Coventry, CV4 7AL, UK

corresponding author: lmhd@soton.ac.uk

Many of the most pathogenic nematodes are microscopic, about 1 mm in length, and in this regard, and also in terms of conserved fundamental biological mechanisms, they are similar to the model genetic organism *Caenorhabditis elegans*. Indeed the nematode worm *C. elegans* has been successfully used as an experimentally tractable platform both to further understanding of parasitic nematodes and to facilitate anthelmintic/nematicide development. A key requirement for progress is the capability for precise and detailed analysis of the impact of drugs on nematode behaviour as this underpins the identification of new chemical identities with nematicidal potential and also is an important indicator of mode of action. A number of approaches have been described for tracking and behavioural analysis of *C. elegans* but these require costly hardware and/or relatively time-consuming post-hoc analysis of videos. We have designed a 3D printed modular system at a cost about 1/40th of current tracking platforms. A robotic arm with a holder for a USB microscope/camera is powered by two servo motors. The arm is controlled by software for worm recognition and is moved over an agar plate keeping a worm in the field of vision, whilst translating the arm movement into the X,Y coordinates of the worm. This delivers an instantaneous read out of the worm's location and velocity and permits capture of metrics to quantify different worm behaviours in the presence of perturbing chemicals. We have capability to extend this to detect hatching and juveniles. The low cost, ease of use, and capability for operator free running, provides the possibility for increasing the throughput of nematode behavioural analyses.

Keywords: Anthelmintic; hatching assay; juvenile; mode of action; nematicide.

Assessing the response of root-knot nematodes to plant and nematode semiochemicals

Valerie Williamson, Rasa Čepulytė-Rakauskienė, Wiseborn B. Danquah and George Bruening

Department of Plant Pathology, University of California, Davis, CA 95616, USA

corresponding author: vmwilliamson@ucdavis.edu

Root-knot nematode (*Meloidogyne* spp.; RKN) second-stage juveniles hatch from eggs in the soil and must locate and invade host roots to complete their life cycle. What attracts nematodes to roots has remained a mystery for decades. In addition, the chemical signals involved in inter-nematode communication are almost entirely unknown for plant-parasitic nematodes. We have developed assays to assess RKN attraction and behaviour using a thermo-reversible gel of Pluronic F127. These assays are being used to identify the chemical signals that RKN utilise to locate appropriate infection sites in the roots and to determine the roles of pheromones in this process. Juveniles are most strongly attracted to a region immediately behind the root tip of healthy seedlings. Cell-free exudate collected from the tomato seedling root tip region is attractive to nematodes and facilitates nematode aggregation. Activity-guided fractionation to identify the responsible host semiochemicals is in progress. This aggregation and other behaviours indicate that RKN communicate chemically with each other. For example, males are strongly attracted to females and egg masses as well as to exudates collected from females and egg masses. Ascarosides, a family of compounds that are glycosides of the sugar ascarylose with a fatty acid-derived side chain, regulate a range of social behaviours and developmental pathways in a range of nematode species. Ascarosides are present in exudates of RKN juveniles and females, and ascaroside#18 is the most abundant species. Synthetically produced ascr#18 attracts *M. hapla* males and juveniles in a concentration-dependent manner. Identification of the semiochemicals that attract RKN to roots and that modify their behaviour has the potential to provide tools for novel and safe strategies of control.

Keywords: Ascaroside; attractant; behaviour; pheromone; pluronic gel.

Nematode infection redirects auxin homeostasis via Rboh-mediated ROS to facilitate feeding site establishment in host roots

Christiane Matera, Divykriti Chopra, Shamim Hasan, Oliver Chitambo, Florian M. W. Grundler and Shahid Siddique

Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

corresponding author: dchopra@uni-bonn.de

Cyst nematodes are obligate parasites that establish syncytial-feeding sites in roots of their host plants. The oxidative burst, a rapid and transient release of huge amounts of reactive oxygen species (ROS), is one of the earliest and vital components of a plant's defence mechanism. ROS during the oxidative burst is mainly produced by plasma membrane-bound NADPH oxidases, named respiratory burst oxidase homolog (Rboh) in plants. Surprisingly, *Arabidopsis* plants lacking ROS production by Rboh (*rbohD/F*) are shown to be less susceptible to nematode attack. A comprehensive microscopic, biochemical and molecular analysis done previously demonstrated that Rboh-dependent ROS are not required for *Arabidopsis* root invasion by *Heterodera schachtii*. However, the absence of Rboh-mediated ROS impairs syncytial establishment and development. Despite the importance of ROS during plant-pathogen interactions, little is known about downstream targets that mediate their pathosystem-specific role. To analyse the mechanistic details of Rboh-mediated ROS in syncytium formation, we carried out comparative transcriptome analyses between Col-0 and *rbohD/F* during the early stages of nematode infection. The data revealed several genes involved in auxin transport, synthesis and/or homeostasis to be down regulated in *rbohD/F* as compared to wild type. The most down-regulated gene encodes for the vacuolar auxin transporter Walls Are Thin (WAT1). The regulation of WAT1 expression by ROS in an infection-specific manner was confirmed by quantitative RT-PCR and GUS assays. *Arabidopsis* loss-of-function mutants of WAT1 showed reduced nematode susceptibility like *rbohD/F*. Furthermore, the auxin (IAA) levels of infected root segments were significantly decreased in both *rbohD/F* and *wat1* as compared to Col-0. Thus we conclude that *H. schachtii* stimulate NADPH oxidase in plants to produce ROS that in turn activate the expression of WAT1 to mediate auxin transport.

Keywords: Auxin; *Heterodera schachtii*; *rbohD/F*; ROS; WAT1.

Are small non coding RNAs involved in the formation of galls induced by root-knot nematodes?

Clémence Medina¹, Marc Magliano¹, Martine Da Rocha¹, Javier Cabrera², Marta Barcala², Carolina Escobar², Carmen Fenoll², Pierre Abad¹, Bruno Favery¹ and Stéphanie Jaubert-Possamai¹

¹ INRA, Université Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

² Universidad de Castilla-La Mancha, Facultad de Ciencias Ambientales y Bioquímica, Avda. Carlos III, s/n 45071 Toledo, Spain

corresponding author: clemence.medina@sophia.inra.fr

Root-knot nematodes are plant-parasitic worms that transform cells from root vascular cylinder into giant, multinucleate and hypermetabolic feeding cells. Transcriptomic analyses showed that redifferentiation in giant cells implies a large reprogramming of gene expression. Small non-coding RNAs have been shown to be key regulators of host gene expression during host/parasite interaction. This work aims to determine if the reprogramming of gene expression within galls is regulated by small RNAs, *i.e.*, small interfering RNAs (siRNA) and/or microRNAs (miRNA). Galls of *Arabidopsis thaliana* inoculated with *Meloidogyne incognita* and fragments from healthy roots were collected 7 and 14 days after infection (dai). From these samples small RNAs (18-25nt) were sequenced by SOLiD technology. We first quantified mature miRNAs expressed in roots and galls and identified 19 miRNAs that are statistically differentially expressed in galls at 7 and/or 14 dai. Some of these miRNAs, *e.g.*, miR390, miR394, miR398, miR167, are known to be involved in responses to biotic and abiotic stresses and in hormone-dependent processes. Using promoter reporter gene strategy, we showed that miR390, miR408 and miR167 genes are expressed in galls. To investigate the role of these miRNAs in galls we performed resistance tests with *Arabidopsis* lines either overexpressing or being depleted for these miRNAs. The results of these tests will be presented. In the second part of our study, we identified the siRNAs expressed in galls and healthy roots by using the Shortstack algorithm that identifies genomic clusters where siRNAs were aligned. Statistical analyses identified siRNA clusters that accumulate siRNAs differentially between galls and healthy roots. Genome annotations corresponding to these differentially expressed siRNA clusters will be presented. The role of miRNAs and siRNAs in the regulation of plant gene expression in response to nematodes will be discussed.

Keywords: *Arabidopsis thaliana*; giant cell; *Meloidogyne incognita*; miRNAs; siRNAs.

A parasitic nematode releases cytokinin that controls cell division and orchestrates feeding site formation in host plants

Shahid Siddique¹, Zoran S. Radakovic¹, Carola M. De La Torre², Demosthenis Chronis², Ondrej Novák³, Eswarayy Ramireddy⁴, Elzbieta Rozanskae⁵, Miroslaw Sobczak⁵, Tatsuo Kakimoto⁶, Miroslav Strnad³, Thomas Schmölling⁴, Melissa G Mitchum² and Florian M. W. Grundler¹

¹ Rheinische Friedrich-Wilhelms-University of Bonn, Department of Molecular Phytomedicine, Bonn, Germany

² Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, USA

³ Laboratory of Growth Regulators, Faculty of Science, Palacký University and Institute of Experimental Botany Academy of Sciences of the Czech Republic, Olomouc, Czech Republic

⁴ Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin, Berlin, Germany

⁵ Department of Botany, Warsaw University of Life Sciences, Warsaw, Poland

⁶ Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka, Japan

corresponding author: siddique@uni-bonn.de

Sedentary plant-parasitic cyst nematodes are biotrophs that cause significant losses in agriculture. Parasitism is based on modifications of host root cells that lead to the formation of a hypermetabolic feeding site (a syncytium) from which nematodes withdraw nutrients. The host cell cycle is activated in an initial cell selected by the nematode for feeding, followed by activation of neighbouring cells and subsequent expansion of feeding site through fusion of hundreds of cells. It is generally assumed that nematodes manipulate production and signaling of the plant hormone cytokinin to activate cell division. In fact, nematodes have been shown to produce cytokinin *in vitro*; however, whether the hormone is secreted into host plants and plays a role in parasitism remained unknown. Here, we analysed the spatiotemporal activation of cytokinin signalling during interaction between the cyst nematode, *Heterodera schachtii*, and *Arabidopsis* using cytokinin-responsive promoter:reporter lines. Our results showed that cytokinin signalling is activated not only in the syncytium but also in neighbouring cells to be incorporated into syncytium. An analysis of nematode infection on mutants that are deficient in cytokinin or cytokinin signalling revealed a significant decrease in susceptibility of these plants to nematodes. Further, we identified a cytokinin-synthesising isopentenyltransferase gene in *H. schachtii* and show that silencing of this gene in nematodes leads to a significant decrease in virulence due to a reduced expansion of feeding sites. Our findings demonstrate the ability of a plant-parasitic nematode to synthesise a functional plant hormone to manipulate the host system and establish a long-term parasitic interaction.

Keywords: Cytokinin; nematode; plant hormone; syncytium.

Pinewood nematode: situation and control measures in the European Union

Pascale Mathes

European Commission, Directorate General Health and Food Safety, Directorate F - Health and food audits and analysis, Grange, Dunsany, Co Meath, Ireland

corresponding author: Pascale.MATHES@ec.europa.eu

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a quarantine harmful organism in the European Union (EU) where its spread would cause significant damage to the pine forests and serious economic impacts on the EU export trade of coniferous wood. Following the first detection of PWN in the EU in 1999, the European Commission has adopted specific emergency control measures that apply to all Member States (the latest of which is Commission Implementing Decision 2012/535/EU). Pinewood nematode is now present under containment in continental Portugal and in Madeira Island. In Spain, four outbreaks of a limited extent have been detected since 2008, which were or are being eradicated. In the rest of the EU territory, pinewood nematode is known not to occur as demonstrated by annual specific surveys performed by all Member States. The European Commission has dedicated a significant budget in order to support the fight against this major pest by co-financing PWN surveys in the EU territory, the implementation of control measures in Portugal and Spain and two research projects (PHRAME and REPHRAME). Additionally, some 25 audits focused on PWN were carried out by the Commission services in Portugal and Spain. In autumn 2014, the Commission set up a Task Force for the control of PWN in order to provide technical support to Portugal and Spain in controlling PWN. The Task Force is a group of 13 experts from 11 Member States, coordinated by the Commission services. It pooled together scientists, technicians and managers who worked together and used their expertise in order to assess the situation on the spot and develop tailor-made proposals with the objective of addressing the problem of pinewood nematode control in its entirety. The work of the Task Force between November 2014 and October 2015 resulted in a number of technical recommendations to the two Member States concerned and the co-financing of scientific studies to enhance the efficiency and reliability of surveillance for PWN.

Keywords: Emergency control measures; European Commission; forest health; pinewood nematode; quarantine.

Heat treatment of wood packaging material and bark to control the spread of pinewood nematode

Maria Clara A. Serra

Direção Geral de Alimentação e Veterinária, Tapada da Ajuda – Edifício 1 1349-018
Lisboa, Portugal

corresponding author: claradealmeidaserra@hotmail.com

Following the introduction of pinewood nematode, *Bursaphelenchus xylophilus*, in Portugal, legislation was enacted establishing phytosanitary measures to control its introduction and spread into new areas within the European Union. One of the main phytosanitary measures is the heat treatment of wood, wood packaging material and bark of coniferous wood. According to the European and national legislations (Commission Decision 2012/535/EU and the Decree law nº 95/2011, changed by the Decree law nº 123/2015), the heat treatment approved follows the specifications of the International Standard Phytosanitary Measures –ISPM-15. In Portugal a system of approval and supervision of the industrial heat treatment facilities was implemented in order to guarantee the efficacy of the heat treatment in the elimination of pinewood nematode from the wood, wood packaging material and bark and to avoid its spread by movement of contaminated wood.

Keywords: Bark; heat treatment; pinewood nematode; wood.

Disentangling Pine Wilt Disease - Roles of the pine endophytic bacteria and of the bacteria carried by the disease-causing pinewood nematode

Diogo Neves Proença^{1,2}, Gregor Grass³ and Paula V. Morais^{1,4}

¹ CEMUC, University of Coimbra, 3030-788 Coimbra, Portugal

² Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

³ Bundeswehr Institute of Microbiology, 80937 Munich, Germany

⁴ Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: pvmorais@ci.uc.pt

Pine wilt disease (PWD) is one of the most destructive diseases in trees of the genus *Pinus* and is responsible for environmental and economic losses around the world. The only known causal agent of the disease is the pinewood nematode (PWN) *Bursaphelenchus xylophilus*. Bacteria belonging to several different genera have been found associated with PWN and their roles in the development of PWD have been suggested. Molecular methodologies and the new era of genomics have revealed different perspectives to the problem, recognising the manifold interactions between different organisms involved in the disease. Here, we reviewed the possible roles of nematode-carried bacteria in PWD, which could be the definition of this group of microorganisms and questioned their origin as possible endophytes, discussing their relation within the endophytic community of pine trees. The diversity of the nematode-carried bacteria and the diversity of pine tree endophytes, reported until now, is revisited in detail in this review. What could signify a synergetic effect with PWN harming the plant, or what could equip bacteria with functions to control the presence of nematodes inside the tree, is outlined as two possible roles of the microbial community in the etiology of this disease. An emphasis is put on the potential revealed by the genomic data of isolated organisms in their potential activities as effective tools in PWD management.

Keywords: Bacteria; biocontrol; *Bursaphelenchus xylophilus*; endophytes; pine wilt disease.

Pine Wilt Disease management in Portugal

Edmundo M. R. Sousa¹, Luís Bonifácio¹, Pedro Naves¹, Maria L. Inácio¹ and José M. Rodrigues²

¹INIAV, Instituto de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras, Portugal

²ICNF, Instituto da Conservação da Natureza e das Florestas, Avenida da República, 16, 1050-191 Lisboa, Portugal

corresponding author: edmundo.sousa@iniav.pt

Strategies adopted to manage pine wilt disease worldwide have taken into account the fact that the expression of the disease results mostly from the interactions among the pathogenic nematode (causal agent), the insect vector (dispersing agent) and the host tree. Therefore, the control procedures can be addressed singly to the nematode, to the insect vector or to both. In Europe, only *Bursaphelenchus xylophilus* is classified as a quarantine organism, and for this reason, object of specific Community legislation. However, its vector, the longhorn beetle, *Monochamus galloprovincialis*, is the main dispersing agent being crucial in the disease dispersion, and the control of its populations is essential in any strategy. The methods for the control of pests can be applied directly on the agents or indirectly in the host tree to improve the development and vigour of the plants and, consequently, their resistance to pests and diseases. These methods can be grouped in six categories: 1) mechanical – removal and destruction of plant material or destruction and death of the agent in the different phases of its life cycle; 2) cultural - forestry management carried out to improve the vigour and vitality of the trees; 3) biotechnical – methods which affect and manipulate the behaviour of the agent or the interactions with the hosts; 4) chemical – application of phyto pharmaceuticals; 5) biological – introduction of living organisms with ability to control pathogens that can be parasitoids (insects), diseases (fungi, nematodes, viruses, bacteria) or predators (insects, birds, mammals); and 6) genetical - increasing of the plant resistance to pests and diseases or to abiotic adverse factors, through the genetic improvement of plants or a careful selection of the plant origin. Most of these methods have been applied over the last years in Portugal and their success or limitations are discussed in this presentation.

Keywords: Control methods; maritime pine; pine sawyer; pinewood nematode.

Pre-establishment of potato cyst nematode results in improved fitness of the peach potato aphid

Grace A. Hoysted¹, Catherine J. Lilley¹, Sue E. Hartley² and Peter E. Urwin¹

¹ Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK

² University of York, Wentworth Way, York, YO10 5DD, UK

corresponding author: bs11gah@leeds.ac.uk

Plants are susceptible to attack from pests and pathogens above and below the ground. *Myzus persicae* and *Globodera pallida* are two major pests that attack the potato crop simultaneously and the relationship between the two organisms remains to be elucidated. The performance of *M. persicae* was tested over a period of 8 days on either potato plants pre-infected with *G. pallida* for 14 days or non-infected control plants. The reproductive rate of aphids was significantly higher when reared on nematode-infected plants. Inducible systemic changes were analysed in leaves of nematode-infected potato plants. RP-HPLC analysis showed a significant increase in salicylic acid (SA) in leaves of potato plants infected with *G. pallida* for 14 days compared to the leaves of non-infected control plants. qRT-PCR analysis of pathogen-related (PR)-gene expression revealed a significant systemic increase in the relative expression of the SA-mediated gene, PR-5. The jasmonic acid signalling pathway was also analysed, however there was no systemic increase in the expression of JAZ-1, suggesting that *G. pallida* does not elicit the jasmonic acid pathway at 14 days post inoculation. Cysts of *G. pallida* were incubated in root diffusate from potatoes that had been infested with different numbers of aphids or that from control plants. Diffusate from the aphid-infested plants stimulated a significantly lower cumulative hatch of second-stage juveniles. In addition, sequence of arrival experiments showed that pre-infestation of *M. persicae* significantly reduced the infection rate of *G. pallida*. Taken together these data suggest an asymmetric plant-mediated interaction between the potato cyst nematode and the peach potato aphid. The molecular response of the host potato plant to infection with *G. pallida* indirectly increases host susceptibility to *M. persicae* while pre-infestation of the host plant with *M. persicae* renders the plant less susceptible to *G. pallida* infection.

Keywords: Aphids; cross-talk; jasmonic acid; nematodes; salicylic acid.

Interactions between the oomycete *Pythium arrhenomanes* and the rice root-knot nematode *Meloidogyne graminicola* in aerobic Asian rice varieties

Ruben Verbeek^{1,2}, Grace Banaay³, Maniruzzaman Sikder¹, Dirk De Waele^{4,5}, Casiana Vera Cruz³, Godelieve Gheysen¹, Monica Höfte² and Tina Kyndt¹

¹ Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium

² Laboratory of Phytopathology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, B-9000 Ghent, Belgium

³ International Rice Research Institute, Los Baños, Laguna, Philippines

⁴ Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), B-3001 Heverlee, Belgium

⁵ Unit for Environmental Sciences and Management, North-West University, 2520 Potchefstroom, South Africa

corresponding author: ruben.verbeek@ugent.be

Rice cultivation is under pressure due to increasing water scarcity. A shift to aerobic rice seems the most promising alternative; however, this shift in cultivation introduced a new array of pathogens that affect rice cultivation. Two predominant root pathogens in aerobic rice are oomycetes (*Pythium* spp.) and root-knot nematode *Meloidogyne graminicola*. We studied the interaction between these root pathogens in the rice roots of two aerobic rice cultivars. In different experimental set-ups and infection regimes, plant growth, rice yield, *Pythium* colonisation, as well as penetration, development and reproduction of *M. graminicola* were studied. We found that penetration, development and reproduction of *M. graminicola* were delayed in the presence of *P. arrhenomanes*. This effect became more prominent with higher inoculation levels of *P. arrhenomanes*. The filled grain weight was reduced after inoculation with either one of the pathogen, but when both pathogens were present it was not significantly different from non-inoculated plants. Our data show that *P. arrhenomanes* and *M. graminicola* antagonise each other in the rice root and that the plant benefits from this interaction. However, the mechanisms of this antagonism remain elusive.

Keywords: Aerobic rice; antagonism; nematode development.

**Influence of maize secondary metabolites on the entomopathogenic nematode
*Heterorhabditis bacteriophora***

Ricardo A.R. Machado, Xi Zhang, Christelle A.M. Robert and Matthias Erb

University of Bern, Institute of Plant Sciences, Altenbergrain 21, 3013 Bern, Switzerland

corresponding author: ricardo.machado@ips.unibe.ch

Plants can influence entomopathogenic nematodes (EPN) through changes in the quality of their insect hosts. We investigated how benzoxazinoids, the major secondary metabolites in maize roots, influence the interaction between the western corn rootworm *Diabrotica virgifera* and the entomopathogenic nematode *Heterorhabditis bacteriophora*. Our experiments demonstrate that benzoxazinoids reduce the infectivity of *H. bacteriophora* towards *D. virgifera*. Through *in vivo* and *in vitro* experiments, we identified several mechanisms that contribute to this effect, including repellency and growth suppression of *H. bacteriophora*. In summary, our results demonstrate that plant secondary metabolites can reduce the predation success of EPN and thereby indirectly benefit insect herbivores.

Keywords: Below-ground tritrophic interactions; benzoxazinoids; nematode foraging behaviour; nematode performance.

A novel root-knot nematode attractant is released from seeds through seed coat mucilage extrusion

Allen Yi-Lun Tsai, Tetsuya Arita, Ryo Kuroda and Shinichiro Sawa

Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo Ward, Kumamoto city, 860-8555 Kumamoto, Japan

corresponding author: allen@sci.kumamoto-u.ac.jp

Root-knot nematodes (RKN; *Meloidogyne incognita*) are ubiquitous plant pathogens that infect many plant species and cause significant agricultural losses annually. RKN are obligate parasites that infiltrate plant roots, feed on plant nutrients while inducing the eponymous root-knot galls at the feeding sites. Newly hatched RKN juveniles must migrate in the soil to locate and infect new hosts to continue growth and development, and they are thought to achieve this by sensing attractants from the exudates secreted by the host plant. However the identities and properties of these attractants have not been extensively characterised. Here we demonstrate that infective RKN juveniles are attracted to seeds of the model plant *Arabidopsis* (*Arabidopsis thaliana*), suggesting that seeds may secrete an uncharacterised RKN attractant. Seeds of many angiosperm plants extrude a layer of gelatinous mucilage from the seed coat upon hydration. Mutant analyses suggest the extrusion of mucilage is required for seeds to attract RKN, and the attraction can be abolished by digesting the mucilage with cellulase. However, extracted mucilage alone is not sufficient to mediate attraction, and mutant seeds with loosely attached mucilage do not show RKN attraction defects. These findings suggest the RKN attractant may be a substance distinct from mucilage. Lastly, wild-type seeds germinated in the presence of RKN juveniles are indeed more prone to RKN infiltration and results in shorter seedlings compared to mucilage-deficient mutants. However, this effect appears to be relevant only for a short period of time during germination before the root emerges. In summary, we propose an RKN attractant is extruded from seeds concurrently with the seed coat mucilage, which is perceived by RKN juveniles to target their hosts. This study highlights a novel mechanism of RKN behaviour regulation, while suggesting the possibility for seed coat mucilage to be involved in soil pathogen interactions.

Keywords: *Arabidopsis*; chemotaxis; plant cell wall; plant-parasitic nematode; seed germination.

Ups and downs of belowground tritrophic interactions

Alan Kergunteuil and Sergio Rasmann

Laboratory of Functional Ecology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

corresponding author: alan.kergunteuil@unine.ch

Belowground tritrophic interactions, particularly involving plants, insect root herbivores, and entomopathogenic nematodes, has seen a surge of interest in the past decade for several reasons. First, the chemical ecology mediating these interactions has been elucidated in several systems. Second, the potential for manipulating the chain of interaction at each trophic level, opens up several possibilities for ameliorating the biological control of root pests. And third, evidence showing that belowground process can influence aboveground community dynamics, has opened the opportunity to integrate fully the two ecological compartments in community ecology research. We will here discuss each one of these points, and propose future venues for research in belowground tritrophic interactions, both for applied and more fundamental research venues.

Keywords: Above-belowground interactions; biological control; chemical ecology.

Differentially expressed small RNAs in *Arabidopsis* galls formed by *Meloidogyne javanica*

Javier Cabrera, Marta Barcala, Ana Cláudia Silva, Carmen Fenoll and Carolina Escobar

Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla La Mancha, Avenida Carlos III, s/n, 45071, Toledo, Spain

corresponding author: javier.cabrerachaves@uclm.es

Plant endoparasitic nematodes cause important agricultural losses estimated in 60 billion euros per year. Root-knot nematodes (*Meloidogyne* spp.) cause the proliferation of vascular cells and the hypertrophy of cortical cells, forming a pseudo-organ called gall. Inside the gall, 5-8 nourishing cells named giant cells are induced. We analysed the transcriptomes of the nematode feeding sites, giant cells and galls, at early infection stages (3 days post infection) induced in *Arabidopsis* and tomato roots. Giant cell transcriptomes in both species presented a massive gene repression, what was not observed in the transcriptomes of the galls containing these giant cells. One of the known mechanisms for gene silencing involves small RNAs function. Hence, we analysed the small RNA population present in early developing galls induced by *M. javanica* in *Arabidopsis* by massive sequencing. Most of the miRNAs detected as differentially expressed were down-regulated while only a few proportion of them were up-regulated in galls compared to the uninfected control roots; among the latter was found miR390a that would be likely mediating the repression of some auxin response factors, such as ARF3, through the regulation of tasiRNA3a in galls (Cabrera *et al.*, 2016). miR390a is induced as well in the syncytia formed by *Heterodera schachtii* in *Arabidopsis* and mutant lines for both small RNAs (miRNA390a and tasiRNA3a) showed a decrease in the infection parameters. Furthermore, in contrast to the miRNAs, the population of small RNAs of 24 nucleotides (usually corresponding to repeat-associated-small-interfering RNAs) were highly induced in galls. These results could explain, at least partially, the massive gene repression observed in the giant cell transcriptomes. Reference: Cabrera, Barcala, García, Rio-Machín, Medina, Jaubert-Possamai, Favery, Maizel, Ruiz-Ferrer, Fenoll, Escobar (2016). *New Phytologist* 209, 1625-40.

Keywords: Galls; giant cells; *Meloidogyne*; miRNAs; rasiRNAs.

EFC7 and EFC15 of *Meloidogyne incognita* are responsible for infection steps in *Arabidopsis*

Chie Shimaoka, Morihiro Oota, Reira Suzuki and Shinichiro Sawa

Kumamoto University, 860-8555, Kumamoto, Japan

corresponding author: sawa@sci.kumamoto-u.ac.jp

Meloidogyne incognita is one of the most detrimental root-knot nematode pests in the world, infecting almost all plant species. Proteome analysis of effector protein identified the EFC7 and EFC15 protein as effector proteins of *M. incognita*. In order to identify the interacting protein of these effector proteins, we performed yeast two hybrid screening using *Arabidopsis* cDNA as a prey. By using EFC7 as bait, a B3 type transcription factor was identified as a candidate of EFC7 binding protein. Overexpression of EFC7 gene induced defence related genes, indicating that EFC7 might inhibit plant defence signals. On the other hand, a MAPKKK and a DCD protein were identified as EFC15 interacting proteins. *Arabidopsis* dcd mutant showed significant reduction of root gall formation rate. Here we will talk about our recent analysis of molecular mechanisms of effector proteins of *M. incognita*.

Keywords: *Arabidopsis*; effector protein; infection steps; *Meloidogyne incognita*.

Modulation of *Arabidopsis* flavonol biosynthesis gene expression during infection by the sugar-beet cyst nematode

Noureddine Hamamouch^{1,2}, Brenda S. J. Winkel³, Chunying Li¹ and Eric L. Davis¹

¹ North Carolina State University, Department of Plant Pathology, Box 7903, 840 Method Road, Unit 4 Bldg, Raleigh, NC 27607, USA

² Polydisciplinary Faculty, University Sultan Moulay Slimane, Beni-Mellal, Morocco

³ Virginia Tech, Department of Biological Sciences, Latham 409, Blacksburg, VA 24060, USA

corresponding author: hamamouch@yahoo.com

Host flavonoids are differentially-regulated during parasitism of plant roots by nematodes. In this study, expression of the *Arabidopsis thaliana* flavonol-specific transcription factor *AtMYB-12*, and the flavonol synthase genes *AtFLS-1*, *-2*, *-3*, *-4*, and *-5*, were examined during infection by *Heterodera schachtii*, the sugar-beet cyst nematode. Target gene expression was monitored in whole infected root systems and in the cyst nematode feeding-sites (syncytia) by quantitative real-time PCR and GUS expression analysis, respectively. Quantitative PCR using whole roots of infected *Arabidopsis* plants showed that expression of *ATFLS-1* was strongly downregulated at 14dpi, while expression of *ATFLS-2*, *-3*, and *-4* increased at 5 dpi and remained high at 9 and 14 dpi. By contrast, expression of *ATFLS-5* did not change during nematode infection. *ATFLS*::GUS expression lines reflect *FLS* expression at the nematode feeding site and showed that expression of *AtMYB-12* and *AtFLS-3* was up-regulated in syncytia, while expression of *AtFLS-1* and *AtFLS-5* was down-regulated. No observable GUS expression for *FLS-2*, and *FLS-4* was observed in the nematode feeding sites. We further investigated if *AtMYB-12* and *AtFLS* are important in nematode feeding site development by screening mutant *A. thaliana* lines that are defective for *AtMYB-12* and each of the *AtFLS* genes. *Arabidopsis Atmyb-12* mutant plants were less susceptible to nematode infection compared to wild-type plants, indicating that *AtMYB-12* may play a regulatory role in syncytia formation. However, *Atfls-1* and *Atfls-5* plants were more susceptible to nematode infection than wild type plants, suggesting that *ATFLS-1* and *ATFLS-5* may be involved in basal host defence response against sugar-beet cyst nematode.

Keywords: *AtMYB-12*; flavonol genes; gene expression; *Heterodera schachtii*; sugar-beet cyst nematode.

NemaWater: A nematode aqueous diffusate elicits immune responses and pathogen resistance in *Arabidopsis*

Badou Mendy¹, Mary Wanjiku Wang'ombe¹, Zoran S. Radakovic¹, Julia Holbein¹, Cyril Zipfel², Shahid Siddique¹ and Florian M. W. Grundler¹

¹ Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

² The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

corresponding author: s7bamend@uni-bonn.de

An effective plant defence against pathogens relies on recognition of pathogen-associated molecular patterns (PAMP) by surface-localised receptors leading to the activation of PAMP-triggered immunity (PTI). Extensive studies have been conducted to characterise the role of PTI in various models of plant-pathogen interactions. However, less is known about the role of PTI in roots in general and in plant-nematode interactions in particular. Our experiments show that treatment of *Arabidopsis* seedlings with a nematode aqueous diffusate (NemaWater) triggers PTI-like responses including ROS burst, specific gene expression, and seedling growth inhibition in a manner dependent on the common immune co-receptor BAK1. Moreover, plants treated with NemaWater prior to infection were considerably more resistant to nematodes as compared to wild-type control. We also found that treatment of NemaWater with proteinase K or heating abolished the ROS burst activation of NemaWater, indicating that the eliciting capacity of NemaWater is due to the presence of a heat-sensitive proteinaceous ligand. A proteome analysis of NemaWater showed the presence of nematode surface proteins inside the NemaWater. The presence of a proteinaceous elicitor in NemaWater triggering PTI responses in a BAK1-dependent manner demonstrates the relevance of PTI during plant-nematode interaction. The role of various conserved signalling molecules present in NemaWater will be discussed.

Keywords: BAK1; nematodes; PAMPs; plant immunity; PTI.

The *Arabidopsis* leucine-rich repeat receptor-like kinase NILR1 is required for induction of innate immunity to parasitic nematodes

Badou Mendy¹, Mary Wanjiku Wang`ombe¹, Zoran S. Radakovic¹, Julia Holbein¹, Cyril Zipfel², Shahid Siddique¹ and Florian M. W. Grundler¹

¹ Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

² The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, UK

corresponding author: s7mawamg@uni-bonn.de

An effective plant defence against pathogens relies on recognition of pathogen-associated molecular patterns (PAMP) by surface-localised pattern-recognition receptors (PRR). Upon PAMP perception, PRR trigger intracellular signalling cascades leading to activation of PAMP-triggered immunity (PTI). A number of PRR perceiving a wide-range of PAMP have now been characterised for various models of plant-pathogen interactions; however, so far none have been identified perceiving a nematode-derived PAMP (NAMP). Here we show that invasion of *Arabidopsis* roots by parasitic nematodes triggers PTI-like responses including an upregulation in expression of a number of plasma-membrane localised leucine rich repeat receptor-like kinases (LRR-RLK). Nematode infection assays with candidate genes demonstrated that loss of NILR1 (for Nematode-Induced LRR-RLK 1) expression enhances the susceptibility of plants to a broad range of nematodes, suggesting that NILR1 is a PRR that perceives a conserved nematode-derived NAMP. This hypothesis is supported by experiments showing that *nilr1* is defective in ROS burst as well as in seedling growth inhibition upon nematode-derived elicitor treatment compared with wild-type control. The identification of NILR1 as a PRR perceiving a NAMP is a major step forward in understanding of the plant basal defence against nematodes. This vital information will not only increase knowledge into plant-nematode interaction, but also provide a resource for molecular breeding of nematode resistant plants.

Keywords: *Arabidopsis*; NemaWater; PAMP-triggered immunity.

Local and systemic hormone-based responses during early infection of the cyst nematode *Heterodera schachtii* affect shoot herbivory in *Arabidopsis thaliana*

Krzysztof Wieczorek¹, Nina Kamerhofer¹, Barbara Eger¹, Zoran Radakovic², Mary Regis¹, Petre Dobrev³, Radomira Vankova³, Florian Grundler², Shahid Siddique², Julia Hofmann¹ and Peter Schausberger⁴

¹ Division of Plant Protection, Department of Crop Sciences, University of Natural Resources and Life Sciences, UFT Tulln, Konrad Lorenz Str. 24, 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 Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 263, 165 02 Prague 6 – Lysolaje, Czech Republic

⁴ Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1190 Vienna, Austria

corresponding author: krzysztof.wieczorek@boku.ac.at

Heterodera schachtii, a plant-parasitic cyst nematode, invades host roots and induces syncytial feeding sites, from which it withdraws all required nutrients, causing severe yield losses. The system *H. schachtii*–*Arabidopsis* is an excellent model for investigating plant defence, which is suppressed in well-established syncytia, whereas it is induced during early parasitism. The host mediates these reactions and physiologically reacts e.g., with local and systemic alterations of endogenous hormone levels coupled with coordinated transcriptional changes. The mechanisms modulating these responses and the roles of phytohormones in these processes are largely unknown. Hence, we elucidated the role of hormone-based defense at the onset of nematode infection in above- and belowground plant parts. In addition, we tested how these responses affect attractiveness and susceptibility during simultaneous nematode root parasitism and shoot herbivory by the thrips *Frankliniella occidentalis* and the spider mite *Tetranychus urticae*. Our results suggest a pivotal and positive role for ethylene during nematode attraction, whereas jasmonic acid triggers early defence responses against *H. schachtii*. Salicylic acid seems to be a negative regulator during later syncytium and female development. Further, we show that nematode infection changes host attractiveness to herbivores or their behavioural and life history performance (below- to aboveground) and that shoot herbivory systemically changes the host attractiveness to nematodes (above- to belowground). Moreover, systemic changes triggered by thrips but not spider mite make *Arabidopsis* roots more attractive for *H. schachtii*. We conclude that nematodes impose specific changes in hormone pools locally and systemically, thus modulating hormone-based defense and signal transduction in strict dependence on their parasitism stage. In tritrophic interactions these changes significantly alter the shoot herbivory by thrips and spider mites.

Keywords: Aboveground-belowground interactions; early infection; herbivores; hormone-based defence responses; plant-parasitic nematodes.

***Meloidogyne incognita* induced cell wall molecular architectures and role of cell wall genes**

Refik Bozbuga, Catherine J. Lilley, J. Paul Knox and Peter E. Urwin

Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, West Yorkshire, UK

corresponding author: bsrb@leeds.ac.uk;

The root-knot nematode, *Meloidogyne incognita*, induces unique cells in host roots, termed giant cells, from which it feeds for the entirety of its parasitic life. *In situ* immunochemical analysis has been carried out to reveal the presence and distribution of a range of glycans, pectin and glycoproteins in the walls of giant cells and surrounding root cells of *Arabidopsis thaliana*, *Vigna angularis* (aduki bean) and *Zea mays* (maize) infected by *M. incognita*. High-esterified pectic homogalacturonan, xyloglucan and pectic arabinan were found to be common glycans within the giant cell walls of all three plant hosts. Xylan, feruloylated xylan and mixed-linkage glucan were found only in giant cell walls of the maize host. The giant cell walls of *Arabidopsis* and maize, but not aduki bean, contained mannan and galactan. Arabinogalactan protein was abundant only in the walls of giant cells induced in maize. *Arabidopsis* cell wall-related mutants were investigated to define the role of specific host genes in giant cell development. *Arabidopsis* knock-out mutants of glycoprotein-related genes supported a greater number of nematodes that were also larger. Conversely, hemicellulose-related gene knock-outs resulted in a decreased nematode burden with less-developed, smaller females and fewer, smaller galls. Pectin-related gene knock-outs revealed more disparate results depending on the specific gene that was mutated. The walls of the giant cells in all mutants were thicker than those formed in wild type roots. Overall, giant cell wall composition differs from surrounding root cells, whilst reflecting the identity of the host plant. Perturbance of cell wall components has an impact on the success of the plant-nematode interaction. The role of cell wall degrading enzymes secreted by the nematode in the development of the nematode-plant interaction is being investigated by using RNAi knock-down.

Keywords: Cell wall genes; cell wall molecular architecture; *Meloidogyne incognita*.

Exploiting natural variation in susceptibility of *Arabidopsis thaliana* to *Meloidogyne incognita* to breed broad-spectrum resistance to root-knot nematodes

Sonja Warmerdam, Casper van Schaik, Jose Lozano-Torres, Anna M. Finkers-Tomczak, Mark Sterken, Jaap Bakker, Aska Goverse and Geert Smart

Laboratory of Nematology, Wageningen University and Research, 6708 PB, Wageningen, The Netherlands

corresponding author: sonja.warmerdam@wur.nl

The root-knot nematode, *Meloidogyne incognita*, is able to parasitise hundreds of plant species and is a major threat in nearly all vegetable-growing regions in the world. Current resistance is based on major dominant resistance (R) genes, which are frequently overcome by the occurrence of resistance-breaking races of *M. incognita*. Allelic variation in genetic loci associated with susceptibility to plant-parasitic nematodes within a single plant species has thus far not been used to breed durable broad-spectrum resistance in crops. We investigated the susceptibility of 350 different ecotypes of *Arabidopsis thaliana* to the root-knot nematode *M. incognita*. A nine-fold difference in susceptibility to *M. incognita* was found between the most and the least susceptible ecotypes. This quantitative variation can be caused by polymorphic levels of gene transcripts, and by allelic variation within transcripts of essential susceptibility genes. We conducted a genome wide association mapping study to determine loci that contribute to susceptibility of *A. thaliana* to *M. incognita*. Genes underlying these loci were investigated with T-DNA insertion mutation analysis to confirm their effect on nematode susceptibility of *A. thaliana*. Our data indicate that allelic variation can be used to breed for broad-spectrum nematode resistance.

Keywords: *Arabidopsis thaliana*; HAPMAP population; genome wide association mapping; plant-nematode interaction; susceptibility factors.

Cyclin-dependent kinase inhibitors of the KRP family perturb the cell cycle in *Arabidopsis* galls: a potential strategy for crop protection against root-knot nematodes

Janice de Almeida-Engler¹, Paulo Vieira², Roberta Ramos Coelho³, Maria Fatima Grossi de Sa³, Gilbert Engler¹ and Pierre Abad¹

¹ INRA, University of Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

² Lab. Nematologia/ ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal

³ Embrapa Genetic Resources and Biotechnology, Parque Estação Biológica - PqEB - Av. W5 Norte, Caixa Postal 02372 – CEP 70770-917, Brasília, DF – Brazil

corresponding author: janice.almeida-engler@Sophia.inra.fr

The cell cycle machinery in galls appears tightly regulated by root-knot nematodes and the expression of a subset of cell cycle genes is essential for the successful establishment of a nematode feeding site. Giant-feeding cells undergo coordinated cell cycle activity initiated by an acytokinetic mitotic phase followed by endoreduplication. During the talk, it will be illustrated that ectopic expression of all members of the *Arabidopsis* KRP family of cell cycle inhibitor genes result in the severe decrease of gall establishment and expansion. With exception of KRP6, ectopic expression of the six remaining *Arabidopsis* KRPs inhibit mitotic activity in galls leading to a severe reduction in neighbouring cell division and giant cell size. While KRP6 induces mitosis upon overexpression in galls leading to a reduced giant cell size, other KRPs, induce cumulative mitotic defects like elongated and apparently connected nuclei. As well, KRP3 overexpressing lines are characterised by exceptionally elongated giant cells. Ectopic expression of all seven AtKRPs induced phenotypes in galls consequently resulting in disturbed nematode development, thereby affecting the offspring. The results presented support the idea that the ectopic expression of cell cycle inhibitors of the KRP gene family in galls can be envisaged as a conceivable strategy to protect crop species attacked by plant-parasitic nematodes.

Keywords: Biotechnology; endocycle; mitosis; nematode feeding sites; plant.

Programmed cell death regulation in *Heterodera schachtii* infected *Arabidopsis thaliana* roots

Mateusz Matuszkiewicz¹, Mirosław Sobczak² and Marcin Filipecki¹

¹ Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences, 02-776 Warsaw, Poland

² Department of Botany, Warsaw University of Life Sciences, 02-766 Warsaw, Poland

corresponding author: matuszkiewicz.mateusz@gmail.com

Plant-parasitic nematodes are common pests of many crops causing substantial losses in agriculture. Their parasitism relies on gland secretion triggered developmental and metabolic reprogramming of plant cells leading to feeding site formation. Reactive oxygen species (ROS) play pivotal role in the regulation of this process. Excessive accumulation of ROS and changes in the distribution of the salicylic acid during infection by parasites leads to the death of plant cells, which may be used to protect resistant plants in case of hypersensitive response or to direct feeding site development in case of susceptible plants. In order to shed light on the role of programmed cell death (PCD) in plant nematode interaction several regulators of PCD were analysed. The infection tests on mutants of genes belonging to LSD regulon showed changes in syncytia size, whereas much less differences in susceptibility were observed. The negative regulator of PCD *LSD1* and positive *LOL1* and *LOL2* revealed slight but antagonistic patterns of susceptibility. The observations of the development and ultrastructure of syncytia induced in *lsd1* lines revealed retarded growth, electron translucent cytoplasm, smaller vacuoles, reduced number of plastids, mitochondria and endoplasmic reticulum structures. To link morphological analysis with molecular mechanisms of PCD control in *lsd1* mutant RNA-seq analysis on infected and non-infected roots was done. These results demonstrate that PCD is a highly regulated process in compatible plant-nematode interaction. This work was supported by National Science Centre (grants no. 50130041000M0007799).

Keywords: *Heterodera schachtii*; *lsd1*; programmed cell death (PCD); RNA-seq.

A genome phylogeny of Nematoda

Mark Blaxter and Georgios Koutsovoulos

Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3TF, UK

corresponding author: mark.blaxter@ed.ac.uk

The phylum Nematoda is speciose and diverse. Many competing systematic treatments of Nematoda have been proposed over the last century, with radically differing conclusions that suggest very different trajectories for the evolution of key morphological characters, marine *versus* terrestrial habit and parasitism. Nearly twenty years ago we published a molecular assessment of the relationships of Nematoda, based on a single gene (the nuclear small subunit ribosomal RNA, nSSU) from 53 taxa selected to span the diversity of the phylum. This phylogeny has proven remarkably robust, but left several deep nodes unresolved. Complete genome sequencing of target species is now easily achieved using next generation sequencing and advanced bioinformatics. Here we present an analysis of nematode phylogeny using not a single gene but nearly one thousand genes from 53 species, derived from large scale genomics and transcriptomics projects. We affirm the core findings made with nSSU and further resolve the deep branching of the phylum.

Keywords: Genomics; parasitism; phylogeny; transcriptomics.

An updated chromadorean nematode phylogeny based on comparative analysis of complete mitochondrial genome information

Joong-Ki Park¹, Jiyeon Kim¹, Taeho Kim² and Steven A. Nadler³

¹ Division of EcoScience, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

² Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, 145 Anamro, Seongbuk-gu, Seoul 02841, Republic of Korea

³ Department of Entomology and Nematology, University of California, Davis, CA 95616, USA

corresponding author: jkpark@ewha.ac.kr

Nematodes are among the most common, abundant and ecologically diverse metazoan groups: they are found in almost every environment (marine, freshwater, and soil) including many parasitic forms in plants and animals that have deleterious effects on agriculture, humans and wildlife. Despite the importance of nematodes to human welfare, their phylogeny remains a subject of active debate. Elucidating nematode phylogenetic relationships can advance understanding of diverse issues in biology such as the frequency of convergent evolution, and the level of complexity involved in changing and adapting to new habitats. Recent molecular phylogenetic hypotheses for chromadorean nematodes have been based on nuclear ribosomal DNA genes, but these have been challenged by trees inferred from complete mitochondrial genome data. In this presentation, we provide an update of phylogenetic hypotheses for chromadorean nematodes based on comparative analyses of complete mitochondrial genomes. The resulting mitochondrial genome trees for 12 protein-coding genes depicted the following relationships: (1) monophyly of Plectida and its basal position within chromadorean clade, (2) sister relationship between Rhabditomorpha and Diplogasteromorpha, (3) non-monophyly of Panagrolaimomorpha, (4) non-monophyly of Tylenchomorpha, (5) monophyly of Aphelenchoidea. For the major chromadorean groups, the most probable gene order and orientation for each hypothetical ancestor was inferred based on the maximum parsimony principle and discussed in conjunction with their phylogenetic relationships

Keywords: Mitogenome evolution; molecular phylogeny; nematoda; Plectida.

Broad phylogenetic analyses of the four major plant-parasitic nematode lineages reveal essentially distinct origins and diversification patterns

Martijn Holterman¹, Akbar Karegar², Paul Mooijman¹, Wilfrida Decraemer³, Sven van den Elsen¹, Mariette T.W. Vervoort¹, Casper W. Quist¹, Gerrit Karssen⁴, Charles H. Opperman⁵, David M. Bird⁵, Geert Smant¹ and Johannes Helder¹

¹ Wageningen University, Laboratory of Nematology, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands

² Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

³ Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

⁴ NPPO, National Reference Centre, P.O. Box 9102, 6700 HC Wageningen The Netherlands

⁵ Department of Plant Pathology, NC State University, Box 7253, Raleigh, NC 27695, USA

corresponding author: hans.helder@wur.nl

Four major nematode clades are discerned within which plant parasitism arose at least once. The distinct levels of diversification within the individual clades are reflected by their systematics: plant parasites in the basal Clades 1 and 2 reside in (just) two families (Trichodoridae and Longidoridae). Meanwhile, in Clades 10 and 12 parasitic nematodes are allocated in a superfamily (Aphelenchoidoidea) and an order (Tylenchida). By taking the coverage of the next lower taxonomic level as a measure, 60, 80, 100 and 85% of the diversity was included in our phylogenetic analyses. Individual clades represented by 93 (Clade 1), 171 (Clade 2), 320 (Clade 10), and 1,089 (Clade 12) nearly full-length SSU rDNA sequences (\approx 1,700 bp each) were analysed using Bayesian and ML-based inferences. Diversification patterns among these Clades are distinct. Clades 1 and 2 exclusively harbour ectoparasites, of which a subset transmits Tobra or Nepo-viruses. Superposition of current virus-transmission data on trees revealed three (Trichodoridae) and > five clusters (Longidoridae) suggesting that minor modifications in the virus-pharynx interface suffice to acquire this capability. Endoparasitism arose exclusively in Clades 10 and 12, resulting in disparate life strategies in these clades. Plant parasitism within Clade 10 arose at least four times from insect-vectored fungivorous lineages (occasionally with secondary loss of the insect association) and resulted almost exclusively in endoparasites of above-ground plant parts, in the absence of (intermediate) ectoparasites. Our analyses point at a single common origin of all below-ground feeding plant parasites in Clade 12, whereas sedentary endoparasitism arose at least five times. Numerous origins and shapes of plant parasitism-relevant characteristics such as the development of plant parasitism per se, of sedentary endoparasitism, and of virus-vectoring capacities point at an unexpected flexibility within this morphologically conserved phylum.

Keywords: Evolution; phoresy; sedentary endoparasitism; SSU ribosomal DNA; virus transmission.

Beet cyst nematodes and sea beets : what phylogeography tells us?

Sylvain Fournet¹, Catherine Porte¹, Eric Petit² and Cécile Gracianne¹

¹ INRA, UMR 1349 IGEPP INRA - Agrocampus Ouest - Université de Rennes 1, BP 35327, 35653 Le Rheu Cedex, France

² INRA, UMR ESE INRA - Agrocampus Ouest, 65 rue de Saint-Brieuc Bât. 4, CS 84215, 35042 Rennes Cedex, France

corresponding author: sylvain.fournet@rennes.inra.fr

Wild populations are often considered as reservoir of genetic variability for agronomical pest species. For instance, the development of the beet cyst nematode, *Heterodera schachtii*, can be very harmful for a large range of crop species and particularly for sugar beet. The use of resistant varieties, introgressed from the wild relative species *Beta maritima*, is thus needed to reduce yield damages. This plant species is largely distributed all along the European Atlantic coast and sometimes close to cultivated fields. The assessment of the occurrence of wild populations of *H. schachtii* on wild beets and the study of the coevolutionary dynamics between the two species may help determine the presence of adapted individuals to plant resistances of *B. maritima* and help evaluate the risk they represent in sugar beet fields. Our study, based on samples collected from the south of Spain to the north of Sweden, shows that *H. schachtii* is largely distributed in the wild but is not the only one cyst nematode species associated with *B. maritima*. Moreover, the study of the spatial distribution of genetic diversity of the nematode and the host-plant suggests that even if *H. schachtii* and *B. maritima* have both persisted in the south of Europe during the last glacial maximum, their evolutionary histories share few similarities indicating that they thus did not strictly coevolve. This suggests that wild populations may not represent a special risk in the case of gene flow between wild and field compartments.

Keywords: *Beta maritima*; *Heterodera betae*; *Heterodera schachtii*.

The effects of an experimental drought on the free-living soil nematode diversity of a protected semi-arid sand steppe in the Kiskunság National Park, Hungary

Judit Szakálas¹, György Kröel-Dulay², Anikó Seres¹, Gábor Ónodi² and Péter Nagy¹

¹ Department of Zoology and Animal Ecology, Szent István University, H-2100 Gödöllő, Páter K. u. 1., Hungary

² Institute of Ecology and Botany, Center for Ecological Research, Hungarian Academy of Science, H-2163 Vácrátót, Alkotmány u. 2-4., Hungary

corresponding author: szakalaszjudit@gmail.com

Studying extreme weather events and their effects on ecosystems can help us forecast ecosystem responses to future climates. Moreover, it is important to predict the sensitivity of rare and protected habitats such as open sand grasslands, which is the characteristic ecosystem type of the Kiskunság, in central Hungary. We established a field experiment to examine the effects of a 5 month drought treatment on the soil nematode community in plant covered and bare soil microhabitats in sand grasslands (EXDRAIN Project – Extreme Drought and Chronic Rain Manipulation Experiment). We excluded the precipitation from 24 April 2014 to 18 September 2014. Our hypothesis was that plant cover would influence the effect of the drought treatment by buffering against its harmful effects. Drought significantly decreased nematode density. The density of the bare areas within the drought plots differed from the other treatments. Furthermore, the densities in the plant covered microhabitats of the control plots differed significantly from the dried and plant covered ones. The drought induced an increase in the densities of *Nothacrobeles* and *Acrobelophis*, and a decrease in the densities of *Aphelenchoides*, *Cephalobus*, *Plectus*, *Geomonhystera*, *Tylenchorhynchus* and *Filenchus*. According to the Rényi's diversity profiles, drought plots were characterised by the least structured nematode community, and the effects of the drought treatment were more pronounced in the bare soil microhabitat compared to the vegetated ones. The diversity patterns differed less between the bare and plant covered samples in the control plots than in the drought treated ones. The bacterial-feeding nematodes appeared to be the most sensitive group to drought, as no effects were observed on the density of the predator and omnivore nematodes.

Keywords: Climate manipulation; community structure; global change; grassland; nematode feeding groups.

Improving the effectiveness and delivery of gene silencing triggers to control plant nematode pests

Fareeha Naz, John Fosu Nyarko and Michael G. K. Jones

Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, Perth, WA 6150, Australia

corresponding author: fareehajaved@hotmail.com

Plant-parasitic nematodes are a major group of crop pests that decrease yields by 7-15%, and locally to 50% or more. The most important groups economically are the endoparasites that invade roots and form distinct feeding sites (sedentary endoparasites), and those that do not (migratory endoparasites). Two genera of sedentary endoparasitic nematodes, with relatively wide host ranges, are major temperate crop pests in Western Australia. These are the beet cyst nematode (*Heterodera schachtii*) and the root knot nematode *Meloidogyne incognita*. Total annual yield loss due to damage caused by sugar beet cyst nematode (BCN) in European countries is around \$95 million. Gene silencing through RNA interference (RNAi) is an emerging field and can play an important part in controlling pests of crop plants. The current study includes, in particular, development of novel strategies for effective delivery of gene silencing triggers, in the form of long double stranded RNA (dsRNA) or small interfering RNAs (siRNAs), to nematodes feeding on standard or transgenic plants. One of the strategies that may be used to introduce long dsRNA and/or siRNAs to plants is ectopic delivery of more stable forms of dsRNA, and monitoring uptake and effects of silencing on nematodes feeding on treated plants. The aim of the approach is to seek alternative modes of delivery of dsRNA that do not require the generation of transgenic plants, and which can be applied when transgenic technology is not acceptable or financially viable.

Keywords: Double stranded RNA (dsRNA); ectopic; plant-parasitic nematodes (PPNs); RNA interference; transgenic.

Imaging free-living nematode feeding behaviour *in vivo* in transparent soil

Felicity O'Callaghan¹, Lionel X. Dupuy¹, Roy Neilson¹, Stuart A. MacFarlane¹ and Roberto Braga²

¹The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK

²Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

corresponding author: felicity.o'callaghan@hutton.ac.uk

Imaging the interactions between free-living nematodes and plants *in vivo* poses a range of challenges. While natural soil is too opaque, gels are unsuitable for natural plant growth and nematode movement. Added to this are the small size, transparency and impermeability of nematodes to stains. We here present a new experimental system in which nematode activity could be visualised live *in situ* and in 3D using transparent soil. Purpose-built growth chambers allowed both plants and nematodes to be cultured within a transparent soil microcosm. The refractive index of the soil particles was matched, and thereby rendered transparent, by the introduction of a silica suspension. The resultant medium allowed plant and nematode survival for several days, enabling plant- nematode interactions to be monitored over an extended period of time. Micro-scale imaging was carried out using confocal laser scanning microscopy for the acquisition of high resolution images of plant-nematode interactions, the filming of nematode behaviour as well as for 3D volume reconstructions of root properties within the soil. For macro-scale imaging, Selective Plane Illumination Microscopy (SPIM) was used to create 3D reconstructions of root architecture. This imaging technique has the advantage of being able to scan wide sections of the root system at high speed and could therefore provide a useful tool in examining overall levels of nematode infection. We are also investigating biospeckle dynamics (the interference pattern generated when irradiating a biological sample with coherent light such as laser) as a novel method for nematode detection and tracking. Preliminary results showed the potential of biospeckle as a quick and stain free screening method for the localisation of pests as well as assessing their biological activity. We are currently investigating the application of these new imaging techniques for observing the effects of nematicides *in vivo*.

Keywords: Biospeckle; free-living nematodes; light sheet microscopy; live imaging; transparent soil.

DuPont™ Vydate™: Novel insights into its compatibility with soil health

Tim Thoden¹, Peggy Link¹, Johan Desaege², Wilbert Flier³, John Wiles⁴, Mariam Alabed Alkader⁵, Sylvie Cranenbrouck⁶ and Stephan Declerck⁶

¹ DuPont de Nemours (France) S.A.S., European Research Center; 68740 Nambenheim, France

² DuPont Crop Protection, Stine-Haskell Research Center, Newark DE 19711, USA

³ DuPont de Nemours (The Netherlands) B.V., 3313 LA Dordrecht, The Netherlands

⁴ DuPont (UK) Limited; 4th Floor Kings Court, London Road, Stevenage, Hertfordshire, SG1 2NG, England, UK

⁵ University of Freiburg, Institute of Forest Botany, 79085 Freiburg, Germany

⁶ University of Louvain, Institute of Applied Microbiology, Louvain, Belgium

corresponding author: tim.thoden@dupont.com

Healthy soils are widely regarded to be an important factor in reducing the incidence of soilborne plant diseases and supporting plant growth. This can be directly linked to a well-developed soil food web (e.g., different nematode feeding groups) as well as the presence of natural antagonists (e.g., nematophagous fungi) as well as plant growth promoting microbial organisms such as mycorrhizal fungi or plant growth promoting bacteria. The management of plant-parasitic nematodes can highly benefit from these natural antagonists and plant symbionts. However, especially when nematode pressure is high, chemical nematicides are needed to guarantee good crop yields. Consequently it is important to know how soil applied nematicides interact with the different organisms that contribute to the overall soil health network. Therefore we investigated the effects of the carbamate nematicide Vydate™ on different feeding groups of soil nematodes, nematophagous fungi, plant-growth promoting rhizobacteria and mycorrhiza as well as entomopathogenic nematodes.

Keywords: Entomopathogenic nematodes; nematicides; nematode feeding groups; nematophagous fungi.

Effect of fluopyram and fluensulfone on initial infection processes of *Meloidogyne* speciesYuji Oka

Gilat Research Center, M.P. Negev 8528000, Israel

corresponding author: okayuji@volcani.agri.gov.il

Fluensulfone and fluopyram are new nematicides probably with new mode of action. These nematicides are highly effective in pot experiments against root-knot nematodes, *Meloidogyne* spp., at low concentrations, which may not be lethal to the juveniles. The present study was conducted to elucidate whether these nematicides affect initial infection processes, such as attraction to roots and penetration, of *Meloidogyne* species. Lettuce seedlings in Petri dish containing pluronic gel were inoculated with second-stage juveniles of *M. incognita* and *M. javanica*, which had been exposed to solutions of fluensulfone (as Nimitz®) and fluopyram (as Velum®) at low concentrations ($\leq 2 \text{ mg a.s. l}^{-1}$). Treated juveniles were successfully attracted to root tips in a few hours if they kept motility similarly to untreated juveniles. Afterwards, root galls were formed on roots, which attracted treated or untreated juveniles. Similar results were obtained when lettuce seedlings were inoculated with juveniles in the gel containing the nematicides at the same concentrations. In another attraction assay on agar plates, fluopyram attracted juveniles of *M. javanica*, *M. hapla* and *M. marylandi*, but not *M. incognita*. Fluensulfone was attractive to *M. javanica* at higher concentrations as compared to fluopyram. The attraction of *M. javanica* to these nematicides was also confirmed in assays in sand. The results suggest that partial or total paralysis or death of the juveniles is the main control mechanism of the nematicides. Involvement of the attractiveness of the nematicides in the nematode control is not clear.

Keywords: Attraction; chemical control; nematicide; root-knot nematodes.

Abamectin as a microbial-derived nematicidal seed coating

Antoon Ploeg and J. Ole Becker

Department of Nematology, UCR, 1463 Boyce Hall, 3401 Watkins Drive Riverside, CA 92521, USA

corresponding author: antoon.ploeg@ucr.edu

Abamectin is a mixture of macrocyclic lactone metabolites produced by the actinobacteria *Streptomyces avermitilis*. It has been known since the early 1980s for its insecticidal, acaricidal and antihelminthic activities. This led to its widespread use in antiparasite medications as well as foliar insecticides and acaricides. Ten years ago the US Environmental Protection Agency registered Avicta® (a.s., abamectin) as a seed coating to protect young seedlings of various agronomic crops against plant-parasitic nematodes. Avicta® caused a paradigm shift in crop protection against nematodes as it provided a relatively inexpensive, effective treatment at extremely low application rates compared to soil-applied nematicides. Moreover, in synergy with various other antimicrobial seed coatings, it significantly lowered the severity of root-knot nematode-enhanced fungal diseases such as Fusarium wilt (*Fusarium oxysporum*) and Rhizoctonia damping-off (*Rhizoctonia solani*). The lack of antimicrobial activity makes abamectin a suitable biorational ingredient in combination products with fungal or bacterial biological control agents. In summary, Avicta® is a valuable tool for economically feasible and ecologically sensible crop protection against plant-parasitic nematodes.

Keywords: Avicta®; biorational; *Meloidogyne* spp.; nematicide; root-knot nematodes.

Life history traits of entomopathogenic nematodes of *Steinernema* spp. and *Heterorhabditis bacteriophora*

Temesgen Addis Desta^{1,3}, Asmamaw Teshome², Nevena Mijušković², Shiferaw Demissie², Olaf Strauch³ and Ralf-Udo Ehlers^{1,2,3}

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

corresponding author: temesgen_addis@yahoo.com

Entomopathogenic nematodes (EPN) are lethal pathogens of insect pests. *Steinernema feltiae* and *Heterorhabditis bacteriophora* are the most widely spread EPN species in the world. On the other hand, *S. riobrave* and *S. yirgalemense* are limited to tropical climates. Although these EPN species are important agents for insect pest control, their availability is limited due to high production costs. Knowledge on the population dynamics of both the nematodes and their associated symbiotic bacteria helps to improve liquid culture production and reduce cost of production. Investigations of the life history traits (LHT) of the four EPN species in hanging drops using semi-solid nematode growth gelrite with their respective symbionts were conducted. The hanging drop method enabled investigation of the LHTs based on individual females/hermaphrodites at different bacterial densities. Offspring production increased with increased bacterial density. For example, at 5×10^9 bacterial cells ml^{-1} of the respective symbiotic bacteria, the number of offspring recorded per female or hermaphrodite of *S. feltiae*, *S. riobrave* and *H. bacteriophora* was 359, 680–792 and 50, respectively. The number of offspring increased by 2–3 fold when the bacterial density increased from 5×10^9 to 20×10^9 cells ml^{-1} . The increase in offspring production was found significantly associated with the body volume of females recorded at the beginning of endotokia matricida. In liquid culture, food is the limiting factor since the culture method does not allow for provision of fresh food every day like in hanging drops. As a consequence of competition, the number of maternal nematodes has a significant negative correlation with offspring production per female ($R = -0.9$). However, it was demonstrated that the total female body volume is a good parameter for DJ yield estimation in liquid culture. This model can be improved further by including bacterial cell numbers at the beginning of endotokia matricida.

Keywords: Bacterial density; biological control; dauer juvenile yield; endotokia matricida; hanging drop.

Recent advances in our understanding of the diversity and co-evolution of entomopathogenic nematode-bacteria complexes

Vladimir Puza, Lucie Faktorova, Jiri Nermut, Martina Zurovcova and Zdenek Mracek

Biology Centre ASCR v. v. i., Institute of Entomology, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

corresponding author: VPuza@seznam.cz

Increased sampling effort together with new molecular techniques in the last decades led to the tremendous increase in the known diversity of entomopathogenic nematodes (EPN). From the year 2000, the number of described steinernematids and heterorhabditids more than tripled from 25 to 92 and from 6 to 18, respectively. A substantial number of these species were reported from Southeast Asia, South Africa and South America. Unfortunately, for many newly described EPN, the information on the identity of bacterial symbiont is missing. Mutualistic microbial symbioses are often hypothesised to have undergone coevolution with hosts, which can eventually lead to parallel speciation or co-speciation in both partners. In the *Heterorhabditis-Photorhabdus* complex a significant coevolutionary relationship was reported. In *Steinernema* and *Xenorhabdus* complex, the only study addressing co-speciation found a high level of incongruence between phylogenies of the species of *Steinernema* and *Xenorhabdus*. A recent study sequenced genomes of nine *X. bovienii* strains and identified cocladogenesis between *Steinernema feltiae* nematode hosts and their corresponding *X. bovienii* symbiont strains, indicating potential specificity within the association. In our study, we compared phylogenies of ca 30 strains of *Steinernema* spp. and their symbiont *X. bovienii* from throughout the world. The nematode and bacteria phylogenies were reconstructed based on the ITS region and the 16S, RecA and GyrB genes, respectively. Our preliminary data show a high level of incongruence between *Steinernema* spp. and *X. bovienii* phylogenies and suggest a geographic pattern in some associations.

Keywords: Co-phylogeny; diversity; new species.

Insect immunity after invasion of entomopathogenic nematodes

Pavel Hyršl¹, Pavel Dobeš¹, Badrul Arefin², Lucie Kučerová^{2,3}, Robert Markus², Zhi Wang², Michal Žurovec³ and Ulrich Theopold²

¹ Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

² Department of Molecular Biosciences, The Wenner-Gren Institute, University of Stockholm, 10691 Stockholm, Sweden

³ Biology Centre of the AS CR, Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

corresponding author: hyrsl@sci.muni.cz

Entomopathogenic nematodes and their associated bacteria comprise together a highly pathogenic complex that is able to invade and kill insect host within two days. Both bacteria and nematodes produce a variety of factors interacting with the insect immune system and help to overcome host defences. The tripartite model (*Drosophila*, nematodes, bacteria) was established and used to show an immune function for candidate genes using different *Drosophila* mutants or RNAi lines with defects in clotting or other branches of the immune system. Microarray analysis was used to compare gene expression of *Drosophila* larvae infected by the entomopathogenic nematode *Heterorhabditis bacteriophora* and its symbiotic bacterium *Photorhabdus luminescens* with non-infected larvae. The role of candidate genes, selected based on genomic comparison, in response towards nematobacterial complex was further evaluated by *in vivo* infection assays. We demonstrated an immune function during nematode infection for known clotting enzymes and substrates, recognition molecules and eicosanoids. In conclusion, we show that the *Heterorhabditis/Photorhabdus* infection model is suitable to identify regulators of innate immunity in insects. Our research is supported by research grants from the Swedish Foundation for International Cooperation in Research and Higher Education (STINT), by grant from Ministry of Agriculture of Czech Republic (NAZV-KUS QJ1210047) and The Technology Agency of the Czech Republic (TA04020318).

Keywords: *Drosophila*; *Heterorhabditis*; *Photorhabdus*.

Insights into the *Heterorhabditis bacteriophora* infective juvenile longevity via genome-wide expression analysis

Carlos Molina, Nanette H. Nellas Sumaya, Verena Dörfle, Mike Barg, Olaf Strauch, Bart Vandenbossche and Ralf-Udo Ehlers

e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany

corresponding author: c.molina@e-nema.de

The nematode-bacterium complex *Heterorhabditis bacteriophora* and *Photorhabdus luminescens* is a safe control agent against insect pests. *H. bacteriophora* infective dauer juveniles (DJ) survive in the soil after application and seek for insect hosts. For industrial production, DJ are propagated in bioreactors in large volumes (up to 40 m³). Thereafter, DJ undergo diverse stresses with a direct influence in their survival during storage, formulation, and after application. Major stress factors are overcrowding, heat, desiccation, and oxidative stress. Accordingly, the study of the genetic regulation of stress tolerance and longevity in entomopathogenic nematodes (EPN) is a compulsory task. In order to gain knowledge about the genetic background of DJ-longevity in *H. bacteriophora*, approaches combining classical breeding and next-generation genomics are combined. In the present work, highly homozygous inbred lines with contrasting DJ-longevity have been generated and their performance has been confirmed for several generations. Subsequently, genetic crosses were made between lines with contrasting DJ-longevity. Parental lines, progeny recombinant inbred lines (RIL) and individuals undergone selection pressure were analysed via MACE (Massive Amplification of cDNA Ends). Here, allele-specific differential expression is being determined. Differentially-expressed genes, as well as a wide palette of single nucleotide polymorphisms have been generated. In a parallel approach, candidate genes derived from previous transcriptomic studies were analysed for polymorphisms along a wide genotype-collection comprising of isolates of different origin. A correlation between genotype and phenotype data is being established to determine phenotype-genotype associations. The present results are an appetiser for the study of *H. bacteriophora* longevity using modern genomic tools. This basic research set the basis for further research activities to prolong shelf life of the nematodes.

Keywords: *Heterorhabditis bacteriophora*; longevity; MACE; oxidative stress.

Phenotyping of virulence and persistence of *Heterorhabditis bacteriophora* strains for control of the western corn rootworm

Bart Vandenbossche¹, Alejandra Centurión Carrera², Mike Barg¹, Verena Dörfler¹, Nanette H Nellas Sumaya¹, Olaf Strauch¹, Carlos Molina¹ and Ralf-Udo Ehlers¹

¹ e-nema, Gesellschaft für Biotechnologie und Biologischen Pflanzenschutz mbH Klausdorfer Str. 28-36, 24223 Schwentinental, Germany

² Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000, Ghent, Belgium

corresponding author: B.Vandenbossche@e-nema.de

The Western Corn Rootworm (*Diabrotica virgifera virgifera*) is one of the most damaging maize pests worldwide. Most European farmers relied on the application of granular soil insecticides and/or chemical seed treatments for several years to counteract this pest. However, serious side effects on honeybees and other beneficial soil organisms stressed the need for alternative control options. The entomopathogenic nematode, *Heterorhabditis bacteriophora*, is a promising biological control agent of this maize pest. These nematodes are applied on the soil together with the maize seeds at sowing time (spring). However, at this time the *Diabrotica* eggs are still in diapause, implying that nematodes need to survive and remain virulent for at least 4-6 weeks until the first host larvae emerge. Hence, improvement of the soil persistence and virulence in *H. bacteriophora* is compulsory. In the present work, the virulence of forty-two *H. bacteriophora* strains and a pooled population (mix) was evaluated. The nematode concentration at which 50% (LC50) of insect larvae were infected ranged between 1.4 to 30.5 infective juveniles per host insect, indicating a large variability. Persistence tests demonstrated that several *H. bacteriophora* strains remain infective against *Diabrotica* larvae after 4 to 6 weeks of incubation in sand bio-assays. Our results document the potential of natural variability in breeding programs aiming to select for improved virulence and persistence against *D. v. virgifera* and other target pests.

Keywords: Biological control; *Diabrotica virgifera virgifera*; entomopathogenic nematodes (EPN); post-application survival.

Impact of nematode plant health management on root architecture and its importance for food security in drought prone climate-change critical production zones

Richard A. Sikora

INRES-Phytopathology, University of Bonn, Nussallee 9, 53115 Bonn, Germany

corresponding author: rsikora@uni-bonn.de

Sustainable production of rainfed crops exposed to moisture stress, for example, under semi-arid conditions is impacted by climate change and hotter and drier soils. In some regions soil temperatures will rise to levels that prevent production of staple crops e.g., maize in southern Africa. In many cases nematodes are ignored by breeders who stress yield, and more recently, water use-efficiency. They fail to recognise that nematodes negatively alter root architecture, thereby impacting moisture use-efficiency and thereby yield. Integrated agronomic approaches using modern cultivars having nematode resistance, technologies that simulate root growth coupled with nematode integrated management are needed for sustainable production in dry climatic zones. Tap root deformation (root-knot and ectoparasites) induction of shallow root architecture due to abnormal branching (cyst nematodes) and root biomass reduction (all nematodes) reduce translocation of moisture and nutrients to the stem and influence grain formation and yield. Bio-assays that incorporate root growth factors, such as speed of development, expanded root architecture, improved biomass, and degree and speed of root depth penetration, need to be incorporated into nematological research programmes. Scanning equipment and software as well as appropriate bio-assays are either available or need to be developed for both glasshouse and field studies. Technologies are available that need to be tested on nematode infested soils under rainfed and moisture stress conditions. These tools include improved seedling vigour through agrochemicals, improved root depth penetration following nematicide seed treatment and improved root biomass production through plant growth and health promoting microorganisms. Data will be presented showing climatic trends and the effects of seed care technologies on root architecture. Deficiencies in breeding programmes and the complexity of multidisciplinary approaches will be discussed.

Keywords: Endophytes; nematicides; rhizobacteria; root system vigour; water use-efficiency.

The potential implications of a changing climate on nematode pest dynamics and the impact of this on nematode management and food security in Africa using *Musa* as an example

Daniel L. Coyne¹, Nessie Luambano², Cornel Massawe³ and Johnathan Dalzell⁴

¹ International Institute of Tropical Agriculture, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya

² Sugarcane Research Institute, Kibaha, Tanzania

³ Horti-Tengeru, Arusha, Tanzania

⁴ University Belfast, Medical Biology Centre, 97 Lisburn Road, BT9 7BL, Belfast, UK

corresponding author: d.coyne@cgiar.org

A change climate has obvious implications to prevailing pest and disease dynamics. Plant-parasitic nematodes are no exception. In Europe and the Americas, where nematode pest characterisation has been relatively well detailed, there are some well documented cases of changing pest distributions in relation to changes in temperature and periods below or above temperature thresholds, and the consequent implications on crop hosts. In sub-Saharan Africa (SSA), it is predicted that the effects of climate change will be felt to a much greater extent than elsewhere, disrupting crop production. The effects of these changes are difficult to predict, however, not least due to the relatively limited knowledge and understanding of many pests and diseases in SSA. Our knowledge and understanding of nematode pest biology, characterisation and distribution is probably less than for most pest or disease groups. However, for some crops there is quite well documented information, such as for *Musa* (banana and plantain), a key staple and food security crop across the region. A major pest of *Musa*, is the burrowing nematode, *Radopholus similis*, a thermophilic species, which in the higher altitude *Musa* growing regions is replaced by the indigenous thermophobic species *Pratylenchus goodeyi*, which is less damaging. Elevating temperatures therefore, will lead to a higher altitude (temperature) threshold for *R. similis*, leading to increased losses of *Musa* from this nematode. Meanwhile, in lowland *Musa* growing areas across SSA there appears to be a gradual shift in the dominance and composition of nematode species occurring on *Musa*, with reducing *R. similis* dominance and replacement by *Pratylenchus* species. Is this in relation to climate change, or other aspects? The implications of this shift and temperature tolerances are discussed in relation to prioritising efforts to manage the evolving threats, such as traditional breeding for resistance and through transgenic options.

Keywords: Banana and plantain; breeding for resistance; sub-Saharan Africa.

Nematode assemblages in conservation agricultural, maize-based cropping systems

Suria Bekker¹, Hendrika Fourie¹, Mieke Daneel² and Andre Nel³

¹ Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2520, South Africa

² Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X1 1208, Mbombela, South Africa

³ Agricultural Research Council – Grain Crops Institute, Private Bag X1251, Potchefstroom, South Africa

corresponding author: driekie.fourie@nwu.ac.za

The main aim of conservation agriculture is to optimise crop production, using practices and principles that promote soil quality by increasing population levels and diversity of beneficial organisms (e.g., non-parasitic nematodes). The abundance and diversity of nematodes (plant-parasitic and non-parasitic) were determined in maize-based cropping systems in fields under conservation and conventional agricultural practices in South Africa. Both a survey was conducted (24 fields) and two trials under conventional agriculture over a 4-year period. Genera of 'large' and 'small' eggs, extracted from maize roots using an adapted NaOCl method, were identified using deoxyribonucleic-acid sequencing. Large eggs belonged to *Meloidogyne* and small eggs to *Rotylenchulus*. Survey results showed that *Rotylenchulus parvus* and *Pratylenchus* spp. were predominant in maize fields under conservation agriculture. By contrast, lower plant-parasitic nematode densities prevailed in the two trials in cropping systems under conservation agriculture than those where monoculture maize was grown under conventional agriculture. However, maize grown in conventional maize-based systems had higher PV values for *Meloidogyne* spp. according to results from the survey. When either cowpea or sunflower were included in the two conservation agriculture trials, a significant decline in *R. parvus* and *Pratylenchus* spp. population densities occurred over the trial period. For non-parasitic nematodes, maize grown in fields where conservation agriculture was practised generally had higher prominence values and diversity than those in conventional fields. Nonetheless, nematode-food web structures showed that most of the soils sampled from both agricultural practices represented resource-depleted nematode communities. Findings from this research are novel and warrant further investigations with regard to the effect of conservation agriculture on nematode assemblages and soil quality.

Keywords: Conservation agriculture; *Meloidogyne*; non-parasitic nematodes; *Pratylenchus*; *Rotylenchulus parvus*.

Performance of non-fumigant nematicides in California vegetables on *Meloidogyne incognita*-infested trial sites.

Antoon Ploeg¹, Ole Becker¹, Joe Nunez² and Scott Stoddard³

¹ Department of Nematology, UCR, 1463 Boyce Hall, 3401 Watkins Drive, Riverside, CA 92521, USA

² UCCE Kern County, 1031 South Mount Vernon Ave. Bakersfield, CA 93307, USA

³ UCCE Merced County, 2145 Wardrobe Ave, Merced, CA 95341-6445, USA

corresponding author: antoon.ploeg@ucr.edu

The state of California is the largest producer of vegetables in the USA. Many of these crops are hosts for one or more species of root-knot nematodes (RKN; *Meloidogyne* spp.). To manage these nematodes, soil fumigants such as metam-sodium, metam-potassium, and 1,3 dichloropropene are important tools. However, continuing public concern about the adverse impact of these pesticides on human health and the environment has led to more regulations that limit their allowed use and increase the overall cost associated with their application. As a result, there is a potential for new products, provided they are less harmful to the environment and human health, effective against the target nematode pest, and economically competitive. Several products that possibly meet these criteria have recently been registered in the USA. We tested their efficacy in field trials over the last few years on *M. incognita*-infested sites in California. Trials have focused on cantaloupe melon and processing tomatoes. In melons, no nematode resistance is currently available. Nematode-resistant processing tomato cultivars are commonly grown, but reports of resistance-breaking populations are becoming more frequent. Additionally, we evaluated new non-fumigant nematicides on the root vegetables carrot and sweet potato, where damage thresholds are low because of the direct impact of RKN nematodes on root yield and quality. Some of the products showed excellent reductions in disease symptoms accompanied with yield and quality improvements. They promise to be very useful tools in RKN management and disease mitigation in California vegetables.

Keywords: Control; melon; root-knot; sweet potato; tomato.

A novel seed-piece nematicide application method shows enhanced protection with ultra-low volume

Charles Opperman¹, Antoine Affokpon², Tim Sit¹ and Saad Khan³

¹ Department of Plant Pathology, NCSU, Raleigh, NC 27695, USA

² Nematology, Phytopathology & Crop Health Improvement, Université d'Abomey-Calavi, Benin

³ Department of Chemical Engineering, NCSU, Raleigh, NC 27695 USA

corresponding author: warthog@ncsu.edu

We developed and validated an innovative, cost-effective method for seed piece treatment for nematode control in sub-Saharan African smallholder farms. We obtained proof-of-concept for our 'wrap and plant' technology, demonstrated enhanced plant-parasitic nematode control in replicated glasshouse trials, and developed a pulping process for banana fibre paper manufacture using locally available components to produce the 'wrap and plant' product. We achieved success using fibre matrix as a delivery system to deliver anthelmintic (abamectin) compounds at low rates. Our approach is to target two crops established by seed piece (yam, *Dioscorea* spp. and potato, *Solanum tuberosum*) for initial field trials in SSA and one established by true seed (pigeon pea, *Cajanus cajan*). This project's (supported by the Bill and Melinda Gates Foundation) purpose is to develop a sustainable, affordable platform technology for crop protection from soil-borne pathogens and pests for smallholder farmers. In 2015, we initiated yam field trials in Benin. In preparation for field trial installation, we manufactured BFP in 12" × 100' (0.31 m × 30.5 m) sheets and introduced perforations to assist in root penetration during plant germination. We treated 400 ft² (122 m²) of paper with abamectin at a rate of 600 ng ft⁻² (600 ng 0.3 m⁻²) active substance (a.s.) (total application = 240 ug a.s.). We established six on-farm (with local growers) and one on-station field trials to determine 'wrap and plant' technology efficacy to manage nematodes infecting yam. Farmers' fields were sampled for nematodes; fields were selected based on populations present (>500 nematodes (250ml soil)⁻¹). We established a research station site to ensure high nematode pressure on the trial by inoculating the field with infested yam tuber peels. Yield and nematode data from December 2015's trial plot harvest indicated that treated banana paper was the superior treatment for protecting yam from plant-parasitic nematode damage.

Keywords: *Meloidogyne*; root-knot nematode; *Scutellonema*; yam.

Heterozygote deficits in cyst forming plant-parasitic nematodes

Josselin Montarry¹, Pierre-Loup Jan¹, Cécile Gracianne¹, Andrew D.J. Overall², Sylvie Bardou-Valette¹, Sylvain Fournet¹, Eric Grenier¹ and Eric Petit³

¹INRA, UMR1349 IGEPP, F-35653 Le Rheu, France

²School of Pharmacy and Biomedical Sciences, University of Brighton, UK

³INRA, UMR985 ESE, F-35042 Rennes, France

corresponding author: josselin.montarry@rennes.inra.fr

The reproduction mode of plant-parasitic nematodes has been poorly investigated in detail, although it will strongly impact the evolutionary potential of these pests and in particular their ability to adapt more or less rapidly to plant resistances. Deviations from Hardy-Weinberg equilibrium due to heterozygote deficits ($FIS > 0$) have been recorded for three cyst forming plant-parasitic nematode species, *Globodera pallida*, *G. tabacum* and *Heterodera schachtii*. However, it has never been determined whether the observed deficits were due: (i) to the presence of null alleles; (ii) to a high level of consanguinity; and/or (iii) to a genetic structuration at a sub-population scale (Wahlund effect). The aim of the present work was to disentangle, using microsatellite markers, consanguinity and Wahlund effect in natural populations of three economically important cyst nematode species: *G. pallida*, *G. tabacum* and *H. schachtii*, which attack potato, tobacco and sugar beet, respectively. Our results suggested that it is restricted dispersal that leads to heterozygote deficits through both consanguinity and substructure, which effects can be linked to soil movement, cyst density, and the number of generations per year. While consanguineous mating leads to homozygosity at all loci, including loci governing avirulence/virulence, which favours the expression of virulence when recessive, the Wahlund effect is expected to have no particular effect on the adaptation of nematodes to resistances.

Keywords: Consanguinity; Hardy-Weinberg equilibrium; microsatellite; null allele; Wahlund effect.

Contrasting evolutionary patterns of 28S and ITS rRNA genes reveal high intragenomic variation in *Cephalenchus* (Nematoda): Implications for species delimitation

Tiago Jose Pereira and James G. Baldwin

Department of Nematology, University of California, Riverside, 900 University Avenue, Riverside, CA 92521, USA

corresponding author: tiagojp@ucr.edu

Concerted evolution is often assumed to be the evolutionary force driving multi-family genes, including those from ribosomal DNA (rDNA) repeat, to complete homogenisation within a species, although cases of non-concerted evolution have also been documented. In this study, sequence variation of 28S and ITS ribosomal RNA (rRNA) genes in the genus *Cephalenchus* is assessed at three different levels, intragenomic, intraspecific, and interspecific. The findings suggest that not all *Cephalenchus* species undergo concerted evolution. High levels of intraspecific polymorphism, mostly due to intragenomic variation, are found in *Cephalenchus* sp1 (BRA-01). Secondary structure analyses of both rRNA genes and across different species show a similar substitution pattern, including mostly compensatory (CBC) and semi-compensatory (SBC) base changes, thus suggesting the functionality of these rRNA copies despite the variation found in some species. This view is also supported by low sequence variation in the 5.8S gene in relation to the flanking ITS-1 and ITS-2 as well as by the existence of conserved motifs in the former gene. It is suggested that potential cross-fertilisation in some *Cephalenchus* species, based on inspection of female reproductive system, might contribute to both intragenomic and intraspecific polymorphism of their rRNA genes. These results reinforce the potential implications of intragenomic and intraspecific genetic diversity on species delimitation, especially in biodiversity studies based solely on metagenetic approaches. Knowledge of sequence variation will be crucial for accurate species diversity estimation using molecular methods.

Keywords: *Cephalenchus*; concerted evolution; polymorphism; rRNA genes; secondary structure.

Novel molecular identification methods for the *Meloidogyne ethiopica* and the tropical group of root-knot nematodes

Barbara Gerič Stare¹, Zübeyir Devran², Gökhan Aydın³, Gregor Urek¹, Sevilhan Mennan⁴ and Saša Širca¹

¹Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

²Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Kampüs, 07058, Konyaalti, Antalya, Turkey

³Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

⁴Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

corresponding author: barbara.geric@kis.si

The tropical group of root-knot nematodes including *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. ethiopica*, etc. are highly polyphagous, remarkably widespread and damaging pests of agricultural crops. *M. ethiopica* has recently spread in Europe and has been reported from Slovenia, Greece, Turkey and Italy. In 2013 Correa *et al.* published a SCAR method for identification of *M. ethiopica* developed on analysis of genetic variability of *M. ethiopica* populations from Brazil, Chile and Kenya. Our attempts failed to amplify a species-specific band with this SCAR method using populations of *M. ethiopica* from Europe and Asia. Therefore, we have developed two primer sets based on the mtDNA sequences from the public database and the in-house database of different populations of *M. ethiopica* (Slovenia, Turkey, Greece, Brazil and South Africa). PCR with the *M. ethiopica* species-specific primers produced a 241 bp long amplicon. The tropical root-knot nematode test was performed with one group specific primer designed in this study and the primer C2F3, a widely used primer for molecular identification of *Meloidogyne* species. PCR with the tropical group specific primer yielded 621 bp long amplicon. Both methods were validated on root-knot nematodes species and populations of diverse geographical origin: 20 populations of *M. ethiopica* (populations from Slovenia, Turkey, Greece, Brazil, South Africa), 4 *M. arenaria*, 9 *M. incognita*, 6 *M. javanica*, 2 *M. hapla*, 1 *M. hispanica*, 1 *M. chitwoodi* and 1 *M. fallax*. The first method developed in this study could be a valuable tool for quick, simple and unambiguous species identification of *M. ethiopica*. As *Meloidogyne* spp. can be challenging for morphometrical identification, the second method could be a quick and simple tool towards determining the RKN sample belonging to the tropical group in the initial steps of the *Meloidogyne* species determination. Acknowledgements: supported by ARRS (P4-0072, BI-TR/15-17-008) and TÜBİTAK (214 O 667).

Keywords: Diagnostics; emerging pest; PCR.

Population genetics of potato cyst nematodes using genotyping-by-sequencing

Benjamin Mimee¹, Pierre-Yves Véronneau¹, Joël Lafond-Lapalme¹ and Éric Grenier²

¹Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu Research and Development Centre, 430 boul. Gouin, St-Jean-sur-Richelieu, QC, J3B 3E6, Canada

²INRA, Institute of Genetic, Environment and Plant Protection, 35653 Le Rheu, France

corresponding author: benjamin.mimee@agr.gc.ca

Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, are among the most economically important pests of potato and quarantine organisms in many countries. The introduction and potential spread of these species in any part of the world has serious implications for potato production and export. PCN management is primarily achieved through the use of resistant varieties. As populations of *Globodera* differ in virulence against the various resistance sources used in potato varieties, it is of paramount importance to be able to identify pathotypes/variants or to be able to identify novel gene pool introductions. To this end, genotyping-by-sequencing has been used to rapidly identify single nucleotide polymorphisms (SNP) and compare allele frequencies between populations. This method allowed the direct comparison of worldwide populations. Phylogeographic results confirmed that both PCN species may share a single common introduction origin into Europe and then to North America. For *G. rostochiensis*, our results showed a clear separation of pathotypes Ro1 and Ro2 from Ro3, Ro4 and Ro5. At a local scale, some populations with different pathotypes (Ro1 vs Ro2) but same origin (NY) were very similar, suggesting a recent differentiation event or a high level of passive gene flow between these populations. Nevertheless, we identified SNP markers that differentiate these two populations and could be used for diagnostics in management programs. For *G. pallida*, several loci were highlighted by GBS that correlated with the development of populations on a set of resistant potato cultivars. This information will be used to recognise the variation that exists inside *G. pallida* and will (i) led the foundation for a revised pathotype classification in this species, and (ii) be used to understand the mechanisms leading to the breakdown of plant resistance by potato cyst nematodes.

Keywords: *Globodera*; molecular diagnostic; pathotypes; virulence.

Intraspecific variation of the potato cyst nematode *Globodera pallida* in the field

Vivian Blok¹, Sebastian Eves-van den Akker², Alex Reid³ and Eric Grenier⁴

¹ The James Hutton Institute, Cell and Molecular Sciences, Invergowrie, DD2 5DA Dundee, UK

² Division of Plant Sciences, College of Life Sciences, University of Dundee, DD2 5DA Dundee, UK

³ SASA - Science and Advice for Scottish Agriculture, Roddinglaw Road, EH12 9FJ Edinburgh, UK

⁴ INRA, Institut de Génétique, Environnement et Protection des Plantes, Université de Rennes, 35653 Le Rheu, France

corresponding author: vivian.blok@hutton.ac.uk

It is recognised that there are several genetically distinct introductions of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* in Europe. This was initially deduced from observations of the different reproductive rates of a set of PCN populations on a differential set of potato genotypes. Phenotypic assays remain a key means by which to differentiate intraspecific variants/pathotypes and are central to the development and deployment of durable resistance to PCN. However, the phenotypic composition of field populations is not routinely monitored and there is uncertainty about the consequences of using PCN resistant cultivars, particularly for *G. pallida*. A recent metagenetics study performed in collaboration with the Scottish statutory agency used a mitochondrial marker, cytochrome B, to assess the composition of *G. pallida* in field populations in Scotland. This revealed that populations were comprised of genotypic mixtures and raised questions of whether intraspecific hybrids may have occurred between them and whether novel phenotypes may have been generated. The genetic diversity within and between cysts determined using microsatellites also revealed that there can be considerable intraspecific diversity in *G. pallida* within a field and even within a single cyst progeny. Managing PCN with resistant cultivars is of increasing importance due to pressures on nematicide use; however, the complexity of field populations is a potential concern and these results strengthen the need for further research regarding how resistant cultivars should be used to preserve their long term durability.

Keywords: Durability; intraspecific variation; potato cyst nematodes resistance.

Biomangement of white grubs and apple root borers in the field by implantation of *Heterorhabditis indica*-infected *Galleria* cadavers in the soil

Sharad Mohan¹, Ackanksha Upadhyay¹ and Keith Davies²

¹ Upadhyay Indian Agricultural Research Institute, Pusa Campus, 110012 New Delhi, India

² Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

corresponding author: sharad@iari.res.in

White grubs (Family: Scarabaeidae) and apple root borer (*Dorysthenes hugelii*, Family: Cerambycidae) are decimating the sugarcane fields and the apple orchards in the Indian states of Uttar Pradesh and Himachal Pradesh respectively. These pests are largely impervious to chemical control. Among the many reasons for their invulnerability, is the innate tendency of the white grubs to evade insecticides by retreating vertically into the soil, and the obvious inaccessibility of the root borer. The control of white grubs and apple root borer by implanting the soil with *H. indica*-infected *Galleria* cadavers has given encouraging results. The application of *H. indica*-infected *Galleria* cadavers at 3000 cadavers per acre (=0.4 ha) of sugarcane and 5-10 cadavers per apple tree has yielded up to 70% reduction in the pest populations. The failure of *H. indica* to recycle in 90% of the dead insects could be traced to co-infection by *B. cereus* and *Sphingobacterium* spp. in white grubs and *Bacillus subtilis*, *Staphylococcus cohnii* and *Acinetobacter* rhizosphaerae in the apple root borer. Competitive *in vitro* bioassays between *Photorhabdus luminescens* and *B. cereus* pointed to the inhibitory effect of the latter. The development of axenic *H. indica* eggs was truncated at the second juvenile stage on exposure to a culture lawn of *B. cereus*. Besides co-infection, the presence of more than 20 species of white grubs in various developmental stages in the afflicted regions, and the resistance of the older instars of the apple root borer to *H. indica* infection, especially in the overwintering stages, pose a formidable challenge to the *H. indica* based biomangement initiatives. An interesting social aspect included in the *H. indica* project for controlling white grub was a capacity-building programme in which villages were involved in the rearing, infecting and application of *Galleria* with especial focus on empowering women and marginal farmers.

Keywords: *Dorysthenes hugelii*; entomopathogenic nematodes; nematode recycle; sugarcane.

Field evaluation of entomopathogenic nematodes for biocontrol of the leopard moth borer *Zeuzera pyrina* (Lepidoptera: Cossidae)Elham Salari¹, Javad Karimi¹, Hussein Sadeghi Nameghi¹ and Majid Fasihi Harandi²¹Department of Plant Protection, School of Agriculture, Ferdowsi University of Mashhad, PO Box 91775-1163, Mashhad, Iran²Research Center for Hydatid Disease in Iran, School of Medicine, Kerman University of Medical Sciences, Kerman 7616914115, Iran

corresponding author: jkb@ferdowsi.um.ac.ir

The leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae) is a key pest of walnut trees in Iran with increasing importance in the last few years. Due to the special habitats of larvae of this cossid, the application of entomopathogenic nematodes (EPN) within an integrated pest management approach may offer an efficient and safe strategy to suppress outbreaks of this pest. In the present work, the efficacy of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against the larvae of *Z. pyrina* in the laboratory and field tests was evaluated. After infectivity assay in plate using a range of EPN concentrations (5, 10, 20, 50 and 100 infective juveniles (IJ) per larva), the virulence experiment was performed in pots containing walnut seedlings in field condition. The applied concentration of EPN was 300 IJ cm⁻² in 25 ml of distilled water that were directly injected into the gallery entrances. The results indicated that both EPN species were capable of reducing populations of *Z. pyrina* larvae in both experiments. In plate assays, larval mortality was significantly influenced by species and concentration. The highest mortality were recorded at the concentration of 20 IJ larva⁻¹ for *S. carpocapsae* (97.1%). Both EPN caused high insect mortality in field experiments with no significant differences in infection of *Z. pyrina* larvae between the EPN species. Five days after EPN application, the infected larvae of the pest were found within branches of walnut seedlings. Dissection of cadavers confirmed nematode infection. These results indicate that EPN could be considered as suitable candidates for application against *Z. pyrina* larvae in IPM programmes. However, a key question concerns the tracking of infection of treated larvae with EPN within the strong trunk of walnut trees. Therefore, further work is necessary in order to find suitable application strategy of these biocontrol agents for controlling *Z. pyrina* in the field within an integrated approach.

Keywords: Biocontrol; insect pathology; field; *Zeuzera pyrina*.

Laboratory bioassay of dose mortality and single dose screening against Colorado potato beetle [*Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomalidae)] with Turkish isolate of entomopathogenic nematodes

İlker Kepenekci¹ and Hayriye Didem Sağlam²

¹Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat, Turkey

²Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Kırşehir, Turkey

corresponding author: saglamhds@gmail.com

Potatoes are the fourth most important agricultural product in the world. Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomalidae) is one of the most important pest damage on potatoes. Use of insecticides against CPB has resulted in development of resistance against the insecticides, which compromises control. Natural enemies are being used to control the pest as an alternative method to reduce damage by this pest. The aim of this study was to evaluate three Turkish isolates of the entomopathogenic nematodes *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* for efficacy as biocontrol agents against the last instar of the CBP under laboratory conditions. For the dose-mortality bioassay, a series of 5, 10, 50, 100, 250, 500 and 1000 infective juveniles (IJ) per 0.2 ml (1.3, 2.6, 13.2, 26.3, 65.8, 131.6 and 260.3 IJ cm⁻²) nematode concentrations were prepared. For the single dose screening; 500 IJ (131.6 IJ cm⁻²) of each nematode species suspended in 0.2 ml water was applied. Mortality was recorded after 5 and 10 days. For both experiment each nematode species was tested at all concentrations: 0.2 ml water was used as the negative control. The plates were labelled and incubated at 25°C. Three independent replicates were performed on separate dates. Approximately 0.2 ml of water was applied to the surface of the soil prior to each test. LC50 values for the three species ranged from 193-345 IJ per 0.2 ml water for *S. feltiae*, 281-473 IJ for *H. bacteriophora*, and 697-1558 IJ for *S. carpocapsae*, and analysis of variance revealed that differences were significant. For LC90, the values ranged from 652-1036, 835-1338 and 1522-3790, respectively. This study indicated that *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* were each able to kill CPB in the single dose screens, and their efficacy increased as the incubation period was extended.

Keywords: Colorado potato beetle; dose mortality; entomopathogenic nematodes; *Leptinotarsa decemlineata*; single dose screening.

***Steinernema carpocapsae* and *Heterorhabditis bacteriophora* for the management of the tomato leaf miner, *Tuta absoluta*, in greenhouse-grown tomatoes**

Shokoofeh Kamali¹, Javad Karimi¹, Albrecht Koppenhöfer² and Fahime Taheri Anaraki³

¹Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, School of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

²Department of Entomology, Rutgers University, New Brunswick, USA

³Management Department of Agricultural Jihad, Fariman, Iran

corresponding author: kshokoofeh@yahoo.com

The tomato leaf miner, *Tuta absoluta*, has emerged as a serious threat to tomato production. Currently, the main control strategies for this pest rely heavily on synthetic insecticides. Entomopathogenic nematodes (EPN) are a potential candidate for the biological control of *T. absoluta*. Since various abiotic and biotic factors can negatively affect EPN efficacy, it is crucial to understand their effect on the efficacy of EPN *versus* *T. absoluta*. In a series of experiments in the laboratory we examined the effect of EPN concentration, soil texture, temperature and exposure period on EPN efficacy against last instar *T. absoluta* larvae. The larvae were highly susceptible to both EPN species with LC50 values of 2 and 1 infective juveniles (IJ) cm² for *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, respectively. Both species caused high mortality in loamy sand (89%). High control rates were observed with both EPN species at 31 ± 1 °C (*H. bacteriophora*, 95%; *S. carpocapsae*, 91%) and at 25 ± 1 °C (*H. bacteriophora*, 96%; *S. carpocapsae*, 89%). After 65 and 240 min exposure to *S. carpocapsae* 77 ± 4% and 96 ± 3% of the larvae were infected. Based on our laboratory observations, both EPN species were evaluated under glasshouse conditions. In the first experiment, EPN were applied directly to tomato leaves, and the leaves were covered with thin plastic covers. *Steinernema carpocapsae* caused higher larval mortality (48%) than *H. bacteriophora* (18%). In the second experiment, in which the EPN were applied to the whole tomato plant surfaces, larval mortality did not differ significantly between *S. carpocapsae* (31%) and *H. bacteriophora* (38%). Our glasshouse results suggest that *S. carpocapsae* could be incorporated as an effective alternative to synthetic insecticides into management programmes for *T. absoluta* in glasshouse tomato production. However, for the introduction of EPN as biocontrol agent against this pest in Iran, some more testing will be required.

Keywords: Entomopathogenic nematode; exposure time; glasshouse; insect pathology; *Tuta absoluta*; soil type; temperature.

Controlling the Western Corn Rootworm *Diabrotica virgifera virgifera* with dianem® (*Heterorhabditis bacteriophora*)

Ralf-Udo Ehlers

e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany

corresponding author: ehlers@e-nema.de

Besides the European Cornborer (Lepidoptera), the Western Corn Rootworm *Diabrotica virgifera virgifera* (Coleoptera, Chrysomilidae) is the major pest of maize. The insect lays eggs in July until September in the maize fields and overwinters in the egg stage. Thus, crop rotation is an effective control measure. However, in many areas crop rotation is not an option and then chemical insecticides are used. Since the ban of neonicotinoid seed treatments most European farmers rely on the application of less effective granular soil insecticides, which have side effects on earthworms and other soil inhabitants and have not been able to reduce the spread of the pest since its introduction in 1992. The entomopathogenic nematode *Heterorhabditis bacteriophora* is a promising biological control agent of this maize pest. For the last 10 years nematodes have been tested in field trials with artificially infested plants to ensure an even population density. Results indicate the high control potential of dianem®. The nematodes are applied as a furrow application with 200-400 l ha⁻¹ into the soil together with the maize seeds. Special injectors have been developed, which are mounted on the seeders and are connected with the water tank in front of the tractor. At the time of application *Diabrotica* eggs are still in diapause. Nematodes survive and remain virulent until larvae hatch at approximately 4-6 weeks later. At a dose of 2 billion ha⁻¹ control results were between 50 and 90%. Although the differences to chemical insecticides in assessment of root damage are remote, nematodes usually provided a higher reduction of adults than chemical insecticides thus, will in the long-term reduce the problems with this invasive pest. The use of dianem® is a sustainable method with no toxic side effects. With selection of genetically improved strains we are aiming at reduction of the application density to 1 billion ha⁻¹.

Keywords: Biological control; Western Corn Rootworm.

Combination of varietal and technical innovations for the sustainable and integrated management of root-knot nematodes: the GEDUNEM project

Caroline Djian-Caporalino¹, Mireille Navarrete², Thierry Mateille³, Amélie Lefevre⁴, Ariane Fazari¹, Nathalie Marteu¹, Marc Tchamitchian², Arnaud Dufils², Alain Palloix⁵, Anne-Marie Sage-Palloix⁵, Johannes Tavoillot³, Laure Pares⁴, Hélène Védie⁶, Claire Goillon⁷, Isabelle Forest⁸ and Philippe Castagnone-Sereno¹

¹ INRA, Université de Nice Sophia Antipolis, CNRS, UMR1355-7254, Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

² INRA, UR0767, Écodéveloppement, 84914 Avignon Cedex 9, France

³ IRD, UMR CBGP, Campus de Baillarguet, 34988 Montferrier-sur-Lez Cedex, France

⁴ INRA, UE0411 Domaine Expérimental de Alénya Roussillon, 66200 Alenya, France

⁵ INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, 84143 Montfavet, France

⁶ GRAB, Groupe de Recherche en Agriculture Biologique, 84 911 Avignon Cedex 9, France

⁷ APREL, Association Provençale de Recherche et d'Expérimentation Légumière, 13210 Saint-Rémy de Provence, France

⁸ Chambre d'Agriculture du Var, 83400 Hyères, France

corresponding author: caporali@sophia.inra.fr

Root-knot nematodes (RKN) are responsible for increasing economic losses on vegetable production in the Mediterranean area. The GEDUNEM project assessed cropping strategies based on the combination of genetic resistance with cultivation practices (crop rotations including susceptible, resistant, and non-host plants, intercropping management such as nematocidal cover crops, solarisation). Their impacts on RKN populations, on resistance gene overcoming, and on soil ecological diversity including other nematode species and pathogens were assessed in a multisite experimental format in research stations and in commercial farms, and their acceptability by farmers was surveyed. The three cropping strategies tested here involved solanaceous crops (tomato, pepper) or cucurbit in summer, salads or chard in winter. They were proven effective after implementation and annual evaluation over a 4-year period. Biofumigant or conventional sorghum as green manure (buried before 3-4 weeks in summer), a resistant Me1-Me3 pepper at high density as a cover trap crop, and solarisation associated with non-host plants, allowed each a sharp decrease of RKN populations in soil (> 90%) and damage reduction on the following crops. These strategies also limited the emergence of virulent nematodes on resistant crops (tomato and pepper) and might increase the free-living species in the soil in some cases. The effect of green manure sanitiser seemed more durable when the nematofauna diversity was high. The pepper cover trap crop produced an organic matter amount equivalent to sorghum, and therefore is also interesting as a green manure. A farmers' survey showed that this innovative cropping system fits for the farms that have enough labour force and where land is available in summer for the duration of the trap crop. Several outlooks to improve the systems are currently being considered. Financial support (2012-2015) from the French INRA Metaprogramme "Sustainable Management of Crop Health" (SMaCH).

Keywords: Agronomic practices; integrated pest management; resistance gene durability; root-knot nematodes; sustainable management.

Successful potato production in Norway despite the presence of potato cyst nematodes

Ricardo Holgado¹, Christer Mangusson¹ and Randi Knudsen²

¹ Norwegian Institute of Bioeconomy Research, Pb 115, NO-1431 Ås, Norway

² Norwegian Food Safety Authority, Moerveien 12, 1430 Ås, Norway

corresponding author: rholcamp@hotmail.com

The Norwegian potato is grown on 14 400 ha, yielding 398 000 tonnes, with an annual value of 600 million NOK (75 million €). Since nematicides have never been used in Norwegian potato production, management of potato cyst nematodes (PCN) has depended on strict phytosanitary measures, resistant cultivars and crop rotation. Farmers need to be well informed about the distribution of PCN, management options, phytosanitary measures and the present regulations. Knowledge on the distribution of species and pathotypes of PCN is of crucial importance for good control. In Norway *Globodera rostochiensis* occurs as pathotypes Ro1, Ro2, Ro3 and Ro4, while *G. pallida* contains pathotypes Pa1, Pa2 and Pa3. Surveys showed that PCN infestation level is low, with about 5% of positive samples. *G. rostochiensis* occurs in southern counties including Nord Trøndelag. *G. pallida* has a more restricted distribution with the highest frequency in the southern counties (Agder and Rogaland). The most common PCN pathotype is Ro1, forming 98% of the total finds. Ro1 is considered non-virulent, and is managed by crop rotations including resistant cultivars. Knowledge on the degrees of susceptibility and resistance of cultivars is important in this context. Infestations by *G. pallida* or virulent *G. rostochiensis* results in a ban of 40-years on growing potato. The current acreage of certified seed potato production is 814 ha, with the major area situated in the middle and eastern parts of the country. Monitoring PCN in certified seed potato started in 1956 and has now been carried out for 60 years without detecting PCN. Norwegian farmers have several advantages with regard to PCN management compared to the situation in many other countries. PCN frequencies are low and the certified seed potato is free of PCN. In addition, Norway has an open policy and provides a register of PCN infested land for free access and use by farmers.

Keywords: Control; *Globodera*; monitoring; Norway; potato.

The use of resistant varieties and nematicides to manage *Globodera pallida* populations in field trials in Scotland

Kim Davie¹, Jon Pickup¹, Yvonne Cole¹ and Eric Anderson²

¹SASA - Science and Advice for Scottish Agriculture, Roddinglaw Road, EH12 9FJ Edinburgh, UK

²Scottish Agronomy, Arlary Farm, Milnathort, Kinross, KY13 9SJ, UK

corresponding author: kim.davie@sasa.gsi.gov.uk

Potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are among the most economically damaging pests in potato production and are responsible for high yield losses in commercial potato production throughout much of the world. Controlling PCN is the highest priority in terms of disease management for the UK potato industry and there are several different management tools that can be adopted. We report on three separate field trials set up to compare the effectiveness of nematicides and resistant cultivars in *G. pallida* infested fields. One trial compares a range of nematicide treatments with the cultivation of the susceptible variety Maris Piper. The second trial investigates the impact of two varieties with differing levels of resistance to *G. pallida* on the resulting PCN populations. The final trial compares the effect of seven varieties with varying levels of resistance with and without a standard nematicide treatment. In all trials the populations of cysts, the egg counts and the values from real time PCR were assessed at before planting and after harvest. Nematicides were able to moderate large population increases but not prevent them; however, highly resistant varieties were able to reduce considerably the final population of PCN. The quantification of the levels of resistance in the varieties used in the field trials using the scoring method set out in the 2007 European Union PCN Directive gave a very close correlation to resistance scores obtained using standard pot tests, as used for National List work in Europe.

Keywords: PCN control; PCN diagnostics; PCN resistance.

Dispersal of potato cyst-forming nematode in the North-West of Russian Federation. Pre- and In-field practices of *Globodera* management

Elizaveta M. Matveeva and Victoria V. Lavrova

Institute of Biology, Karelian Research Centre of Russian Academy of Sciences, 185910 Petrozavodsk, Russia

corresponding author: elizaveta.matveeva@gmail.com

Dispersal of potato cyst nematode (PCN) *Globodera rostochiensis* Woll. through the territory of Northwest Russia (former USSR republics where PCN was detected for the first time, Leningrad Region, the Republic of Karelia) were investigated. PCN expansion followed two pathways: gradual northwards spread (an average 1-2° over 2-5 years), and intensive expansion around the sites of the first findings, where PCN rapidly increased in numbers and established in the soil nematode communities in local potato fields. In Karelia PCN was first found in 1976 in south-western part (4 cysts (100 g of soil)⁻¹). The nematode had spread 2° northwards within the 3 years since the previous detection (Leningrad Region, 1973). During 10 years the nematode was observed in southern, south-western and central Karelia (2-400 cysts (100 g of soil)⁻¹). In the northern part of Karelia gradual PCN dispersal lagged behind (2° northwards over 12-15 years). The latest pest finding was recorded from 66° N. Intensive PCN expansion is limited by severe climatic conditions for potato growing and small crop areas, mostly private fields. Management practices for potato growing and protection from PCN were suggested. Low temperature pre-treatment of potato tubers for short periods increased plant tolerance to subsequent low field temperatures, decreased nematode multiplication and increased crop productivity. Technologies based on the use of wastes from the logging and wood industries (freshly-crushed conifer bark and sodium lignosulphonates) were found to possess nematicidal properties and significantly reduced nematode multiplication. Application of these products to the infested plants decreased the number of newly-formed cysts, the total number and viability of eggs and juveniles inside cysts, and changed the sex-ratio in the nematode population. These technologies are recommended for PCN control in Karelia. Study was carried out under state order (№ 0221-2014-0004) and partially supported by RFBR (№ 16-34-00650).

Keywords: Agroecosystems; control measures; *Globodera rostochiensis*; pest; potato.

A routine test for relative susceptibility estimation of potato genotypes with resistance to *Meloidogyne chitwoodi*Thomas Been¹, Corrie Schomaker¹ and Misghina Goitom Teklu^{1,2}¹ Wageningen University and Research Centre, Agro-systems-research, 6700 AP, Wageningen, The Netherlands² Laboratory of Nematology, Wageningen University, 6708 PD Wageningen, The Netherlandscorresponding author: misghina.goitomteklu@wur.nl

Several aspects of the Relative Susceptibility (RS) of seven potato genotypes, one susceptible and six partially resistant against *Meloidogyne chitwoodi*, were studied in four successive glasshouse experiments, in 10, 5, 3 and 2 kg pots. The cultivar Désirée was used as a susceptible control. The initial nematode densities (P_i), consisted of a 2 log series: 2^x , with x ranging from - 4 to 8. The final population density (P_f) was estimated in the roots, soil and, in one experiment, also in the tubers. The proportion of nematode free tubers was estimated in all experiments. Seinhorst's logistic model of population dynamics was fitted to the data to estimate the maximum multiplication rate (a), maximum population density (M), RS_a and RS_M . Apart from one genotype, resistant to *Globodera pallida* but susceptible to *M. chitwoodi*, the averaged estimates for RS_a and RS_M of all tested genotypes were smaller than 0.29% and 0.23%, respectively, with coefficients of variation of 0.21 and 0.09, respectively. The M/a ratio for six out of seven genotypes and cv. Désirée was 1.3, indicating that for these genotypes RS is independent of P_i ($RS_a = RS_M$). RS can, therefore, be tested at one well-chosen P_i . Although a and M were sometimes influenced by pot size, both RS and M/a were not. A choice of $24 \text{ J2 (g dry soil)}^{-1}$ as a P_i and pot sizes of 2 or 3 kg for routine testing is discussed. Tuber infestation levels after 8-10 months of storage, only estimated in Exp. 2, was $0.35 \text{ J2 (g dry soil)}^{-1}$ for cv. Désirée and less than $0.0015 \text{ J2 (g dry soil)}^{-1}$ for four tested genotypes, on average 2% of the total P_f .

Keywords: Maximum multiplication rate; maximum population density; partial resistance; tuber infestation.

Inundation to control *Meloidogyne chitwoodi*

Leendert P.G. Molendijk and Johnny H.M. Visser

Applied Plant Research, Wageningen UR (PPO), Edelhertweg 1, 8200 AK Lelystad, The Netherlands

corresponding author: leendert.molendijk@wur.nl

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, such as potato, carrots, black salsify and gladiolus, cause a strong demand for control methods that can prevent yield losses and spread of this species as much as possible. Inundation (flooding) is a technique used by flower bulb growers since 1945, to control *Pratylenchus penetrans* and *Ditylenchus dipsaci*. In cooperation with a farmer organisation in 2015 a project was started to measure the effects of inundation on *Meloidogyne chitwoodi*. To accomplish this, a field of 13 ha with a natural infestation, was flooded for 14 weeks. Next to the natural infestation, heavily infested test samples were buried within the field. After the flooding period the field and test samples were processed to look for free-living juveniles and living eggs. Next to these direct measurements of the final population a bioassay was performed. Tomato, *Solanum esculentum*, a good host for *M. chitwoodi*, was grown for 12 weeks. Roots of these plants were checked for the development of eggs and juveniles as being the offspring of *Meloidogyne* that had survived the flooding. In this large scale experiment, no surviving *M. chitwoodi* could be found neither in the directly processed soil samples nor in the root systems of the bioassay. This is the first time a technique showed 100% control of a *M. chitwoodi* infestation. For farmers inundation will be developed into a new tool to be integrated in their nematode control strategy to cope with this quarantine species. PPO develops Anaerobic Soil Disinfestation as a possible alternative to flooding for areas where flooding is not feasible. Results and opportunities for implementation will be discussed.

Keywords: Management; non-chemical control; flooding.

Are nematode tolerant sugar beet varieties resistant or susceptible to the beet cyst nematode *Heterodera schachtii*?

Marie Reuther^{1,2}, Christian Lang¹ and Florian M.W. Grundler²

¹ Verband der Hessisch-Pfälzischen Zuckerrübenanbauer e.V., Rathenaustraße 10, 67547 Worms, Germany

² INRES-Molekulare Phytomedizin, Karlrobert-Kreiten Straße 13, 53113 Bonn, Germany

corresponding author: reuther@ruebe.info

Heterodera schachtii is an important parasite compromising yield of sugar beet in many sugar beet growing areas of the world. Resistant varieties reducing initial infestation are available but have only low yield potential. A new option is the use of so-called tolerant sugar beet varieties, since their yield potential is equal to susceptible varieties with and without nematode infestation. Although tolerant varieties are currently well established in the market, it is not known whether they increase or reduce nematode population levels. Therefore, we determined nematode population levels before and after sugar beet cultivation during three years' field trials at 15 locations. Five tolerant, one resistant and one susceptible variety were tested. Nematode population levels were determined by taking soil samples at 0-30 cm and 30-60 cm depth. The number of juveniles was analysed through hatching tests from the soil samples. The results demonstrate that nematode propagation in all tested tolerant varieties amounts to levels between those of the susceptible and the resistant variety. The tolerant varieties tend to respond as moderately resistant rather than susceptible or highly resistant. Since the level of resistance is an important trait in sugar beet production, we suggest to include a compulsory resistance test within the official registration.

Keywords: Partial resistance; population level.

Laboratory and field resistance and tolerance of some onion varieties to *Ditylenchus dipsaci*

Elif Yavuzaslanoglu

Karamanoglu Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey

corresponding author: eyavuzaslanoglu@kmu.edu.tr

Onion is economically important crop grown especially in temperate regions. Unfavourable environmental conditions and biotic stress agents reduces the onion yield. Stem and bulb nematode is one of the most important constraints of onion production. The current study was conducted to investigate the resistance and tolerance reaction of four commercially produced onion cultivars, Kantartopu, Betapanko, Valenciana and Pan88, in Turkey under growth room and field conditions. Two-year experiments were set up in Karaman province on a naturally infected field during 2012 and 2013 growing seasons. Valenciana cultivar showed significant negative relationship between initial and final nematode populations while Betapanko and Pan88 cultivars were positively correlated. Dry onion yield parameters of Betapanko and Pan88 cultivars were significantly negatively correlated with initial nematode populations, indicating intolerance to *Ditylenchus dipsaci*. Kantartopu and Valenciana cultivars were tested for three times and Betapanko and Pan88 cultivars for four times in growth room conditions for resistance reactions. There was no significant difference among runs; therefore, data were evaluated together. *D. dipsaci* populations multiplied 5.21 and 3.86 times on cvs Betapanko and Pan88, respectively, 5 weeks after nematode inoculation; they are susceptible to *D. dipsaci*. On cvs Kantartopu and Valenciana *D. dipsaci* multiplied relatively less; 1.41 and 0.99 times, respectively. Growth room resistance reactions of tested cultivars supported field observations. There was no significant differentiation among cultivars for tolerance reactions. Resistance and tolerance to *D. dipsaci* at both seedling and bulb stage of dry onion is important for development of effective control strategies. Kantartopu and Valenciana are possible cultivars to reduce *D. dipsaci* populations in combination with other control methods.

Keywords: Onion; resistance; stem and bulb nematode; tolerance.

Microbiomes of potato cyst nematodes: the relationship between laboratory and environmental conditions

Diogo Neves Proença¹, Soraia Perpétuo², Isabel Luci Conceição^{2,3} and Paula Vasconcelos Morais^{1,3}

¹ CEMUC, University of Coimbra, 3030-788 Coimbra, Portugal

² CFE, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

³ Department of Life Sciences, FCTUC, University of Coimbra, 3000-456 Coimbra, Portugal

corresponding author: diogo.proenca@gmail.com

Potato is an important staple crop worldwide with an estimated production of million tons every year. Portugal has a lower average potato yield compared with the average in the European Union and may be attributed to the presence of plant-parasitic nematodes, for example, potato cyst nematodes (PCN), *Globodera* spp. In some European collections, PCN isolates kept in lab cultures started to die out and were not able to multiply after the first generation obtained from potato cultures in pots that were also shared by the Portuguese collections. This work is aimed to assess the microbiome of wild PCN (W-PCN) and PCN maintained in laboratory collections (L-PCN). Cysts from *Globodera* spp. were extracted from soil infested with PCN. DNA was extracted from W-PCN, immediately after their isolation from the soil, and from L-PCN stored in closed glass tubes for 6 months. Molecular methods (DGGE and Illumina MiSeq) were used to assess the microbiomes (Bacteria, Archaea and Fungi) present in cysts. DGGE profiles showed that the microbial community was different between wild and laboratory cysts. Comparing W-PCN with L-PCN, by the analysis of Illumina Miseq, it was possible to verify in L-PCN the increase of classes Gammaproteobacteria (30% to 60%, namely *Acinetobacter*) and Bacilli (8% to 20%, namely *Bacillus* and *Staphylococcus*) and a decrease in the percentage of microorganisms of the classes Actinobacteria (18% to 8%), Sphingobacteriia (7% to 1%), Betaproteobacteria (13% to 1%) and Alphaproteobacteria (20% to 7%). The number of bacteria and archaea involved in nitrification pathways in L-PCN was lower than in W-PCN. Moreover, we found the yeast *Saccharomyces* (30%) in L-PCN microbiome. The microbiomes of L-PCN and W-PCN are different and these differences might be involved in the loss of viability of the isolates in lab cultures. *Saccharomyces* was previously shown to kill nematodes and its presence in the L-PCN microbiome could also be related to the death of PCN maintained in lab conditions.

Keywords: Diversity; *Globodera*; Illumina; microbiome; potato cyst nematodes.

NemaDecide GEO, a web-based decision support system for nematodes

Thomas Been¹, Corrie Schomaker¹ and Leendert Molendijk²

¹ Plant Research International, Wageningen University and Research Centre, Plant Sciences Group, P.O. Box 16, 6700 Wageningen, The Netherlands

² Applied Plant Research, Wageningen University and Research Centre, Plant Sciences Group, Edelhertweg 1, 8219 PH Lelystad, The Netherlands

corresponding author: Thomas.been@wur.nl

During the past 4 years an effort was made to translate the advisory system for potato cyst nematodes (*Globodera* spp), root knot nematodes (*Meloidogyne chitwoodi*) and the root-lesion nematode (*Pratylenchus penetrans*), NemaDecide, from a PC-based version into a web-based version. The web-based version should combine geographic information of fields and their sampling units with the results provided by the soil sampling agencies and the scientific knowledge collected from nematological research, resulting in the possibility to compare different control scenarios and provide the grower with the possibility to make the most profitable economic decision. The first step taken was the development of a GEO platform, named Akkerweb, which provides the tools for data-exchange with soil sampling agencies and visualisation of GEO-information. A second step was the development of applications on the Akkerweb platform to facilitate the usefulness for farmers. A 'Digital Sampling Request' App was built for farmers to select a field on screen and request soil sampling for nematodes. A 'Sampling App' provides the possibility to split fields in various ways into sampling units according to the selected sampling method. A 'Potato information' App provides the farmer with all the necessary information, including variety resistance, of more than 400 potato varieties. A 'Granular nematicides' App helps the farmer to put nematicides, if needed, only where nematodes are present. The 'GeoNema' App with its science based decision tools supplies the farmers with yield loss predictions, population development and cost/benefit calculations of control treatments. GeoNema will be available on the net for farmers in The Netherlands, and also in other countries. Translations into English and German are planned. A free version and an extended licenced version will be available.

Keywords: Control measures; cost/benefit; partial resistance.

The use of cover crops to manage plant-parasitic nematodes in field vegetable production: promises and pitfalls

Wim M.L. Wesemael^{1,2,3}, Alexander Mbiro³, Pieter-Jan De Temmerman⁴ and Nicole Viaene^{1,3}

¹ Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

² Laboratory for Agrozoology, Faculty of Bioscience engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

³ Nematology Unit, Department of Biology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

⁴ Veterinary and Agrochemical Research Centre (CODA), Groeselenberg 99, B-1180 Uccle, Belgium

corresponding author: wim.wesemael@ilvo.vlaanderen.be

Major nematode problems in open field vegetable production in Belgium are the root-lesion nematode *Pratylenchus penetrans* and the quarantine root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*. Crop rotation, cover crops and black fallow are the most used management options. The use of cover crops is stimulated by local and European policy through subsidies. About 75% of vegetable farmers are using cover crops in their rotation basically to improve the soil structure and to control the nitrogen balance. Most farmers are aware that cover crops can have an effect on plant-parasitic nematodes but they lack reliable and objective support. Only 14% is using cover crops to manage nematode problems. Cover crops with resistance to *M. chitwoodi*, *M. fallax* and *Heterodera schachtii* have been developed. European policy (CAP) requires 'greening' to strengthen environmental sustainability of agriculture. As a result Belgian farmers need to use mixtures of cover crops. This makes it even more challenging to tackle nematode problems, especially when *P. penetrans*, *M. chitwoodi* and *M. fallax* are present together, cropping options become very limited. More knowledge on host plant status of cover crops for different plant-parasitic nematodes, their potential as trap crops and how they can fit in an economic sustainable rotation scheme is needed. Different strategies, based on results on host plant status and crop features, will be discussed.

Keywords: Green manure; management; root-knot nematodes; trap crops.

Evaluation of 21 brassicaceous plants as biofumigants for *Meloidogyne arenaria* and *M. incognita*

Sevilhan Mennan¹ and Gökhan Aydınli²

¹ Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

² Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

corresponding author: smennan@omu.edu.tr

Biofumigation using brassicaceous crops has great potential for sustainable nematode management strategy. *Brassica* species can produce significant amounts of glucosinolates that are sulfur-containing compounds. When brassicaceous plant tissue is incorporated into soil, glucosinolates are hydrolysed to a variety of volatile products. Isothiocyanates are the main active compounds in biofumigation and are responsible for nematode suppression. In this study, 21 different genotypes in family Brassicaceae as biofumigant were evaluated for their suppressive effect on *Meloidogyne arenaria* and *M. incognita* in pot experiments. Seedlings of each genotype were inoculated with 5000 egg after transplanting (one plant per pot) and were grown in a glasshouse. At approximately halfway to flowering stage, plants were harvested and all part of plant were cut and incorporated in soil. The pots containing amended soil were then covered with polyethylene plastic for 4 weeks. After removal of the plastic cover, susceptible tomato was grown for 60 days. Experiment were conducted in a completely randomised design with five replicates and repeated once. Among all genotypes tested for biofumigation potential after incorporation into soil, eight genotypes caused a marked reduction of galling index ($GI < 2$ based on 0-5 scale) on the roots of tomato plant caused by both nematode species. The most effective plants were Brussel sprouts, broccoli (Verdia and Batavia) and *Arugula* for both *M. arenaria* and *M. incognita*. Some plants surprisingly have different effects on the two root-knot species, like some kale varieties (S8). Acknowledgement: this study was supported by TÜBİTAK-TOVAG (109O544).

Keywords: Biofumigation; *Brassica*; *Meloidogyne arenaria*; *Meloidogyne incognita*.

Evaluation of different cultivation measures combined with soil fumigation on controlling root-knot nematodes *Meloidogyne incognita*

Wenkun Huang, Deliang Peng, Huan Peng, Kong Lingan and Jiangkuan Cui

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

corresponding author: wkhuang2002@163.com

Root-knot nematode *Meloidogyne incognita* has been a serious problem in continuous cropping system of Northern China. To get safe and efficient control measures, four mechanical cultivation methods included shallow tillage, deep tillage, shallow tillage or deep tillage in combination with dazomet fumigation were carried out 1 month before transplanting of watermelon. Control effects on nematode and the micro minerals were evaluated 3 months later. Compared with the common cultivation method, all treatments significantly reduced the nematode number in soil, improved the control effect, and increased the yield of crops. The best treatment for controlling nematodes and improving watermelon yield was deep tillage in combination with dazomet, which also increased the level of exchangeable magnesium and effective selenium but had no significant impact on the level of exchangeable calcium and effective copper. Based on the present investigation, deep tillage in combination with dazomet can not only decrease the dosage of chemical nematicides, but also improve the soil quality, which was applicable to the areas with severe root-knot nematode infestation. Considering the cost and convenience of operation, treatment with shallow tillage was effective for the areas where the nematode disease is not very serious. In conclusion, deep tillage in combination with fumigation would be useful in decreasing the dosage of chemical and fertiliser, and in controlling root-knot nematodes in continuous cropping system.

Keywords: Continuous cropping; mechanical deep tillage; soil fumigation.

Temperature-induced potato resistance in potato cyst-forming nematode management

Victoria Lavrova, Elizaveta M. Matveeva and Daryia S. Kalinkina

Institute biology, Karelian Research Centre of RAS, 185910 Petrozavodsk, Russia

corresponding author: VVLavrova@mail.ru

The potato cyst-forming nematode, *Globodera rostochiensis* Woll., is a serious pest of potato, the subject of strict world-wide quarantine regulations. The search for environmentally friendly methods for the nematode management is on-going. It is known that temperature can modulate plant resistance to pathogens. In this work daily temperature fluctuations (temperature drop from 23 to 5°C for 2 h at the end of the night for 6 days) were used and their effect on potato metabolism and the nematode reproduction was investigated. Results have shown differences in physiological parameters and transcription profile of the main response genes between susceptible, resistant and temperature-treated susceptible plants. Susceptible plants exhibited low fitness and inactive immunity system under nematode invasion. Temperature-treated susceptible plants had similar traits with resistant ones. The plants had increased fitness and expressed R-genes (*H1*, *Gro1-4*) at pre-invasion state. After nematode invasion of roots, these plants rapidly generated a strong signal for enhanced expression of R- and defence genes (*PI*, *PAL*, *PRs*). Temperature treatment of susceptible plants led to a considerable reduction of final cyst population ($k=2,7$ vs 4,0 in control), cysts contained fewer eggs and second-stage juveniles (J2) (198 and 304, respectively) with decreasing viability (49% vs 99%). Taking into account egg viability, multiplication rate was decreased to 1.05. Thus, temperature fluctuations at the pre-invasion stage provided high plant fitness and active immune system for subsequent nematode invasion and had a negative impact on nematode reproduction: plants triggered in time defence responses that disrupted the nematode feeding sites in roots, which became insufficient for J2 development. Some of the J2 that successfully avoided a cascade of plant immune responses and completed their life-cycle had reproductive disfunctions. In summary, temperature can be used in nematode management. The study was supported by RFBR (№16-34-00650 mol_a).

Keywords: *Globodera rostochiensis*; nematode reproduction; plant metabolism; *Solanum tuberosum*; temperature fluctuations.

Xenobiotic metabolism of *Globodera pallida* in the context of biofumigation

John W.H. Lennon, Laura M. Jones, Catherine J. Lilley and Peter E. Urwin

FBS, University of Leeds, Leeds LS2 9JT, UK

corresponding author: bs09j2l@leeds.ac.uk

Potato cyst nematodes (*Globodera* spp.) are economically important pests of potato. *Globodera pallida* is the most prevalent species in the UK, found in approximately 58% of potato fields in England and Wales. Cyst nematodes persist for decades in affected fields, and infections reduce yield and marketability of susceptible crops. Many effective chemical nematicides have been withdrawn or face increasing regulation due to environmental concerns and off-target effects. Growers are therefore increasingly looking to alternatives such as biofumigation. Biofumigation is the management of soil-borne pests through the application of brassica green manures, releasing glucosinolates which are hydrolysed by myrosinase to generate isothiocyanates. Isothiocyanates are toxic to many phytopathogens, including nematodes. In spite of this, consistent, effective application of biofumigation for nematode control has yet to be achieved, perhaps due to short periods of exposure to the volatile chemicals. Dazomet is a chemical nematicide that breaks down on application to form methyl isothiocyanate, its active component. Genes up-regulated in *G. pallida* second-stage juveniles under Dazomet exposure have been identified through transcriptome analysis, including genes with homology to *Caenorhabditis elegans* glutathione-S transferases, enzymes typically involved in detoxification of xenobiotic compounds. Genes and associated promoter regions have been cloned and changes in expression have been confirmed by qPCR. Generation of transgenic *C. elegans* reporter lines that express green fluorescent protein under control of *G. pallida* promoters demonstrate conservation of transcription factor activity across distant nematode lineages. Reporter lines allow the specificity of the regulatory response to biofumigation to be tested, as shown with reporters for *C. elegans* *gst-31*, which produce a specific response to brassicas when incubated in green tissue extracts from several plant species.

Keywords: Biocontrol; biofumigation; detoxification; reporters; xenobiotic.

An approach to soil response to different organic amendments and crop management

Antonio Lopez-Perez¹, Carlos García-Villarrubia¹ and Sara Sánchez-Moreno²

¹ Centro Agrario Marchamalo, INCRECYT, JCCM, Camino de San Martín s/n, 19180 Marchamalo, Guadalajara, Spain

² Unidad de Productos Fitosanitarios (DTEVPF), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Crta. Coruña km 7,5 28044 Madrid, Spain

corresponding author: Jalopezp@jccm.es

With the reduction in the use of pesticides, several control alternatives have emerged. The knowledge about the soil microcosm has become increasingly important. Biodiversity of agrarian soils is becoming a positive value, as opposed to the biological emptiness that soil fumigants used to produce. Chemically treated soils usually show low diversity and unbalanced properties that may enhance the apparition of pests and diseases. Several agrarian practices have been developed, and even rescued from the past, such as the use of organic amendments. This practice improves soil fertility but can also be used as biodisinfestation or biofumigation technique for controlling phytoparasitic organisms through its decomposition. The goal of this study was to check the effect on the soil nematode and on the soil food web related to the organic amendments applied. The study was developed in three different cropping systems, two of them under glasshouse conditions. Soil sampled from the three trials was studied. The results obtained show differences, not only on the soil food web, but also on the maturity indexes. Several aspects related to the farm practices applied are involved in such changes, and it becomes evident that the selection of a certain combination of cropping management techniques may contribute to the reduction of soil disturbance in agrarian systems and may help to increase biodiversity of the soils.

Keywords: Biodisinfestation; biofumigation; green manure; *Meloidogyne incognita*; trophic group.

Nematology and environmental change: perspectives, challenges and the future

Thomas Kakouli-Duarte

Molecular Ecology and Nematode Research Group, enviroCORE, Dargan Centre, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

corresponding author: Thomae.Kakouli@itcarlow.ie

Many anthropogenic factors have a negative impact on the quality of soil, air and water leading to detrimental environmental changes. The field of environmental nematology was born by a few colleagues a few decades ago, realising the potential of nematodes as environmental and ecological indicators and key ecosystem players. Since the pioneering work of Aldo Zullini, Gregor Yeates, Tom Bongers and Howard Ferris, to mention a few, the planet has witnessed environmental change of a high magnitude. Our field has considerable applied, economic and societal significance, as scientific awareness grows of the potential consequences of global warming, pollutants and environmental contaminants, and the need to decompose and recycle human, urban, and industrial wastes. In relation to soil health alone, soil degradation costs the EU at least €38 million annually. These costs are mainly borne by the European society. This keynote address will aim to provide an insight on the evolution of the field of environmental nematology, on the current state of the art, and taking into account latest developments, yet also highlighting deficiencies and areas that need improvement. It will be a point of thought and reflection on important aspects, such as industry demand, education and policy, and will dwell on future challenges and how the European, and indeed the global, community of nematologists can be an influential body for environmental sustainability.

Keywords: Bioindicators; biomonitoring; ecosystem service; environmental risk assessment.

Soil type, soil group and ecoregion based strategies for scalable soil health management in Michigan agriculture

Sevilhan Mennan^{1,5}, ZinThuZar Maung¹, Jenni Gronseth², Pablo D. Reeb³, Alvin J.M. Smucker⁴, Jiaguo Qi² and Haddish Melakeberhan¹

¹ Agricultural Nematology Laboratory, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA

² The Center for Global Change and Earth Observations, Michigan State University, East Lansing, MI 48823, USA

³ Statistical Consulting Center, College of Agriculture and Natural Resources

⁴ Department of Plant Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA

⁵ Ondokuz Mayıs University, 55139 Samsun, Turkey

corresponding author: melakebe@msu.edu

The need for developing integrated soil health management using different agronomic practices in cropping systems, that nematode assemblage analysis is an excellent indicator of soil health, and that soil conditions and temperature affect soil health-driven ecosystem services are well established. Scaling up soil health management within and across cropping systems across soil types (texture-based classification) and soil groups (orders), however, remains challenging. Different soil groups could have overlapping textures, but have different horizons influencing biological and physiochemical processes therein. Two studies were conducted. First, we tested over two growing seasons how cover (mustard and oilseed radish), rotation (maize and soybean) and sugar beet crops affect soil health in sandy clay loam and loam types. Neither soil type had optimum soil food web structure for agroecosystem, the sandy clay loam soil was more stressed than the loam soil. Across crops the results were not the same within or in both soil types, suggesting distinct interactions. The second study focused on exploring basis for scalability across Udalfs, Psammets and Sapristis soil sub-orders (major agricultural soils with contrasting properties) within two temperature zones, 40.1-45.0°F (northern half) and 45.1-50.0°F (southern half) of Michigan. Soil samples were collected from natural (pristine forests and other vegetation) and disturbed (agricultural soils with altered biological functions and soil nutrients) landscapes in the northeast and southwest regions, which are separated by about 300 miles (= 483 km). Within each landscape, 2-3 fields were selected and 5-10 geo-referenced samples per field were collected from 0-30 cm depth. Soil food web structure varied by temperature zone and/or soil group, suggesting ecoregional patterns. Overall, the data suggest that the soil groups may have to be treated differently within or across ecozones when considering soil health management strategies.

Keywords: Cropping systems; nematode community; soil food web; temperature zones.

Conservation agriculture and soil food web condition in extensive Mediterranean crops

Sara Sánchez-Moreno¹, Diana Martín-Lammerding², Mar Albarrán² and José L. Tenorio²

¹ Plant Protection Products Unit (DTEVPF). National Institute for Agriculture and Food Science and Technology, Crta Coruña km 7.5, 28040 Madrid, Spain

² Environmental Science Department, National Institute for Agriculture and Food Science and Technology, Crta Coruña km 7.5, 28040 Madrid, Spain

corresponding author: sarasm@inia.es

In Mediterranean extensive agroecosystems, conservation agriculture techniques, mainly reduced tillage, have significantly spread in the last years as a means to reduce soil erosion, increase soil fertility and reduce input costs. The effects of such management on soil organisms' diversity and functioning have been seldom studied in such systems, so an experiment including standard, reduced and no-tillage in a wheat monoculture vs rotation system was established in Central Spain in 1994. 21 years after the establishment of the tillage/rotation experiment, soil physical-chemical properties, microbial activity and soil food web condition were assessed. The composition, structure, and functioning of the nematode community were used as indicators of food web condition. Reducing tillage intensity improved soil organic matter accumulation in soil the surface. Total enzymatic activity (FDA hydrolysis), microbial biomass carbon (MBC) and microbial basal activity were also significantly higher under no tillage compared to conventional practices. Metabolic quotients ($q\text{CO}_2$ and $q\text{CO}_2/\text{SOC}$) indicated that the microbial community was more mature and C use was more efficient under no tillage than under conventional tillage. Our results showed that both soil food web structure and functioning clearly varied among systems. The absence of tillage maintained more structured food webs and more functional higher trophic links. Standard tillage favoured enrichment indicators, and reduced tillage, which improved food web structure, was the most conducive system to herbivore nematodes. Total nematode biomass was much higher under wheat monoculture than under the rotation system, mainly due to large abundances of *Pratylenchus*. Structural equation modelling was used to infer the magnitude of the relationships existing among soil food web components.

Keywords: Diversity indices; indicators; no-till; reduced tillage; rotation.

Differences between species matter: A plea to move away from traditional nematode guild classifications

Tom Moens¹, Luana da Costa Monteiro^{1,2}, Anna-Maria Vafeiadou¹, Katja Guilini¹,
Sofie Derycke³, Walter Traunspurger² and Nele De Meester¹

¹ Ghent University, Biology Department, Marine Biology Lab, Krijgslaan 281/S8, 9000 Gent, Belgium

² University of Bielefeld, Department of Animal Ecology, Morgenbreede 45, 33615 Bielefeld, Germany

³ Royal Belgian Institute of Natural Sciences, Operational Directory Taxonomy & Phylogeny, Vautierstraat 29, 1000 Brussels, Belgium

corresponding author: tom.moens@ugent.be

Changes in nematode assemblages can be powerful indicators of the recent disturbance history of soils and sediments. Because detailed assemblage analyses at species or genus level are tedious, the use of life-history guilds defined at family level, or morphology-based functional guilds defined at even lower resolution, and of indices based on these guilds, is very popular in biomonitoring. Such approaches implicitly assume that phylogeny provides a measure of similarity in life history, which in turn would reflect the tolerance of nematodes to disturbance. In addition, they assume that mouth morphology reflects nematode resource use and trophic position in food webs. Here we provide evidence based on natural stable isotope ratios that nematode species belonging to the same feeding guild and even to the same family can differ substantially in their resource use and even trophic level. We then use next generation sequencing to analyse the microbiomes of individual nematodes and demonstrate that even cryptic nematode species, belonging to the same morphospecies complex, display species-specific resource use. Moreover, population assays in heavy-metal polluted media show that these cryptic species exhibit differential sensitivities to pollution. In a final experiment, we subjected a variety of bacterivorous nematodes from marine and freshwater sediments to water-soluble oil fractions, and found that the differences in sensitivity between cryptic species were as substantial as differences between genera and even families. We conclude that phylogenetic relatedness is not always a reliable proxy of similarity in sensitivity to pollution, and that mouth morphology is a poor predictor of actual resource use. As a consequence, the use of life-history or mouth-morphology-based guilds to characterise shifts in nematode assemblages in the context of disturbance and/or resource use, hides potentially important trends and should be carefully reconsidered.

Keywords: Biomonitoring; functional guilds; life-history groupings; pollution sensitivity; species-specific differences.

Nematodes in compost: indicator potential for Ecological Soil Engineering

Gisele L. Herren, Lisa Joos, Isabelle Binnemans, Wim Bert and Hanne Steel

Ghent University, Department of Biology, Nematology Research Unit, Ledeganckstraat 35, 9000 Ghent, Belgium

corresponding author: gisele.herren@ugent.be

Soil Ecological Engineering is a newly defined approach of human land-use system management to enhance biological diversity together with targeted manipulations of soil biota to drive desired ecosystem processes. We propose that this exciting new frontier of soil research can be aided by the use of nematodes as bio-indicators, and more specifically used in the creation of high biological quality composts and monitoring of these manipulations in the soil food web and ecosystem functioning. Nematode community successional patterns in compost have been shown to be more informative of composting processes than microbial community patterns or any physio-chemical characteristics alone. Analysis of nematode communities during composting has been used to create an Index of Compost Maturity, combining four nematode based criteria (*i.e.*, abundance, fungivore: bacterivore ratio, presence of more than one fungal-feeding taxon and presence of diplogasterids). Carefully controlling compost processes and/or inoculating composts with biological control organisms allows for the creation of so called 'Designer Composts' that would target specific needs in the field. We present the nematode community changes during (vermi) composting and evaluate the validity of the Index of Compost Maturity for composts produced in alternative methods. This index is further evaluated by inoculating biological control agents (the nematode *Steinernema feltiae* and the fungus *Trichoderma harzianum*) into composts of variable maturity in order to assess the suitability of composts to be enhanced (*i.e.*, 'Designer Composts'). Finally, compost application combined with managed grazing has led to significant increases in carbon storage in California grassland soils. We will discuss our future research in this framework and describe how nematode indices can be used to investigate the influence of compost quality on carbon sequestration rates and changes in the soil food web.

Keywords: Biological control; carbon sequestration; compost maturity; soil food web; sustainable agriculture; vermicompost.

Detailed mapping of spatial distribution patterns of terrestrial nematodes at landscape scale reveals distinct, ecologically relevant patterns

Casper Quist¹, Gerrit Gort², Paul Mooijman¹, Sven Van den Elsen¹, Dick J. Brus³, Jaap Bakker¹ and Johannes Helder¹

¹ Laboratory of Nematology, Wageningen University, P.O. Box 8123, 6700 ES, Wageningen, The Netherlands

² Biometris, Wageningen University and Research Centre, PO Box 16, 6700 AC Wageningen, The Netherlands

³ Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands

corresponding author: casper.quist@wur.nl

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 mainly technical reasons; the microscopic analysis of nematode assemblages is labour intensive and requires extensive expertise. With the availability of relatively large molecular frameworks, it became possible to design molecular assays that allow for the quantitative analysis of individual taxa against complex DNA backgrounds. Quantitative (q) PCR assays were used to explore spatial distribution patterns of 45 individual taxa (at family, genus and species level) across The Netherlands at mesoscale (plot or field – scale dimensions: 1 – 1,000 m). Fields were located in two systems (nine arable fields and three grasslands with high plant diversity) and three soil types (marine clay, river clay and sand). In each field 100 composite samples were collected by using a sampling grid (100 × 100 m) optimised for geostatistic analysis. Each sample (n >1,200) was analysed with about 25 - 30 nematode taxon-specific molecular assays. In total over 35,000 qPCR reactions were run. Spatial patterns of nematode taxa were mapped by using Integrated Nested Laplace Approximation (INLA) in combination with geostatistical modelling. Patchiness might be related to either nematode traits, such as feeding strategy, body size and coloniser-persister (c-p) scale, or the characteristics of the environment, such as system, soil type and other abiotic conditions. The results that will be presented show a great variation in distribution patterns among nematode taxa, not only on the basis of their traits, but also based on environmental characteristics. These data are essential for the design of scientifically sound sampling schemes for nematodes in agricultural and natural soil at hectare scale and above.

Keywords: Bioindicator; distribution patterns; geostatistics.

Impact of agricultural management on nematode communities

Roy Neilson¹, David M. Roberts¹, Tom Brown² and Tim J. Daniell¹

¹ Ecological Sciences, The James Hutton Institute, Dundee, DD2 5DA, Scotland, UK

² University of Dundee, Dundee, DD1 4HN, Scotland, UK

corresponding author: roy.neilson@hutton.ac.uk

Soil is a non-renewable asset and a key component of natural capital. Thus, protection and maintenance of soils is fundamental to achieving the global challenges of food security. It has been estimated that in Europe, 16% of all soils are damaged and approximately 40% of agricultural soils are degraded. Consequently, there is a requirement primarily driven by policymakers for effective soil monitoring. With fundamental roles in soil processes, biota are considered an effective biological proxy measure of soil health/quality. It is well established that nematode communities are effective biological indicators of perturbation across a range of ecosystems. Molecular techniques have offered the opportunity to increase sample throughput, analyse the total nematode community (*cf.* manual identification of 100 random nematodes) and reduce the reliance on skilled taxonomists. Using an established T-RFLP method we have analysed whole nematode communities from 670 agricultural soil samples representing a range of management systems (conventional, organic and integrated), soil types and crops from a farm ($n > 50$) network in Scotland. Underpinning farm management and environmental data were analysed to identify the drivers of the nematode communities. Using nematodes as an exemplar, this project has demonstrated that cost effective national scale soil monitoring of agricultural soils can be readily achieved.

Keywords: Indicators; intensive farming; management; sustainable production.

Management strategies to manipulate soil biodiversity and disease suppressiveness

Gerard W. Korthals¹, Wim v.d. Berg², Leendert P.G. Molendijk² and Johnny H.M. Visser²

¹ Centre for Soil Ecology (CSE), NIOO-KNAW, Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

² Applied Plant Research, Wageningen UR, Edelhertweg 1, 8200 AK Lelystad, The Netherlands

corresponding author: gerard.korthals@wur.nl

There is an urgent need to develop sustainable management strategies to increase disease suppressiveness. 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 treatments (compost, chitin, marigold, grass-clover, biofumigation, anaerobic soil disinfestation, a physical control method and a combination of marigold, compost and chitin) and two reference treatments (chemical control with 300 l ha⁻¹ Metam sodium and untreated control). These ten treatments were studied for their effects in conventional and organic farming systems during 8 years. The present study demonstrated that in comparison to chemical control, additions of chitin, anaerobic soil disinfestation and marigold are excellent alternatives to improve disease suppressiveness and that some of these treatments performed better in the organic farming systems. 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. Yield increases were probably less influenced by changes in chemical soil properties but were the consequence of changes in the soil biota. In addition, it was shown that most of these treatments could already be implemented in arable crop rotations throughout the world and that nematodes are very good bioindicators of soil health.

Keywords: Biocontrol; bioindication; long-term field experiment; *Pratylenchus penetrans*; soil health.

Four years later: The effect of cover crops on the nematode community in an apple orchard

Caro Kapp¹, Sheila G. Storey² and Antoinette P. Malan¹

¹ Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, 7602, South Africa

² Nemlab, C/O R44 and Answortelrug Road, Klapmuts, 7625, South Africa

corresponding author: ckapp@sun.ac.za

The addition of organic soil amendments has become common practice in apple orchards in the Western Cape province of South Africa. Organic amendments (e.g., green manures, composts, cover crops, etc.) play a vital part in preserving and cultivating soil health and quality and directly influence soil biology, structure and fertility. Also, organic amendments impact the dynamics of certain soilborne pathogens, e.g., plant-parasitic nematodes, through the stimulation of antagonistic organisms. It is imperative to evaluate critically the effect these amendments have on soil health. Thus, for the past four years, the nematode community structure and function in an apple orchard has been evaluated, using nematodes as a bio-indicator. To facilitate the effect that cover crops can have on a nematode community and on trophic shifts over time, soil samples were collected from the tree and working rows of five treatments in an apple orchard. Each treatment had five replicates. Nematodes were extracted using Cobb's sieving and decanting method, followed by the use of a modified Baermann funnel. The nematodes obtained were then counted and identified, mostly to family level, using their morphological characteristics. The families were then assigned feeding groups, and coloniser-persister values. Various indices were then applied to the findings including the Enrichment, Basal, Structure, Channel, and Maturity indices. To determine the existing enrichment, structural and basal conditions, the nematode faunal analysis for each treatment was evaluated. Results continually indicated a nematode community dominated by bacterial-feeding nematodes (e.g., Cephalobidae and Rhabditidae). Such plant-parasitic nematodes as *Pratylenchus* spp. comprised a significant portion of the nematode population. Different cover crop treatments implied discernible differences regarding community composition and structure.

Keywords: Bio-indicators; food webs; nematodes; soil health; trophic shifts.

Host sex and phenological state influence the nematode community associated with a dioecious coastal shrub

Ana Martins¹, Daniela Tavares¹, Helena Freitas¹ and Sofia R. Costa^{2,3}

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000- 456 Coimbra, Portugal

² Mountain Research Center (CIMO), Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

³ CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

corresponding author: anafdmartins@gmail.com

Nematodes are frequently used for environmental monitoring of terrestrial communities. We analyse the nematode communities associated with *Corema album*, a dioecious coastal shrub, endemic to the Iberian Peninsula, to examine its interactions with the rhizosphere. In dioecious plant species, female and male individuals have different physiological requirements according to phenological stage, and may interact differently with soil organisms. We sampled the rhizosphere of 150 plants in three populations along the West coast of Portugal, in the semi-fixed dune and the hind dune woodland. Nematodes were extracted, identified and quantified using an inverted microscope, and community indices calculated. Bacterial-feeding nematodes were the most abundant trophic group, followed by fungal feeders. The Nematode Channel Ratio was higher for males during fruiting and the Food Channel Index was significantly higher in males during flowering, possibly due to higher biological activity in their rhizosphere. Composition and abundance of plant-parasitic nematode (PPN) communities were assessed through a Correspondence Analysis, revealing significant differences between female and male plants during fruiting but not during flowering, which suggests physiological requirements according to plant sex are drivers in the ecological relations with the associated biota. Of the 13 PPN genera identified, three (*Criconema*, *Hemicriconemella* and *Meloidogyne*) showed significant differences according to the Generalized Linear Model analyses, depending on host sex during fruiting, but not during flowering. *Criconema* and *Hemicriconemella* (ectoparasites) were more abundant in the rhizosphere of male plants, whereas *Meloidogyne* (sedentary endoparasite) were more abundant in females, suggesting that host suitability depends on feeding strategy. We conclude that plant sex, depending on phenological stage, of a dioecious plant species influences its relationship with the associated nematode community.

Keywords: Above-belowground; *Corema album*; *Meloidogyne*; rhizosphere; sand dune.

Genetic analysis of parasitism by *Meloidogyne hapla* reveals isolate-specific alleles responsible for modulating host gene expression

David Bird^{1,2}, Dahlia Neilsen^{1,2}, Soyeon Cha^{2,4}, Peter Digennaro^{1,5} and Valerie Williamson³

¹ Dept. Entomology and Plant Pathology, NC State University, Raleigh NC 27695, USA

² Bioinformatics Research Center, NC State University, Raleigh NC 27695, USA

³ Department of Plant Pathology, UC-Davis, Davis CA, USA

⁴ Current address: Samsung Genomic Institute, Seoul, Korea

⁵ Current address: Dept. Entomology and Nematology, University of Florida, Gainesville Florida, USA

corresponding author: bird@ncsu.edu

Individual field isolates of root-knot nematodes exhibit striking differences in their parasitic ability, presumably reflecting underlying genetic diversity. Because genetic analysis of the host-parasite interaction has the power to reveal causal relationships, we developed the tools necessary for linkage analysis and fine structure mapping. Using an approach based on Quantitative Trait Locus (QTL) analysis, we identified 213 distinct genes in the model legume *Medicago truncatula*, with expression levels significantly influenced by genetic variation at one (or more) *Meloidogyne hapla* locus. A single *M. hapla* locus on LG4 is responsible for half of the observed transcriptional changes in the host, which include a disproportionate number of transcription factors. Other hotspots are discernable on *M. hapla* LG 8 and 21. We named these parasite hotspot loci Host Expression Modulator (HEM). HEM-1 has been localised to a 84kb region, and appears to involve three distinct *M. hapla* genes. To understand properly the precise role for these genes will require a reverse genetics approach. To this end, we have begun to design a suite of CRISPR/Cas9-based genome editing tools. Beyond that, we propose to use CRISPR/Cas9 to develop a Gene Drive in which a transdominant allele of a gene deleterious to the population structure of the targeted nematode species is introduced into the wild. Driven by the growing affluence of a growing human population, the demand for food will soon outstrip supply. In the absence of new farmlands, controlling pests is one of the few avenues open to increasing yield. Plant-parasitic nematodes are the major source of biotic damage to staple and cash crops alike. They contribute to malnutrition in subsistence farming, and broadly diminish agricultural profitability; losses are estimated to approach \$170 billion per year. Eliminating one of the major species of PPN would help ameliorate this impact.

Keywords: CRISPR/Cas9; eQTL; Gene Drive, HEM1; root-knot nematode.

Identification of new *Meloidogyne javanica* proteins involved in rice-nematode interactions

Maira Grossi de Sa¹, Anne-Sophie Petitot¹, Itamara Mezzalira^{1,2}, Magda Beneventi^{1,2}, Maria Eugênia Lisei de Sa^{1,2}, Deisy Amora², Hugues Baimey³, Janice de Almeida-Engler^{2,4}, Erika Freire², Maria Fatima Grossi-de-Sa² and Diana Fernandez^{1,2}

¹ UMR IPME, Institut de Recherche pour le Développement (IRD), 34394 Montpellier, France

² Embrapa Recursos Genéticos e Biotecnologia, 70770-917 Brasília-DF, Brazil

³ Université de Parakou, Benin

⁴ UMR IBSV, Institut National de la Recherche Agronomique (INRA) 06903 Sophia Antipolis, France

corresponding author: diana.fernandez@ird.fr

Root-knot nematodes are endoparasites with a wide host range, encompassing mono- and dicotyledonous plant crops. *Meloidogyne javanica* is responsible for rice (*Oryza sativa*) production losses in Brazil and Africa (Benin). Successful infection is likely achieved by effector proteins produced in the nematode oesophageal gland cells and released in the host plant cells. The aim of this study was to assess the functional role of three oesophageal gland cell proteins of *M. javanica* in rice - nematode interactions. RT-qPCR assays showed that *Mj-SP2*, *Mj-SP18* and *Mj-SP19* genes are over-expressed all along the infection cycle in rice roots. Immuno-detection experiments using specific antibodies raised against synthetic peptides showed that the nematode proteins are localised in circular granules structures within the nematode body. Subcellular localisation experiments in onion cells showed that that *Mj-SP2* may be addressed to the nucleus, and *Mj-SP18* and *Mj-SP19* to the cytoplasm and nucleus. Transgenic rice (*O. sativa* Nipponbare) plants expressing the candidate proteins or artificial micro-RNAs (amiRNAs) able to silence the cognate genes in the nematode were produced. Assessment of nematode reproduction on homozygous transgenic lines allowed the identification of rice lineages with altered susceptibility, indicating that these proteins may be involved in *M. javanica* virulence. The role of these proteins in suppressing host immunity is now investigated in tobacco cell death-induced transient assays. Data obtained may help deciphering nematode-rice molecular interactions and open new avenues for nematode control strategies.

Keywords: amiRNA; functional analysis; immunolocalisation; transgenic rice; virulence effector.

Identification and characterisation of effector candidates from root-knot nematodesNathanon Leelarasamee¹ and Cynthia A. Gleason^{1,2,3}¹ Georg-August-University, Göttingen, Schwann-Schleiden-Forschungszentrum, 37077 Göttingen, Germany² Georg August University, Goettingen Center for Molecular Biosciences (GZMB), Department of Plant Molecular Biology and Physiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany³ Washington State University, Department of Plant Pathology, Pullman, WA 99164, USAcorresponding author: cynthia.gleason@wsu.edu

Some of the most economically damaging plant-parasitic nematodes belong to the group of root-knot nematodes (*Meloidogyne* spp). Root-knot nematodes have an extremely broad host-range. They infect plant roots and form feeding sites called 'giant cells'. During infection and formation of the giant cells, the nematode secretes a cocktail of molecules called effectors. The effectors must suppress plant defences and/or alter the host plant physiology for a successful nematode infection. My lab is interested in identifying and characterising root-knot nematode effectors. Our efforts have led to the discovery of two new root-knot nematode effectors called Mh265 and MiPFN3. Both Mh265 and MiPFN3 are transcriptionally up-regulated during the parasitic life-stages and both appear to have roles in nematode parasitism. First, Mh265 is specific to root-knot nematodes and encodes a novel secreted protein. Ectopic expression of Mh265 in *Arabidopsis* leads to enhanced nematode susceptibility. In addition, the transgenic plants were compromised in elicitor-induced callose deposition, suggesting the Mh265 is modulating the plants' basal immune responses. The second effector we identified, MiPFN3, is a profilin protein. We show that MiPFN3 can bind and sequester actin monomers and in doing so, alter the actin cytoskeleton in plant cells. Plants that express MiPFN3 at low levels are more susceptible to root-knot nematodes, suggesting that MiPFN3 is facilitating infection processes. Overall, it is vital to unravel mechanisms of nematode pathogenicity in the efforts to engineer new nematode resistance in plants.

Keywords: Actin; callose; defence; parasitism.

Love me not: a *Globodera pallida* unique effector with affinity for several potato NAC-transcription factors

Sophie Mantelin¹, Mirela C. Coke², Kathryn M. Wright¹, Peter Thorpe^{1,2}, Peter J. Cock¹, Ailsa Smith¹, Peter E. Urwin² and John T. Jones^{1,3}

¹ Cell and Molecular Sciences Group, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

² Department of Plant Sciences, University of Leeds, Leeds LS2 9JT, UK

³ Biology Department, University of St Andrews, St Andrews, Fife KY16 9TZ, UK

corresponding author: Sophie.Mantelin@hutton.ac.uk

During a compatible interaction, the potato cyst nematode, *Globodera pallida*, induces complex changes in its host, resulting in the formation of its feeding site, the syncytium. Effector proteins, which are mainly produced in the pharyngeal gland cells of the nematode and delivered to the plant cells through the stylet, are thought to be important in promoting establishment and maintenance of the syncytium. The completion of the *G. pallida* genome sequence has allowed identification of the full effector complement of this species. Most effectors are pioneers with no similarity to other sequences in databases. In order to shed light onto the putative biological function of such effectors, we have undertaken a large scale analysis of their subcellular localisation *in planta* using transient expression of fluorescent reporter fusions. In addition, many effectors were used as bait in yeast two-hybrid (Y2H) screens to identify potential host target proteins. More than 50 effectors were investigated but only one localised to the endoplasmic reticulum. It represents a unique gene in *G. pallida*, which is specifically expressed in pre-parasitic and parasitic stage juveniles. Screening different potato Y2H libraries identified several transcription factors from the NAC family that showed variable strength of interaction and different subcellular localisation patterns *in planta*. NAC transcription factors are resident in the endoplasmic reticulum from where they can be released upon stress and translocated to the nucleus. Cell biological and chromatin binding analyses suggest that the effector stabilises the NAC proteins *in planta*. Concomitantly, overexpression of the nematode effector in plants seems to compromise plant immunity.

Keywords: Chromatin associated proteins; nuclear re-localisation; plant immunity; potato cyst nematode; yeast two-hybrid.

GpRpb-1 from *Globodera pallida* manipulates plant post-translational modification in host cells to promote nematode infection

Amalia Diaz Granados, Hein Overmars, Erik Slootweg, Wiebe Postma, Jaap Bakker, Aska Goverse and Geert Smart

Department of Nematology, Wageningen University, Wageningen, The Netherlands

corresponding author: amalia.diazgranadosmunoz@wur.nl

In persistent nematode infections plant cell morphology and physiology are manipulated by the nematodes to establish sophisticated feeding structures. Modifications to plant cells are largely attributed to the activity of nematode secreted effectors. SPRYSECs are a remarkably expanded family of effectors identified initially in potato cyst nematodes. While SPRYSECs have been implicated in suppression of plant immunity, their intrinsic role in nematode virulence remains unexplored. *GpRpb-1* is a 'type' SPRYSEC from *Globodera pallida* with virulent and avirulent variants present in field populations of the nematode. Y2H screening of a nematode-infected susceptible potato library yielded interacting candidates for a virulent *GpRpb-1* that are involved in post-translational modification in the plant. We have independently confirmed that two ligases involved in post-translational modification can interact with virulent and avirulent variants of *GpRpb-1* in yeast. A localisation study also shows that the candidate interactors localise to the nucleus, which allows interaction with *GpRpb-1*. Furthermore, upon silencing of the corresponding ligase genes in *Arabidopsis thaliana*, we observed significant differences in the amount of developing females present in the roots of nematode-infected plants. These candidate interactors of *GpRpb-1* suggest that the intrinsic role of the effector is carried out through manipulation of the plant post-translational modification machinery. Our findings suggest that nematodes are able to use this family of effectors to control different aspects of the plant cell to establish a feeding site. Therefore, our results may provide further insight into the basis of virulence of nematodes in plants.

Keywords: *Globodera* spp.; effectors; plant-nematode interactions; SPRYSECs.

C-terminally encoded plant peptide (CEP) hormone domains from *Rotylenchulus reniformis* have dual roles *in planta*

Catherine Lilley^{1*}, Sebastian Eves-van den Akker^{2*}, Hazijah B. Yusup¹, John T. Jones^{3,4}
and Peter E. Urwin¹

¹ Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK

² Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

³ Cell and Molecular Sciences Group, Dundee Effector Consortium, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

⁴ School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9TZ, UK

*These authors contributed equally

corresponding author: c.j.lilley@leeds.ac.uk

The interaction between sedentary plant-parasitic nematodes and their host is mediated by nematode effectors. These secreted proteins are responsible for the induction and maintenance of the nematode feeding site within the host root. We have discovered a large and diverse family of effector genes, encoding C-terminally Encoded Peptide (CEP) plant hormone mimics (RrCEP), in the syncytium-forming, reniform nematode *Rotylenchulus reniformis*. Unlike CEP genes from all other organisms, whether plant or nematode, those cloned from *R. reniformis* contain one intron per domain sequence, regardless of the number of tandem domains that are present. Together with the distant phylogenetic relationship of *R. reniformis* to the only other CEP-encoding nematode genus identified to date (*Meloidogyne*), this suggests CEPs likely evolved *de novo* in *R. reniformis*. We have characterised the first member of this large gene family (*RrCEP1*), demonstrating its expression in the effector-producing pharyngeal gland cell and significant up-regulation during the biotrophic phase of the life cycle. *RrCEP1* encodes a functional CEP domain, which significantly up-regulates expression of an *Arabidopsis* nitrate transporter, while simultaneously reducing primary root elongation. The same CEP domain also limits syncytium expansion in *Arabidopsis* for the non-CEP containing cyst nematode *Heterodera schachtii*. CEP effectors of *R. reniformis* may therefore represent a two-fold adaptation to sustained biotrophy by increasing host nitrate uptake whilst limiting the size of the syncytial feeding site produced.

Keywords: Effector; evolution; nitrate; syncytium.

The role of polygalacturonase (PG), PG-inhibiting proteins (PGIP), and oligogalacturonides (OG) in plant–nematode interaction

Syed Jehangir Shah, Muhammad Arslan Anwer, Muhammad Shahzad Anjam, Florian Grundler and Shahid Siddique

Rheinische Friedrich-Wilhelms Universität Bonn, INRES-Molecular Phytomedicine, Karlrobert-Kreiten Strasse 13, 53115 Bonn, Germany

corresponding author: jehangir@uni-bonn.de

Host perception of microbial- or damage-associated molecular patterns (MAMP or DAMP) via surface-localised receptors leads to the activation of antimicrobial defence responses in all multicellular organisms, including plants. Infective second-stage juveniles (J2) of endoparasitic cyst and root-knot nematodes invade the roots close to the elongation zone and migrate through different tissue layers to reach the vascular cylinder forming a feeding site and becoming sedentary. The invasion of roots by nematodes and their subsequent migration inside the roots results in extensive cell wall damage, which is likely to generate cell wall fragments that can act as DAMP (oligogalacturonides; OG) and may activate host defence responses against nematodes. Here we characterise the role of a group of cell wall receptor proteins—polygalacturonase-inhibiting protein (PGIP)—in *Arabidopsis* during infection with beet cyst nematode (BCN) *Heterodera schachtii* and root-knot nematode (RKN) *Meloidogyne incognita*. PGIP is encoded by a small gene family of two genes (PGIP 1 and PGIP2) in *Arabidopsis* and is involved in perception of OG upon pathogen infection. We have used promoter reporter lines (GUS and GFP), microarrays analysis and real-time RT-PCR to study expression of PGIP genes upon infection with nematodes. All these methods showed that PGIP genes are particularly strongly induced upon infection with cyst nematodes. Loss-of-function mutants for PGIP1 and PGIP2 also showed a significant increase in the susceptibility to cyst nematodes when compared with the wild-type. However, there was no change in susceptibility to root-knot nematodes. The mechanistic details into role of PGIP during cyst as well as root-knot nematode infection will be presented.

Keywords: Cyst nematode; defence response; PGIP.

Apoplastic venom allergen-like proteins of plant-parasitic nematodes modulate the activation of plant innate immunity by cell surface receptors

Jose Lozano-Torres, Ruud Wilbers, Sonja Warmerdam, Anna Finkers-Tomczak, Amalia Diaz-Granados, Casper Schaik, Johannes Helder, Jaap Bakker, Aska Goverse, Arjen Schots and Geert Smart

Wageningen University and Research, Laboratory of Nematology, P.O. Box 8123 6700 ES, Wageningen, The Netherlands

corresponding author: jose.lozano@wur.nl

Despite causing considerable damage to host tissue during parasitism, nematodes establish persistent infections in both animals and plants. It is thought that an elaborate repertoire of nematode effectors suppress damage-triggered immune responses of the host. However, the nature and mode of action of most of nematode immunomodulatory compounds are not well understood. We have recently discovered that the effector venom allergen-like proteins (VAP) of plant-parasitic nematodes selectively suppress host immunity during the onset of parasitism. VAP are uniquely conserved in secretions of all animal- and plant-parasitic nematodes, but their role in parasitism has remained elusive. Knocking-down the expression of Gr-VAP1 severely hampered the infectivity of *Globodera rostochiensis*. By contrast, heterologous expression of Gr-VAP1 and VAP from *Heterodera schachtii* in plants resulted in the loss of basal immunity to multiple pathogens. Surprisingly, these VAP only affect the defence responses mediated by surface-localised immune receptors. The modulation of basal immunity by ectopic VAP involves extracellular protease-based host defences and chloroplast-localised non-photochemical quenching. The delivery of VAP into host tissue coincides with the enzymatic breakdown of plant cell walls by migratory nematodes. We, therefore, conclude that parasitic nematodes most likely utilise VAP to suppress the activation of defences by immunogenic breakdown products in damaged host tissue.

Keywords: Immunosuppression; membrane-bound immune receptors; nematode effector proteins; VAP.

Identification and characterisation of *Heterodera schachtii* effector proteins

Samer S Habash¹, Miroslaw Sobczak², Shahid Siddique¹, Abdelnaser Elashry¹ and Florian M.W. Grundler¹

¹ Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

² Department of Botany, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159, PL-02787 Warsaw, Poland

corresponding author: Habash.samer@yahoo.com

The beet cyst nematode, *Heterodera schachtii* is an important pathogen of sugar beet. Protein effectors produced by the nematodes facilitate penetration, migration and feeding site initiation and maintenance inside the host roots. There is emerging evidence that nematodes also secrete effectors that manipulate the redox status in the plant and protect them from plant-delivered reactive oxygen species (ROS). Analysing the *H. schachtii* transcriptome, we found a sequence matching thioredoxin-like proteins (Hs-Trx). As revealed by real time qPCR of samples from different developmental stages, the gene is up-regulated in the early parasitic phase. *In situ* hybridisation of the transcript localised Hs-Trx specifically to the oesophageal gland of the second-stage juveniles. Silencing of Hs-Trx with specific dsRNA-constructs led to reduced transcription and decreased parasitism in *Arabidopsis thaliana*. The structure and ultrastructure of feeding sites induced by silenced nematodes showed profound differences compared to that of untreated nematodes. Transgenic *Arabidopsis* plants, overexpressing Hs-Trx were found to be more susceptible to *H. schachtii*. After treatment with the PTI- inducing peptide flg22, the level of ROS burst was lower in the transgenic plant than in the control. The function of Hs-Trx as a scavenger of ROS was further supported by H₂O₂ exogenously applied to silenced and control nematodes. Silencing induced higher sensitivity to H₂O₂. The analyses show that Hs-Trx is important factor for successful parasitism and survival of *H. schachtii*.

Keywords: *Arabidopsis thaliana*; effector; *Heterodera schachtii*; plant-nematode interaction; reactive oxygen species (ROS); thioredoxin.

Host-specific gene induction in the plant-parasitic nematode *Pratylenchus coffeae*

Christopher A. Bell, Catherine J. Lilley, Howard J. Atkinson, James McCarthy and Peter E. Urwin

¹Centre for Plant Sciences, University of Leeds, Leeds, UK

²Research and Development, Nestle, Tours, France

corresponding author: bscb@leeds.ac.uk

Pratylenchus coffeae is of widespread economic importance but interaction with its hosts is ill-defined, both before and during parasitism. For instance, roles of cell wall degrading enzymes are well characterised for other plant-parasitic nematodes but not *P. coffeae*. Four β -1,4-endoglucanase genes from this nematode were identified from published data. Three of these genes cluster together with orthologues from other migratory nematode species but the fourth gene has greater sequence similarity to bacterial genes. *In situ* hybridisation revealed that all four genes are expressed in the pharyngeal glands and may be secreted into the host roots. One gene is also expressed in the intestine. Relative expression of the four genes differed significantly for *P. coffeae* recovered from the roots of either banana, carrot, coffee, maize or potato. Similar differential, host-specific expression was also detected for orthologous genes from *P. vulnus* and *Radopholus similis*. Root exudates from these host plants stimulated stylet thrusting of *P. coffeae* and induced differential expression of the four β -1,4-endoglucanase genes. Exposing this nematode to serotonin stimulated stylet thrusting and up-regulated the expression of all four genes. Pre-treatment of *P. coffeae* with levamisole, an agonist of cholinergic signalling in nematodes, inhibited both stylet thrusting and induction of gene expression in response to root exudate exposure. This suggests detection of a nearby host is perceived via cholinergic neurons and results in the induction of β -1,4-endoglucanase genes in a host specific manner. It also suggests that the serotonin pathway, previously shown to stimulate stylet thrusting, is involved in gene regulation. Current work is focused on identifying the host factors responsible for these differential responses and pathways involved in host detection. Determining the plant signals and their perception by the nematode may underpin novel approaches to plant defence.

Keywords: Endoglucanase; gene expression; levamisole; *Pratylenchus*.

The microbiome of sympatric cryptic nematode species reflects resource differentiation which alters by ecological interactions

Sofie Derycke¹, Nele De Meester² and Tom Moens²

¹ Royal Belgian Institute of Natural Sciences (RBINS), Vautierstraat 29, 1000 Brussels, Belgium

² Marine Biology Laboratory, Ghent University, Krijgslaan 281 (S8), 9000 Ghent, Belgium

corresponding author: sofie.derycke@naturalsciences.be

Differences in resource use or in tolerances to abiotic conditions are often invoked as potential mechanisms underlying the sympatric distribution of cryptic species. Additionally, the microbiome can provide physiological adaptations of the host to environmental conditions. We determined the intra- and interspecific variability of the microbiomes of three cryptic nematode species of the *Litoditis marina* species complex that co-occur, but show differences in abiotic tolerances. Roche 454 pyrosequencing of the microbial 16S rRNA gene revealed distinct bacterial communities characterised by a substantial diversity (85 – 513 Operational Taxonomic Units (OTU)) and many rare OTU. The core microbiome of each species contained only very few OTU (2–6), and four OTU were identified as potentially generating tolerance to abiotic conditions. A controlled experiment in which nematodes from three cryptic species (Pm1, Pm3 and Pm4) were fed with either an *Escherichia coli* suspension or a bacterial mix was performed and the 16S rRNA gene was sequenced using the Mi Seq technology. OTU richness ranged between 1118–7864. This experiment confirmed the existence of species-specific microbiomes, a core microbiome with few OTU, and high interindividual variability. The microbiome of starved nematodes was clearly different from that of nematodes offered food. The offered food source affected the bacterial community and illustrated different feeding behaviour between the cryptic species, with Pm3 exhibiting a higher degree of selective feeding than Pm1. A second experiment in which the three species were incubated together illustrated that Pm1 and Pm4 feed on the same bacteria, while Pm3 had a very distinct microbiome. Similar resource use may explain the largely allopatric distribution of Pm1 and Pm4 in the field. Morphologically similar species belonging to the same feeding guild (bacterivores) can thus have substantial differences in their associated microbiomes and feeding strategy, which in turn may have important ramifications for biodiversity–ecosystem functioning relationships.

Keywords: 16S metagenomics; competition; feeding ecology; resource differentiation.

A marine epiphytic nematode (Cyatholaimidae) reveals low genetic structure but significant morphological variation over more than 1000 km coast line

Daniel Apolônio Silva de Oliveira^{1,4}, Sofie Derycke², Tom Moens³, Giovanni A.P. dos Santos⁴ and Wilfrida Decraemer^{1,2}

¹ Department of Biology, Ghent University, Ghent, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

² Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium

³ Department of Biology, Marine Biology Laboratory, Ghent University, Krijgslaan 281, S8, 9000 Gent, Belgium

⁴ Federal University of Pernambuco, Av. Professor Moraes Rego, 1235 - Cidade Universitária, 50670-901 Recife, Brazil

corresponding author: apoloniobio@gmail.com

Determining population isolation, gene flow and dispersal capacities of organisms in marine environments can be challenging. Because of their lack of planktonic larvae, nematodes are generally believed to have a limited dispersal capacity compared to organisms with a planktonic life stage. Nematodes can use drifting seaweeds to disperse passively, and water currents may provide natural barriers. Along the northeastern coast of Brazil, a split of two main currents occurs that could potentially function as such a barrier to dispersal. We studied the genetic structure of a new *Paracanthochus* species, a nematode living on seaweeds, in four locations distributed over more than 1000 km of coastline and on opposite sides of these two major currents, using the mitochondrial *COI* gene. Additionally, we compared genetic diversity in two populations in regions with and without historical exploitation of seaweed. A total of 17 haplotypes were found, with a high dominance of one haplotype representing 6 to 83% of the individuals. Surprisingly, the AMOVA analysis showed little genetic structure ($F_{st} = 0.05204$; $P = 0.00391$). The pairwise analysis showed no significant differences between the populations under the influence of the two different water currents. Historical seaweed exploitation did not affect the population genetic diversity. Morphometrically, the populations significantly differed in some characters, whereas the genotypes were very conserved. We discuss the possibility of alternative dispersal mechanisms for marine nematodes that could explain the wide distribution of some haplotypes between populations. Our results suggest that dispersal over large distances of nematodes associated with seaweeds is more common than previously expected.

Keywords: *COI*; connectivity; morphometry; population genetics.

What molecular tools bring to our understanding of entomopathogenic nematodes ecology?

Raquel Campos-Herrera

MeditBio, Universidade do Algarve, Campus Gambelas, 8005-139 Faro, Portugal

corresponding author: rcherrera@ualg.pt

A major goal in ecology is unravelling the factors that drive multitrophic interactions, in space and time. When these interactions occur among organisms of agronomic interest, resolving the key players that involve natural plant protection can reveal more rational ways to manage the agroecosystem. For decades, this pursuit has been neglected due to challenges in the ability to measure complex soil communities. However, advances in PCR-based techniques are allowing more accurate assessment of the diversity and function of soil food webs. Entomopathogenic nematodes (EPN) provide a good example because they are ubiquitous in agricultural and natural soils and their populations are modulated by both abiotic and biotic factors. The development of more than 35 species-specific, qPCR molecular probes to identify and quantify organisms involved in EPN soil food webs has expanded our ability to understand critical species assemblages in various regions in the world. In citrus groves on the Florida peninsula, two eco-regions with different patterns of herbivory comprise edaphic properties that modulate the EPN-assemblages differently in each. Similarly, qPCR tools were required to detect EPN in a mining-polluted region of southern Spain and demonstrated the apparent capacity of EPNs to colonise any habitat capable of supporting a host. The limited abundance of EPN in Swiss annual crops may be a consequence of competition by certain free-living nematodes (FLN) for the cadaver. Ongoing laboratory experiments are elucidating the extent to which EPN reproduction is modulated by coexistence with FLN and sympatric EPN species in the host.

Keywords: Cadaver competition; multitrophic interactions; real time qPCR; soil food web.

Ecology of *Caenorhabditis* sp. 34

Ryusei Tanaka and Taisei Kikuchi

Division of Parasitology, University of Miyazaki, 5200 Kihara, Kiyotake-cho, Miyazaki-city, Miyazaki, Japan

corresponding author: caenorhabditisjaponica@gmail.com

Caenorhabditis sp. 34 is a nematode that was recently discovered from a fresh fruit (syconium) of *Ficus septica*, which is known to have a mutualistic relationship with a pollinator wasp *Ceratosolen bisulcatus*. Ribosomal RNA and genome sequence analyses revealed *C. sp. 34* is the most closely related to *Caenorhabditis elegans* among known species (a sister species). However, *C. sp. 34* shows multiple interesting traits that differ from the model organism, including the large body size, the gonochoristic reproduction and the complex lifecycle. In this study, we sought to understand the basic ecology of *C. sp. 34* by investigating foods, vectors and natural diversity of the species. To identify foods of *C. sp. 34* in the natural condition, nematodes isolated from fresh fig syconia were washed vigorously with M9 buffer containing antibiotics to remove bacteria on the surface. Then, the washed nematodes were crushed and plated on bacterial growth media. Multiple species of bacterium including *Stenotrophomonas* sp., *Enterobacter* sp., and *Siccibacter* sp. were detected on the media, suggesting *C. sp. 34* uses various bacteria as a food source. Nematode growth tests on these bacterial species revealed *C. sp. 34* has different growth rate by each bacterial species. Because *C. sp. 34* was identified only from fresh syconia on *Ficus* trees, it is suggested they are vectored by other organisms. We tried to detect *C. sp. 34* from the coexisting organisms and found the pollinator wasp *C. bisulcatus* harboured *C. sp. 34*, as the dauer form, with high frequency, suggesting the wasp is a main vector of the nematode. Finally, we tried to clarify natural diversity of the nematode by a large scale sampling in five islands in south Japan in which *Ficus septica* grows. *C. sp. 34* was detected from all five islands and the preliminary sequence analysis suggested *C. sp. 34* has high level of diversity and there seem to be two subpopulations in the area.

Keywords: Diversity; interaction.

Below-ground effects of related exotic and native plant species on primary decomposers as well as their main grazers; bacterivorous and fungivorous nematodes

Paula Harkes and Johannes Helder

Laboratory of Nematology, Graduate School of Experimental Plant Sciences, Wageningen University, 6709 PD Wageningen, The Netherlands

corresponding author: paula.harkes@wur.nl

Introduction of non-indigenous species is one of the major drivers of changes in biological communities. Above-ground effects of exotic plant species on the vegetation composition are generally easily scorable and relatively well documented. Although its relevance in terms of ecological functioning is not disputed, far less attention has been paid to below-ground effects of invasive plant species on the rhizosphere food web. Here, we compared the rhizosphere community of an exotic plant species, narrow-leaved ragwort (*Senecio inaequidens*), with the community surrounding the roots of a native relative, tansy ragwort (*Jacobaea vulgaris*). In this study, the primary decomposer community (bacteria and fungi), as well as the nematode grazing on these groups (bacterivorous and fungivorous nematodes), are taken into consideration. Rhizosphere samples were collected from three semi-natural grassland sites with high plant diversity in which both ragwort species were growing in close proximity. In total 96 rhizosphere samples were collected from two phenological stages of *S. inaequidens* and *J. vulgaris*. A set of newly developed quantitative PCR (qPCR) assays was used for a quantitative analysis of bacterial and fungal communities at phylum-level. In addition, changes among bacterivorous and fungivorous nematode taxa were monitored. To our surprise the effect of plant developmental stage was shown to be stronger than the native versus exotic plant species effect. This was not only true for the bacterial and fungal community, but also for the 15 most prominent nematode taxa present in these habitats. Correlation between changes in primary decomposer community and the nematode community grazing on it will be highlighted.

Keywords: Invasive plant species; molecular analysis; rhizosphere biodiversity; soil free-living nematodes; soil microbiota.

Vertical distribution of soil nematode communities in coastal Japanese pine forests

Yudai Kitagami¹, Natsumi Kanzaki² and Matsuda Yosuke¹

¹ Laboratory of Forest Mycology, Graduate School of Bioresources, Mie University, Tsu, Mie, 514-8507, Japan

² Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

corresponding author: 515M109@m.mie-u.ac.jp

Nematodes are supposed to be a key bio-indicator of food web, and thus their community structures have been characterised at various ecosystems, e.g., soil or water. In the forest ecosystems, the nematode species richness is very high, and many environmental factors can be involved for regulating its community structure. In this respect, we focused on coastal forests, which are harsh environmental conditions with poor nutritional distribution on the soil surface and with mono-dominance by pine trees. Thus, coastal ecosystems enable us to determine the assemblage pattern of soil nematodes as a model system to understand tree-nematode interactions. The aim of this study was to clarify the soil nematode community structure in coastal pine forests. We chose experimental sites at four coastal pine forests in the central part of Japan, which were 50-100 km apart from each other. In each site, we collected sandy soil samples from 0-20 cm depth, separating between 0-10 cm and 10-20 cm, within 1 ha plot. Water suspensions of 100 g fresh soil samples were decanted onto 75 and 25 μm mesh sieves and the soil remaining on the sieves was used to retrieving nematodes by the Baermann funnel technique. Fifty individuals were randomly chosen in each plot from both the 0-10 cm and 10-20 cm depths, examined under a stereomicroscope, lysed individually for PCR templates, and the partial 18S ribosomal RNA was sequenced for each individual. A total of 400 nematodes were successfully sequenced and were divided into 37 molecular operational taxonomic units (MOTU; 98% identity). *Acrobeloides* sp. 1 (20-25%), *Aporcelaimellus* sp. 1 (15-20%), and *Prismatolaimus* sp. 1 (12-22%) were dominant taxa at both soil depths. The number of MOTU as well as diversity index, i.e., the Shannon index and Chao dissimilarity values, were not significantly different between 0-10 cm and 10-20 cm depths, suggesting that the nematode community in coastal pine forests is rather stable down to 20 cm in depth.

Keywords: Diversity index; DNA sequencing; molecular operational taxonomic unit; *Pinus thunbergii*.

Effects of land-use intensity on nematode communities of tropical soils in Vietnam

Duong Thi Anh Nguyen^{1,2,3}, Reyes Peña-Santiago³ and Michael Bonkowski¹

¹ Institute for Zoology, Department of Terrestrial Ecology, University of Cologne Zùlpicher StraÙe 47 b, D-50674 Kùln, Germany

² Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam

³ Department of Animal Biology, Plant Biology and Ecology, University of Jaén, 23071 Jaén, Spain

corresponding author: nad2807@yahoo.com

Increasing land-use intensity has likely strong impacts on soil biodiversity and fertility in the tropics. Nematodes are known to respond rapidly to soil disturbance and changing resources. Therefore, the functional composition of the nematode community offers a reliable measure for the biological assessment of the quality and functioning of tropical soils. In Northern Vietnam we investigated the diversity of soil nematode communities of three spatially separated karst mountain systems. The island-like character of the karst mountain systems enables biogeography comparisons between regions. To investigate how local land management interferes with species richness, we took soil samples on each location along gradients of increasing land-use intensity: i) primary forest, ii) secondary forest, iii) slash and burn agriculture, and iv) intensive agriculture, to investigate changes in nematode community composition, soil carbon storage and nutrient cycling. Nematode community composition differed between regions, but land-use intensity had general and distinct impacts on the functional composition and food web structure of nematodes. Indicators of these changes, such as the Maturity Index, Channel Index or Plant-Parasitic Index will be correlated to and discussed in relation to changes in soil carbon and nutrient levels.

Keywords: Land-use intensity; nematode communities; tropical soil; Vietnam.

Statistical approach assessing the impact of cultural practices and environmental variables on the structure and functioning of the plant-parasitic nematode communities in various agrosystems in France

Nathan Garcia^{1,2}, Laurent Folcher¹ and Eric Grenier²

¹ ANSES, Plant Health Laboratory, Nematology Team, Domaine de la Motte au Vicomte BP 35327 35653 Le Rheu Cedex, France

² INRA, UMR 1349 IGEPP, Domaine de la Motte au Vicomte BP 35327 35653 Le Rheu Cedex, France

corresponding author: nathan.garcia@anses.fr

Nowadays, one of the major concerns in agriculture is to improve both quality and quantity of the production, while preserving the environment. In the context of a reduction of pesticide, finding alternative management practices to control pests constitute a key challenge for the future. Many studies have highlighted that it is important to consider not only interactions between a plant and a pest, but also interactions with and within pests communities. Plant-parasitic nematodes feed on plant tissues and can have very important economic impacts. Thus, it is important to know the diversity and structure of the local communities and to assess the impacts of the cultural practices (such as crop rotations, pesticides uses, tillage etc.) and soil physico-chemical proprieties on those communities. In order to achieve that, we developed a statistical approach associating descriptive and inferential analysis. The Multiple Correspondence Analysis allowed us to select only several contributive variables, among the cultural practices and environmental variables, describing the population level of each genus of plant-parasitic nematodes. Those variables were then put in General Linear Models to test the relations highlighted by the descriptive analysis. Thus, this statistical strategy allowed us to determine the impact of agricultural management practices on the structure and functioning of the nematode communities. Two years' samplings in an agricultural landscape where field crops are grown have resulted in the identification of eleven genera of plant-parasitic nematodes. To date, even though analysis needs to be pushed forward, we were able to highlight the important role of the soil management practices (for instance the presence of tillage) and use of various pesticides (excluding nematicides) on the abundance of the identified genera. We also showed that communities are not always conserved from one year to another suggesting a strong impact of the recent practices and/or climate on some nematode genera.

Keywords: Agroecology; community ecology; general linear model; multiple correspondence analysis.

Diversity and ecology of plant-parasitic nematode communities in olive in Spain

Juan E. Palomares-Rius, Blanca B. Landa, Juan A. Navas-Cortes, Miguel Montes-Borrego, Carolina Cantalapiedra-Navarrete, Antonio Archidona-Yuste and Pablo Castillo

Instituto de Agricultura Sostenible (IAS), Department of Plant Protection, Consejo Superior de Investigaciones Científicas (CSIC), Avda. Menéndez Pidal s/n, 14004 Córdoba, Spain

corresponding author: palomaresje@ias.csic.es

This work has studied the diversity of plant-parasitic nematodes infecting olive in a wide-region in southern Spain (Andalusia). Our data indicate that there is a high diversity of plant-parasitic nematodes (including root-knot = *Meloidogyne* spp., root-lesion = *Pratylenchus* spp., cyst-forming = *Heterodera mediterranea*, stubby-root = *Trichodorus* spp., reniform = *Rotylenchulus* sp., dagger and needle = *Longidorus* spp., *Xiphinema* spp., ring = *Criconemoides* spp., *Ogma* spp., and other migratory ectoparasitic nematodes viz. *Amplimerlinius* spp., *Helicotylenchus* spp., *Neodolichorhynchus* spp., *Paratrophurus* spp. *Paratylenchus* spp., *Rotylenchus* spp., *Trophurus* sp., *Tylenchorhynchus* spp.) associated with olive in Southern Spain that can exert different damage to olive roots depending on the olive variety and their abundance. The highest number of species was detected in *Xiphinema* and *Longidorus*, with description of eight and seven new species, respectively, apart from several new records for Spain. In addition, seven *Meloidogyne* species were detected in wild and cultivated olive, from which one is being described as a new species. Multivariate analyses allowed determining the agronomic and environmental factors that drive the structure and diversity of plant-parasitic nematodes infecting olive in Southern-Spain, that included: soil physicochemical factors (texture, pH, sand and clay, and extractable K), climatic variables (minimum and maximum temperatures) and the olive cultivar. Nematode abundance and diversity indexes (Richness, Shannon, Evenness and plant-parasitic index) were influenced by olive cultivar, and orchard and soil management practices. The plant-parasitic nematode abundance was significantly higher in orchards managed organically compared to those managed conventionally. These findings could be the result of the influence of the herbaceous plants that compose the soil cover, which may affect plant-parasitic nematodes at the species level.

Keywords: Diversity indexes; *Longidorus*; *Meloidogyne*; *Pratylenchus*; *Xiphinema*.

Complex of plant-parasitic nematodes in the rhizosphere of introduced trees of insular and mainland botanical gardens

Daryia S. Kalinkina, Elizaveta M. Matveeva and Anna A. Sushchuk

Institute of Biology of Karelian Research Centre Russian Academy of Sciences, 185910, Petrozavodsk, Russia

corresponding author: dania_22@mail.ru

Nematodes are sensitive to any changes that occur in the environment due to natural factors and anthropogenic impact. For example, the introduction of new plant species leads to restructuring of soil nematode communities and dispersal of dangerous plant parasite species. The aim of this research was to study plant-parasitic nematodes in the rhizosphere of introduced trees (coniferous - *Abies sibirica*, *A. balsamea*, *Pinus sibirica*; deciduous - *Tilia cordata*, *Quercus robur*) growing on the territory of the insular (Valaam island) and the mainland (Petrozavodsk) botanical gardens, located in one geographical zone. Results showed that general and plant-parasitic nematode abundance under the majority of trees had middle values for the investigated region. Taxonomic diversity was higher under the introduced trees on the island compared to the mainland (27-40 vs 25-34). Diversity of plant parasites (3-7) also increased in insular garden while 1-4 taxa were revealed in mainland one. The rhizosphere of coniferous trees was characterised by more diverse plant-parasitic nematodes as compared with deciduous trees. The most frequently occurred taxa were *Paratylenchus* and *Pratylenchus*; also observed were *Tylenchorynchus*, *Helicotylenchus*, *Paratrichodorus*, *Nagelus*, *Mesocriconema*. The last three taxa were found singularly (mainly in the insular botanical garden) and they are rare for the Republic of Karelia. Age structure of populations of most plant parasite species was characterised by the presence of all groups with a predominance of juveniles. This ratio indicates that these populations are in favourable habitat conditions. However, some sex groups were absent in *Nagelus*, *Gracilacus*, *Mesocriconema* populations. Thus, it was demonstrated that the botanical gardens are characterised by a high representation of plant-parasitic nematodes and the presence of regionally rare plant parasite taxa under the introduced trees. The study was supported by the RFBR (№ 15-04-07675).

Keywords: Abundance; age structure; anthropogenic impact; taxonomic diversity.

Genomics and transcriptomics in *Pristionchus*: A balanced view of conserved, fast-evolving and horizontally acquired genes

Ralf J. Sommer and Christian Rödelesperger

Max-Planck Institute for Developmental Biology, Tübingen Spemannstrasse 37, 72076 Tuebingen, Germany

corresponding author: ralf.sommer@tuebingen.mpg.de

Genomics has revolutionised biology and together with functional tests, such as gene knockout by CRISPR/Cas9, allows novel insight into non-model organisms including nematodes. One unexpected finding in genomics was that essentially all organisms harbour a large number of genes that are not conserved over larger evolutionary distances. Three mechanisms can be responsible for the evolution of taxonomically restricted genes. First, genes might evolve rapidly, for example, under the influence of positive selection; second, genes might also evolve *de novo*. Indeed, recent studies provide evidence for both mechanisms. Finally, genome-sequencing projects revealed widespread horizontal gene transfer between eubacteria and eukaryotes. However, little is known about the function of taxonomically restricted and horizontally acquired genes. I will provide insight into genomics and transcriptomics of *Pristionchus pacificus*, a species that we have established as model system in evolutionary biology to integrate development with ecology and population genetics. *P. pacificus* lives in association with scarab beetles. One key feature of its life style is a mouth dimorphism that enables predatory feeding. The development of teeth-like denticles of two discrete forms represents an example of developmental plasticity and we test if plasticity is a facilitator of phenotypic diversification. Using forward and reverse genetics and genome-wide association studies in *P. pacificus*, we made the surprising finding that taxonomically restricted genes seem to be functionally overrepresented in regulating plasticity and I will provide case studies to highlight this finding. Similarly, we found that a horizontally acquired gene was developmentally integrated into *P. pacificus* by co-option of genetic regulators of plasticity. I argue that a full understanding of biological processes in any organism requires a balanced view of conserved, taxonomically restricted and horizontally acquired genes.

Keywords: Developmental plasticity; genomics; horizontal gene transfer; *Pristionchus*; taxonomically-restricted genes.

Polyploid hybrid genomes of devastating plant pests allow plasticity without sex and meiosis

Romain Blanc-Matthieu¹, Laetitia Perfus-Barbeoch¹, Jean-Marc Aury², Martine Da Rocha¹, Jérôme Gouzy³, Erika Sallet³, Cristina Martin-Jimenez¹, Philippe Castagnone-Sereno¹, Jean-François Flot⁴, Djampa Kozłowski¹, Julie Cazareth⁵, Arnaud Couloux², Corinne Da Silva², Julie Guy², Corinne Rancurel¹, Thomas Schiex⁶, Pierre Abad¹, Patrick Wincker² and Etienne Danchin¹

¹ INRA, Université Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

² Commissariat à l'Energie Atomique (CEA), Institut de Génomique (IG), Genoscope, Evry, BP5706, 91057, France

³ INRA, CNRS, UMR 441-2594, Laboratoire des Interactions Plantes-Microorganismes (LIPM), Castanet-Tolosan, F-31326, France

⁴ Université Libre de Bruxelles (ULB), Evolutionary Biology & Ecology, C.P. 160/12, Avenue F.D. Roosevelt 50, 1050 Brussels, Belgium

⁵ CNRS, Université Nice Sophia Antipolis, Institute of Molecular and Cellular Pharmacology, 06560 Sophia Antipolis, France

⁶ INRA, Unité de Mathématiques et Informatique UR 875, F-31320 Castanet Tolosan, France

corresponding author: etienne.danchin@sophia.inra.fr

Root-knot nematodes (genus *Meloidogyne*) show an intriguing diversity of reproductive modes ranging from obligatory sexual to fully asexual reproduction. Intriguingly, the most damaging species to world agriculture are those that reproduce without meiosis and without sex. To understand the paradox of this parasitic success despite the absence of sex and genetic exchanges, we have sequenced and assembled the genomes of three obligatory ameiotic asexual *Meloidogyne* species and have compared them to those of relatives with facultative or obligatory meiotic asexual reproduction. Our comparative analysis shows that the genomes of obligatory asexual root-knot nematodes are made of duplicated regions with a high within-species average nucleotide divergence of 8%. Furthermore, they show a higher abundance of transposable elements (TE) compared to their facultative sexual relatives. Phylogenomic analysis of the genes present in these duplicated regions suggests that they originated from multiple hybridisation events. The average nucleotide divergence in the coding portions between duplicated regions is ~5-6% and we detected diversifying selection between the corresponding homeologous gene copies. Genes under diversifying selection covered a wide spectrum of predicted functional categories, which suggests a high impact of the genome structure at the functional level. Contrasting with high within-species nuclear genome divergence, mitochondrial genome divergence between the three ameiotic asexuals was very low, suggesting that these putative hybrids share a recent common maternal donor lineage. The intriguing parasitic success of mitotic root-knot nematodes in the absence of sex may be partly explained by their TE-rich composite genomes resulting from multiple allo-polyploidisation events and promoting plasticity in the absence of sex.

Keywords: Comparative-genomics; parthenogenesis; positive selection.

Identification of new putative effectors by investigating *Heterodera schachtii* transcriptome

Abdelnaser M. Elashry¹, Samer S. Habash¹, Nahal Ahmadinejad², Heiko Schoof² and Florian M.W.Grundler¹

¹INRES Molecular Phytomedicine, University of Bonn, Karlrobert-Kreiten-Str.13, Bonn 53115, Germany

²INRES Crop Bioinformatics, University Bonn, Katzenburgweg 2, 53115 Bonn, Germany,

corresponding author: nasserelashry@hotmail.com

Beet cyst nematodes (*Heterodera schachtii*) depend on a set of secretory proteins (effectors) for the induction and maintenance of their syncytial feeding sites. In order to understand the relationship between *H. schachtii* and its host, the identification of *H. schachtii* effectors is a crucial step. Aiming to identify *H. schachtii* putative effectors, we sequenced its transcriptome using next generation sequencing. Analysing the resulted sequences led us to identify a subset of sequences representing the putative secretory proteins. Comparing of the identified subset with oesophageal gland-related cyst- and root-knot nematodes resulted in identifying a subset of oesophageal gland related sequences and common putative effectors across the tested species. Structural and functional annotation led to identify nearly 200 putative effectors in *H. schachtii*. In order to validate the resulted putative effectors, we analysed their expression level (by qPCR) and localisation (by *in situ* hybridisation). Sequences that have shown upregulation in post-infective stages and specific localisation in oesophageal gland were further analysed. Especially, putative effectors that had their functions related to reactive oxidative species, carbohydrate binding, or metabolic processes. We used RNAi to silence our genes of interest and analysed the effect of knocking them down on the level of infection. Interestingly, all genes that were tested have shown a significant effect on parasitism level in one or more of the tested parameters. We expect that our investigation can help understanding more about the processes of cyst nematode parasitism.

Keywords: Cyst nematodes; next generation sequencing; oesophageal glands; RNAi; transcriptome.

***Meloidogyne* comparative genomics: Comparison of 22 genomes reveals origins, species structure, diversity and ploidy of globally important crop pests**

Amir Szitenberg¹, Laura Salazar², Vivian Blok³, Soumi Joseph⁴, Dominik Laetsch^{2,3}, Valerie Williamson⁵, Mark L. Blaxter² and David H. Lunt¹

¹ Evolutionary Genomics Group, University of Hull, UK

² Institute of Evolutionary Biology, School of Biological Sciences, The University of Edinburgh, UK

³ The James Hutton Institute, Dundee, UK

⁴ Entomology and Nematology Department, University of Florida, USA

⁵ Department of Entomology and Nematology, UC Davis, USA

corresponding author: dave.lunt@gmail.com

Root-knot nematodes (RKN) are amongst the world's most important crop pests, infecting all the major agricultural crops, and causing very significant reduction in yields. The tropical RKN of the *Meloidogyne incognita* group are particularly serious pests, with global distributions covering areas on all continents with mild winter temperatures, and also pose a threat to current temperate regions as ranges expand with climate change. We have sequenced the genomes of 21 RKN isolates from six species and released these data as a community resource. We describe comparative genomic analyses of these 21 genomes and the previously published *M. incognita* to clarify the origins and speciation pattern in this important group. We discuss in the context of a resolved species phylogeny the remarkable genome structure found, genomic diversity, and ploidy. The intraspecific genomic diversity of globally-distributed isolates of *M. incognita*, *M. arenaria* and *M. javanica* are interpreted in terms of their origins and spread with modern agriculture. Lastly we discuss the exciting prospects of comparative *Meloidogyne* genomics for understanding both the complex biology of RKN and also their pathogenicity and polyphagy.

Keywords: Asexuality; genomics; hybridisation; phylogenomics; root-knot nematodes.

The transcriptome of *Meloidogyne indica*: a root-knot nematode that infects neemManisha Sajnani, Prakash Banakar, Vishal Somvanshi and Uma Rao

Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

corresponding author: umarao@iari.res.in

Meloidogyne indica is commonly known as the citrus root-knot nematode and is confined to the Indian subcontinent. Unlike other root-knot nematodes, *M. indica* possess an extraordinary ability to infect woody perennials. In a recent survey in India, it has been found to infect citrus, Bt-cotton and, most surprisingly, the neem (*Azadirachta indica*) trees. Neem and neem products are well known to possess anti-nematode metabolites (azadirachtin, nimbin, nimbidin, salanine etc.) and are traditionally used for the management of nematodes and other plant pests/ pathogens. A transcriptome sequencing of mixed stages (eggs, second-stage juveniles and feeding female) of *M. indica* was carried out using Illumina (platform) technology. The sequencing resulted in 6.42 GB sequence of 76 read length, generating 71.3X coverage. *De-novo* assembly was performed by rnaSPAdes and velvet on 28,921,889 high quality reads with 55 k-mer length, which resulted in 33,310 and 69,094 contigs, respectively. After scaffolding and duplicate removal, 23,622 transcripts were obtained at N50 size of 1,575 bases. The preliminary analysis of the transcriptome shows that ca 69% of *M. indica* transcripts could be annotated by comparing the *M. incognita* sequences against non-redundant protein sequences available in GenBank using blastx. The blast search identified genes known to be important for plant-parasitism, such as FMRFamide-like peptides (0.13%), neuropeptide-like proteins (1.32%) and secretory peptides. A search with the CAZymes database for enzymes involved in carbohydrate metabolism resulted in 1683 hits. Several homologues of *C. elegans* gene families important for nematode development, viz., *unc* (4.47%) and *daf* (1.35%) and various RNAi pathway genes were found. Further analysis of the transcriptome is currently underway to identify the genes that enable this nematode to parasitise neem and other woody perennials.

Keywords: Bt-cotton; *Meloidogyne indica*; neem; transcriptome; woody perennials.

The genome of the yellow potato cyst nematode, *Globodera rostochiensis*, reveals insights into the bases and regulation of parasitism and virulence.

Sebastian Eves-van den Akker¹, Dominik R. Laetsch², Peter Thorpe³, Catherine J. Lilley⁴, Etienne G.J. Danchin⁵, Martine Da Rocha⁵, Corinne Rancurel⁵, Nancy E Holroyd⁶, James A Cotton⁶, Amir Szitenberg⁷, Eric Grenier⁸, Josselin Montarry⁸, Benjamin Mimee⁹, Marc-Olivier Duceppe⁹, Ian Boyes¹⁰, Jessica M.C. Marvin⁴, Laura M. Jones⁴, Hazijah B. Yusup⁴, Joël Lafond-Lapalme⁹, Magali Esquibet⁸, Michael Sabeh⁹, Michael Rott¹⁰, Hein Overmars¹¹, Anna Finkers-Tomczak¹¹, Geert Smant¹¹, Georgios Koutsovoulos², Vivian Blok³, Sophie Mantelin³, Peter J.A. Cock¹², Wendy Phillips¹³, Bernard Henrissat^{14,15}, Peter E. Urwin⁴, Mark Blaxter² and John T. Jones^{3, 16}

¹ Division of Plant Sciences, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

² Institute of Evolutionary Biology, University of Edinburgh, EH9 3FL, UK

³ Cell and Molecular Sciences Group, Dundee Effector Consortium, James Hutton Institute, Dundee, DD2 5DA, UK

⁴ Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK

⁵ INRA, Université Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

⁶ Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK

⁷ School of Biological, Biomedical and Environmental Sciences, University of Hull, Hull, HU6 7RX, UK

⁸ INRA, UMR1349 IGEPP (Institute for Genetics, Environment and Plant Protection), F-35653 Le Rheu, France

⁹ Agriculture and Agri-Food Canada, Horticulture Research and Development Centre, 430 Boul. Gouin, St-Jean-sur-Richelieu, Quebec, J3B 3E6, Canada

¹⁰ Sidney Laboratory, Canadian Food Inspection Agency (CFIA), 8801 East Saanich Rd, Sidney, BC V8L 1H3, Canada

¹¹ Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

¹² Information and Computational Sciences Group, James Hutton Institute, Dundee, UK

¹³ USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon, USA

¹⁴ CNRS UMR 7257, INRA, USC 1408, Aix-Marseille University, AFMB, 13288 Marseille, France

¹⁵ Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

¹⁶ School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9TZ, UK

corresponding author: s.evesvandenakker@dundee.ac.uk

A community-wide consortium was launched in 2014 to analyse the genome of *Globodera rostochiensis*. This devastating plant-pathogen of global economic importance is classified into

pathotypes of different plant resistance-breaking phenotypes. *G. rostochiensis* secretes effectors from pharyngeal glands into the host to manipulate host processes and promote parasitism: some effectors were acquired by horizontal gene transfer. We have generated a draft genome assembly for *G. rostochiensis* to identify putative effectors and HGT events, to map gene expression through the life cycle focusing on key parasitic transitions, and to explore the genetic variation underlying eight populations including four additional plant resistance-breaking pathotypes. Horizontal gene transfer contributed 3.5% of the predicted genes, ~8.5% of which are deployed as effectors. We identified a putative regulatory motif consistent with the highly tissue-specific expression pattern of effectors. The six base pair DORSAL Gland motif (DOG box) is present in the promoter region of representatives from 26 of the 28 dorsal-gland effector families. Using the DOG box, we predicted a superset of putative effectors associated with this motif, validate gland cell expression for two novel genes by *in situ* hybridisation, and catalogue DOG effectors from available cyst nematode genomes. Comparison of effector diversity between pathotypes highlights polymorphisms that correlate with plant resistance-breaking pathotypes. These *G. rostochiensis* genome resources will facilitate major advances in understanding nematode plant-parasitism. DOG effectors are at the front line of the evolutionary arms race between plant and parasite, and the ability to predict gland cell expression a priori promises rapid advances in understanding their roles and mechanisms of action. The *G. rostochiensis* consortium has rapidly established a model to study pathogenicity and virulence in plant-parasitic nematodes.

Keywords: Effector regulatory motif; effectors; genome sequence; plant-parasitic nematode; virulence.

The rice root-knot nematode *Meloidogyne graminicola* through the genomics looking glass

Vishal Somvanshi¹, Praseon Thakur¹, Sandeep Kaushik², Pawan Jayaswal³, Manisha Sajjani¹, Prakash Banakar¹ and Uma Rao¹

¹ Division of Nematology, ICAR-Indian Agricultural Research Institute, PUSA Campus, New Delhi 110012, India

² Genome Institute of Singapore, 60 Biopolis St, #02-01, Singapore-138672, Republic of Singapore

³ Genoinformatics Lab, ICAR-National Research Centre on Plant Biotechnology LBS Building, PUSA Campus, New Delhi 110012, India

corresponding author: vssomvanshi@iari.res.in

Rice is the most important staple food crop in the world and about 53% of the world's rice is grown under irrigated conditions. Reduced water availability is forcing rice farmers to cultivate rice under aerobic conditions. Under the 'reduced water' situation, the root-knot nematode, *Meloidogyne graminicola*, has emerged as a devastating pest of rice in Southeast Asia where the majority of the world's rice is grown and consumed. However, the options for the management of this nematode are rather limited. To enable basic and applied research on this important agricultural pest, we sequenced the genome of *M. graminicola* to obtain a high-quality reference sequence along with the transcriptome of two populations of the species. We achieved 88.93 % genome completeness, and predicted 14,823 protein-coding genes, of which ~10,494 genes could be annotated. Almost 79% of the annotated genes showed similarities to animal-parasitic nematodes, followed by free-living nematodes. The *M. graminicola* genome revealed 39.79% repetitive elements, the majority of which could not be associated with known transposable element (TE) families. The fraction of repetitive sequences in the *M. graminicola* genome is comparatively higher than *M. incognita* (36%), *M. hapla* (15%), *Heterodera avenae* (23%) and *Caenorhabditis elegans* (14%). Search for orthologues of *C. elegans* RNAi lethal phenotypes revealed 203 nematode specific genes which can be utilised as targets for its management. Six hundred and sixty-four predicted secreted proteins were identified. The *M. graminicola* genome showed the presence of 123 CAZyme families that might be involved in plant parasitism, including GT64 family of glycosyltransferases, which is absent in *M. incognita*, *M. hapla* and *M. floridensis*. The *M. graminicola* genome data is being made available through the HATDB (<http://insilico.iari.res.in/>).

Keywords: *Meloidogyne graminicola*; genome; rice; rice root-knot nematode; transcriptome.

AnisakisDB: A database hosting the transcriptomes of the *Anisakis simplex* s.s., *A. pegreffii* and their hybrid haplotype parasitic complex

Carlos Llorens¹, Susana C. Arcos², Lee Robertson², Ricardo Ramos³, Ricardo Futami¹, Sergio Ciordia⁴, Mercedes Careche⁵, Micol González-Muñoz⁶, Yolanda Jiménez-Ruiz², Juan Pablo Albar⁴, Mark Blaxter⁷ and Alfonso Navas²

¹ Biotechvana, Valencia, Edificio CEEI, Avd Benjamin Franklin 12, Parque Tecnológico Paterna 46980, Spain

² Museo Nacional de Ciencias Naturales. C/José Gutiérrez Abascal 2, Madrid 28006, Spain

³ Unidad de Genómica, Fundación Parque Científico de Madrid, C/ Faraday, 7 planta baja, Campus de Cantoblanco, Madrid 28049, Spain

⁴ Centro Nacional de Biotecnología, CSIC, C/ Darwin 3, Campus de Cantoblanco, Madrid 28049, Spain

⁵ Instituto de Ciencia y Tecnología de Alimentos y Nutrición (CSIC), C/José Antonio Novais 10, E-28040 Madrid, Spain

⁶ Servicio de Inmunología, Hospital Universitario La Paz, Paseo de La Castellana, 261, 28046-Madrid

⁷ The Institute of Evolutionary Biology, The School of Biological Sciences, The King's Buildings, Edinburgh EH9 3JT, UK

corresponding author: anavas@mncn.csic.es

Anisakis is a genus of parasitic nematodes known to be etiological agents of Anisakiasis a human gastrointestinal infection caused by consumption of raw or undercooked fish containing larvae. With the aim to support research in the field we have created an online database devoted to *Anisakis*. This release is based on the transcriptomes of the *Anisakis* parasitic complex constituted by three taxa: *A. simplex* s.s., *A. pegreffii* and their hybrid haplotype taxon. We have sequenced and assembled (*de novo*) the three transcriptomes, which respectively consist of 36645 (*A. simplex* s.s.), 31988 (*A. pegreffii*) and 29656 (hybrid) unigenes represented by 121907, 91541 and 76848 transcripts or isoforms. Transcriptomes were annotated using several classificatory databases and systems and used to reconstruct a consensus transcriptome useful as reference in further RNAseq studies. The database has been created to host the three assembled transcriptomes and their annotations and their consensus transcriptome. This database, we call AnisakisDB, can be accessed via a friendly-to-use interface giving access to distinct sections and search engines to retrieve sequences, motifs and annotations according to different filters and criteria. Aspiring to bring some light on their evolutionary origins of the hybrid haplotype taxon, the functional and/or expression profiles of the three transcriptomes have also been compared. Aiming to provide an example of the database utility, we also include the results of a blast search to our database using as queries, most of the distinct food allergens and their possibly phylogeny by using step matrices approach. Finally, comparison of expressed proteomes by iTRAQ analysis of the three taxa resolved a set of 20 proteins which were unambiguous and significantly differentially regulated and their metabolic pathways are explored considering the transcriptomics data. Public access to AnisakisDB is at www.anisakis.mncn.csic.es.

Keywords: *Anisakis simplex* complex; food allergens; proteomes; transcriptomes description

Effectors of root-lesion nematodes

Michael G K Jones, Jo-Anne Tan, Sameer Khot and John Fosú-Nyarko

Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, Perth, WA 6150, Australia

corresponding author: m.jones@murdoch.edu.au

Root lesion nematodes (RLN; *Pratylenchus* species) are the major nematode pests of dryland rain-fed wheat production in Australia and similar environments elsewhere. To understand the host-parasite interactions better and to identify new gene targets for their control we have sequenced the transcriptomes of *P. thornei* and *P. zaeae* and genomic sequence data for *P. coffeae* has also been published. Using comparative and functional genomics and available data from other plant-parasitic nematodes, a picture is emerging of which effectors are common between migratory and sedentary plant endoparasites, and which are not. There are many genes encoding enzymes that appear to be involved in migration through plant tissues common to migratory and sedentary endoparasites, and the migratory nematodes also appear to be able to suppress or evade host defences. By contrast, the effectors involved in the formation of giant cells or syncytia, so far identified in root knot and cyst nematodes, appear to be absent from RLN. Genes encoding wall modifying enzymes of *Pratylenchus* spp. include pectate lyases, polyglacturonases, xylanase, cellulases, cellulose binding proteins and annexins. Similarly, modulation and protection from host defences appears to involve genes encoding thioredoxin, peroxiredoxin, superoxide dismutase, glutathione-S-transferase, SPRY domain-like proteins, Sec-2/FAR and venom-allergen like proteins. Some putative effectors that target regulation of signalling pathways are also common, as are some proteases thought to be involved in feeding. By contrast, genes required for initiation and maintenance of feeding cells appear to be absent. In general, these data support the view that those effectors present in sedentary endoparasites but not migratory endoparasites, are involved in feeding site formation and function. They also suggest that the majority of wall modifying enzymes, particularly expansins, are required for nematode migration through plant tissues.

Keywords: Effectors; gene silencing; *Pratylenchus*; RNA interference; transcriptome.

Analysing patterns of gene family evolution within the phylum Nematoda

Dominik R. Laetsch^{1,2,3}, Vivian C. Blok², Peter J. Cock³ and Mark L. Blaxter¹

¹ University of Edinburgh, Institute of Evolutionary Biology, Edinburgh, UK

² The James Hutton Institute, Cell and Molecular Sciences Group, Dundee, UK

³ The James Hutton Institute, Information and Computational Sciences Group, Dundee, UK

corresponding author: dominik.laetsch@gmail.com

The field of nematode genomics has thrived in recent years due coordinated sequencing efforts, such as the 50 Helminth Project (coordinated by the Wellcome Trust Sanger Institute) and the *Caenorhabditis* Genome Project, as well as contributions of individual laboratories. Here, we present the results of a clustering analysis of >2.6 million proteins derived from 112 nematode next generation sequencing datasets, and 9 outgroup species. The putative gene families obtained through this analysis can now serve as a resource for the study of the phylum Nematoda, such as the identification of protein clusters unique to a species or group of species, which can then be associated with features of the species' biology. We will present an estimate of the protein sequence diversity of Nematoda and demonstrate the value of this resource based on the analyses of single gene families, such as the nematode-specific "Worm ARGonauts" (WAGOs). In addition, we will show results regarding patterns of gene family evolution within genomes of plant-parasitic nematodes of the suborder Tylenchina (Aphelenchoidea and Heteroderidae).

Keywords: Evolution; gene families; genomics; phylogeny.

Imported plants as a possible pathway for introducing harmful nematodes into the EU region; a survey in The Netherlands

Anne Sophie van Bruggen, Gerrit Karssen and Loes J.M.F. den Nijs

National Plant Protection Organization, Geertjesweg 15, 6706 EA Wageningen, The Netherlands

corresponding author: a.s.vanbruggen@nvwa.nl

In The Netherlands high numbers of plants are imported and distributed through the EU. This is a potential pathway for introducing harmful organisms. The standard procedure in The Netherlands is that consignments are rejected when quarantine organisms are found. To assess the possible risk of other nematode species a Risk List of potential harmful plant-parasitic nematodes was prepared based on a literature review. On the Risk List are harmful nematodes that are not present in the EU and that are not already on the EU and EPPO lists of regulated pests. A survey on adhering soil from imported plants was conducted in 2010-2013 to study the presence of risk list nematodes. In this survey 258 soil samples were analysed. These samples came from 20 countries and from 54 different plant species. In 91% of the samples plant-parasitic nematodes were found and 17% of the samples were infested with species from the Risk List or the EU quarantine list. Part of the findings in the soil concerned endoparasitic nematodes. Therefore, following on this survey, in 2015 and 2016 a survey on roots from imported plants was carried out. In 2015 110 root samples were collected originating from 14 different countries and from 49 ornamental crops. In 52% of the samples plant-parasitic nematodes were found and 16% of the samples were infested with Risk List or quarantine nematodes. In 2016 this survey is still in progress. In conclusion, adhering soil is clearly a pathway for harmful nematodes. Our findings confirm the opinion of the EU and EFSA. New legislation for adhering soil is prepared in the EU. However, also in samples of plants without soil many findings were done. This indicates that plants traded without soil, but with roots have not been produced in pest free soil. Proposed legislation on the production place is intended to block this pathway.

Keywords: Legislation; quarantine; risk list.

Pest risk analysis as applied to nematodes: the example of *Meloidogyne mali*

Thomas Prior¹, Giovanna Curto², Emre Evlice³, Jose Maria Guitian Castrillon⁴, Gerrit Karssen⁵, Loes den Nijs⁵, Christer Magnusson⁶, Wim Wesemael⁷ and Fabienne Grousset⁸

¹ Fera, National Agri-Food Innovation Campus, Sand Hutton, York, UK

² Plant Protection Service - Servizio Fitosanitario Regione Emilia-Romagna, Bologna, Italy

³ Ministry of Food Agriculture and Livestock, Ankara Plant Protection Central Research Institute, Yenimahalle-Ankara, Turkey

⁴ Tecnologias y Servicios Agrarios, S. A. - TRAGSATEC, C/Julian Camarillo, Madrid, Spain

⁵ National Plant Protection Organization, P.O. Box 9102, 6700 HC Wageningen, The Netherlands

⁶ Norwegian Institute of Bioeconomy Research, Hogskoleveien 7, Norway

⁷ Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, Merelbeke, Belgium

⁸ European and Mediterranean Plant Protection Organization, 21 boulevard Richard Lenoir, 75011 Paris, France

corresponding author: thomas.prior@fera.co.uk

The European and Mediterranean Plant Protection Organization (EPPO) is a regional standard-setting organisation created in 1951. One of EPPO's main priorities is to prevent the introduction of dangerous pests from other parts of the world, and to limit their spread within the region should they be introduced. Measures adopted by countries to protect their territories from these introductions should be technically justified and based on International Standard. Since the 1990s, EPPO has been involved in developing schemes for PRA and the scheme currently mostly in use is PM 5/5 Decision-Support Scheme for an Express Pest Risk Analysis (available on the EPPO website www.eppo.org). A system has also been established to provide early warning on emerging pests and to perform PRA at the EPPO level. Expert Working Groups are convened to conduct PRAs on specific pests. This system is presented with the example of a recent PRA prepared for *Meloidogyne mali*, a polyphagous root-knot nematode described from Japan. *M. mali* has been introduced into the EPPO region in The Netherlands and Italy. It has been mostly reported in relation to *Malus* spp. and *Morus* spp. in Japan, and *Ulmus* spp. in The Netherlands and Italy, but it has a much wider host range of trees and shrubs. Uprooted trees in The Netherlands led to concerns on the potential impact of *M. mali* in the EPPO region. An EPPO Expert Working group conducted a pest risk analysis on *M. mali* in May 2016. The main elements of the biology of *M. mali* and the outcomes of the PRA will be presented.

Keywords: Quarantine; risk; root-knot nematode.

First report of *Meloidogyne luci* in Portugal - a potential threat to the potato crop

Carla Maleita¹, Ivânia Esteves², Joana M.S. Cardoso², Maria José Cunha³, Regina M.D.G. Carneiro⁴ and Isabel Abrantes²

¹ CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

² CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

³ CERNAS, Department of Agronomic Sciences, High School of Agriculture, Bencanta, 3045-601 Coimbra, Portugal

⁴ EMBRAPA – Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-979 Brasília, DF, Brazil

corresponding author: carlammaleita@hotmail.com

Meloidogyne luci, a tropical root-knot nematode (RKN) species, was described in 2014 from isolates obtained from lavender (*Lavandula spica*) infected roots collected in Brazil. Isolates with similar esterase phenotype (EST) have been recorded since 1985 in South America (Argentina, Bolivia, Brazil, Chile, Ecuador, and Guatemala), Iran and Europe (Turkey and Slovenia). In 2013, during a field survey conducted in Portugal on potato (*Solanum tuberosum*), an unusual EST was detected in one RKN isolate obtained from potato roots collected in Coimbra. This isolate was maintained on tomato and its morphological, biochemical and molecular characteristics were studied. Perineal pattern morphology was highly variable, similar to *M. ethiopica* and not useful for identification. The EST obtained from young egg-laying females protein extracts revealed three bands correspondent to the *M. luci* L3 phenotype. Concerning the molecular analysis, a cytochrome oxidase subunit II region from mitochondrial DNA (mtCOII) was amplified by PCR, cloned and sequenced. Phylogenetic analysis obtained from the multiple sequence alignment of Portuguese isolate mtCOII sequences and those of *M. ethiopica*, *M. arabicida*, *M. hispanica*, *M. incognita*, *M. izalcoensis*, *M. javanica* and *M. luci* isolates revealed that the Portuguese isolate grouped with *M. luci* isolates, close to *M. ethiopica*, confirming its identification as a *M. luci* isolate. The host-*M. luci* relationships were also assessed in 16 commercial potato cultivars. All potato cultivars were classified as susceptible to *M. luci* with reproduction factor values varying from 12.5 to 63.6 and gall index of 5. *Meloidogyne luci* constitutes a potential threat to farmers and potato production. Therefore, a survey for evaluation of *M. luci* distribution is desirable to decrease the risk of spread. *Meloidogyne luci* is reported for the first time in Portugal and parasitising potato.

Keywords: Esterase phenotype; mitochondrial DNA region; pathogenicity; perineal pattern; root-knot nematodes.

Soil sampling and testing for detecting and estimating populations of potato cyst nematodes

Jon Pickup¹, Kim E.F. Davie¹ and Adrian M. I. Roberts²

¹ Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh EH12 9FJ, UK

² Biomathematics and Statistics Scotland (BioSS), JCMB, The King's Buildings, Peter Guthrie Tait Road, Edinburgh EH9 3FD, UK

corresponding author: jon.pickup@sasa.gsi.gov.uk

Potato cyst nematodes *Globodera pallida* and *G. rostochiensis* are among the most economically damaging pests of potato production and are considered by British growers to present the greatest pest and disease threat to sustainable commercial potato production. With the continued use of the remaining active ingredients for the chemical control of nematodes under greater scrutiny, it is increasingly important to ensure that nematicide treatments are used responsibly. Currently, a wide variety of sampling and laboratory testing methodologies are used in Britain without any consistent rationale presented as to whether the method chosen is most appropriate for the intended purpose. We reviewed the available mathematical models for describing the spatial distribution of PCN in a field. We examined whether these different mathematical models adequately described a suite of known PCN distributions from British fields. Selected models were used to investigate different approaches to soil sampling. The likelihood of detection of cysts was estimated under different intensities of infection, along with the precision of the estimate of population density, varying the number and size of soil cores, pattern of sampling and the amount of soil tested in the laboratory (subsampling). Where it is uncertain if a field is infested or not, priority should be given to detection. In this case testing as large a soil sample as is practical is recommended; increasing the number of cores above a minimum of 50 cores ha⁻¹ is of minimal advantage and sampling using a grid pattern is more effective than using a 'W pattern'. If a field is believed to be infested, growers are generally more concerned with accurately estimating the level of the PCN population. In this case the amount of soil tested is not as important and subsampling by the laboratory can save costs without unduly impacting upon accuracy. However, increasing the number of sampling points across the field will improve accuracy.

Keywords: Detection; field distributions; population estimation.

An alternative way of soil sampling for detection of field infestations with *Globodera* spp.

Nicole Viaene¹, Anne-Marie Deeren¹, Marc Goeminne², Pascal Dupont², Kürt Demeulemeester³, Déborah Lanterbecq⁴ and Michel De Proft⁵

¹ ILVO (Institute for Agricultural and Fisheries Research), Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

² PCA (Centre for Applied Research and Extension on Potato), Karreweg 6, B-9770 Kruishoutem, Belgium

³ Inagro, Department Arable Crops, Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium

⁴ CARAH (Research Centre for Agronomy and Agro-industry, HEPH-Condorcet), 11 rue Paul Pastur, Ath, Belgium

⁵ CRA-W (Centre Wallon de Recherches Agronomiques), 9 Rue de Liroux, 5030 Gembloux, Belgium

corresponding author: nicole.viaene@ilvo.vlaanderen.be

Field sampling is the recommended method to detect cyst nematodes in a field. The EU directive on potato cyst nematodes (*Globodera* spp.) prescribes a 1500 ml soil sample taken per hectare, consisting of at least 100 soil cores, prior to potato seed production. More intensive field sampling methods can be applied to increase the detection probability of cysts. With the AMI100 method, for example, a total of 6600 ml soil ha⁻¹ (11 samples of 600 ml taken from 40 soil cores ha⁻¹) is extracted to find out if cysts are present in the field. In a previous study we found that soil tare, i.e., soil that is taken from the field together with potato tubers at harvest is an important way of passive spread of nematodes, particularly cysts. Putting this knowledge together, we wondered if sampling soil tare could be an alternative for the rather intensive field sampling. For 11 fields, with varying levels of *Globodera* infestation, we compared the detection of cysts when using the field sampling methods (EU and AMI100) and when sampling 1500 ml of soil tare at harvest. The detection of cysts was as good using the soil tare method as performing field sampling, especially after growing a susceptible potato variety. In addition, more than 700 samples consisting of soil tare or soil adhering to potato tubers were checked for cysts. Soil still adhering tubers was brushed off from 200 tubers to collect a sample; this adhering soil did not result in higher detection levels than 1500 ml soil tare. In cases where only a few cysts were found in soil at harvest, it was not always possible to detect cysts in soil samples of the corresponding field. This observation suggests that the alternative method is as sensitive, or even better at detecting cysts than field sampling. Provided that soil tare samples are very well linked to the corresponding field, this new method can be a fast and easy alternative to field sampling for farmers who want to assess the infestation status of their fields.

Keywords: Cyst; potato cyst nematode; soil tare; waste soil.

Gibberellin mediates rice susceptibility to *Meloidogyne graminicola*

Henok Zemene Yimer^{1,2}, Kamrun Nahar¹, Tina Kyndt¹, Monica Hofte² and Godelieve Gheysen¹

¹ Department of Molecular Biotechnology Coupure, Links 653, 9000 Ghent, Belgium

² Department of Crop Protection Coupure, Links 653, 9000 Ghent, Belgium

corresponding author: HenokZemene.Yimer@UGent.be

Rice is the most important staple food grain and the second most widely produced cereal crop worldwide. The rice root-knot nematode, *Meloidogyne graminicola*, is an obligate endoparasitic plant pathogen and a major problem in many rice growing areas. The widespread adoption of anaerobic rice cultivation is favouring the rapid population buildup of *M. graminicola*. The plant hormones salicylic acid, jasmonic acid and ethylene are known to elicit and mediate an array of plant defence responses against invading pathogens. Other plant hormones such as gibberellins are known to regulate plant growth and development. Compared to the wealth of accumulated knowledge on the regulatory role of gibberellins in plant growth and development, relatively little is known about its role in plant immunity, and more specifically against nematode infection in rice. The aim of this study was to decipher the role of gibberellin and its signalling components in rice upon infection with the root-knot nematode *M. graminicola*. The role of gibberellin biosynthesis and signaling components was examined by using several gibberellin biosynthesis and signalling mutant lines of rice, and treating plants with hormone and biosynthesis inhibitors upon compatible interactions between rice and *M. graminicola*. Our study reveals that exogenous treatment of rice plants with gibberellin results in increased susceptibility to *M. graminicola*. By contrast, depletion of endogenous gibberellin levels in rice plants using the gibberellin biosynthesis inhibitor treatment or the use of gibberellin deficient rice lines resulted in an increased resistance to *M. graminicola*. In addition to gibberellin biosynthesis, our research with gibberellin-signalling mutants demonstrates that gibberellin signalling also plays a role in disease resistance against *M. graminicola* in rice. Our study further tries to unravel the mechanism behind gibberellin-dependent susceptibility of rice plants to *M. graminicola*.

Keywords: Nematodes; plant hormones; plant immunity; yield decline.

**Screening of synthetic wheat lines for resistance to the cereal cyst nematode
*Heterodera filipjevi***

Fateh Toumi^{1,2}, Abdelfattah A. Dababat⁴, Jan De Riek³, Alexey Morgounov⁴,
Ellen De Keyser³, Gul Erginbas-Orakci⁴, Nicole Viaene^{1,5}, Julie M. Nicol⁶, Francis
Ogbonnaya^{7,8}, Lieven Waeyenberge^{1,2} and Maurice Moens^{1,2}

¹ Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium

² Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

³ Institute for Agricultural and Fisheries Research (ILVO), Caritasstraat 39, 9090 Melle, Belgium

⁴ International Maize and Wheat Improvement Centre (CIMMYT), Ankara, Turkey

⁵ Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium

⁶ International Maize and Wheat Improvement Centre (CIMMYT)

⁷ International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria

⁸ Grains Research and Development Corporation (GRDC), Level 1, 40 Blackall Street, Barton ACT 2600, Australia

corresponding author: fateh.toumi@ugent.be

Wheat (*Triticum aestivum* L.) is the main food crop worldwide in terms of production, acreage and source of nutrition. Cereal cyst nematodes (*Heterodera* spp.) are considered to be the most important group of nematodes on this crop; within the group, *H. avenae*, *H. filipjevi*, and *H. latipons* are the most significant species. The use of host resistance for nematode management is cost-efficient, environmentally friendly and easy for farmers to apply. Therefore, we screened a total of 217 synthetic winter wheat lines representing three nurseries for resistance to *H. filipjevi* in the growth chamber. These lines were also genotyped by using the Amplified Fragment Length Polymorphism (AFLP) technique. Genomic DNA extracted from the young leaves of all lines was digested with three restriction enzymes used in two combinations (*EcoRI* + *MseI* and *PstI* + *MseI*). AFLP was performed using two sets of primers for each enzyme combination. The average cyst number on 217 breeding lines ranged between 0 and 40 cysts per plant. Within each nursery, some of the lines had relatively lower numbers of cysts compared to the others. The statistical analysis resulted in a significant, but weak, positive correlation between the number of developed cysts and root length ($r^2 = 0.136$), and root surface ($r^2 = 0.107$). Interestingly, the biplot based on the two functions of the discriminant analysis of the AFLP DNA markers, clearly evidenced the genetic diversity of the resistant lines in all three nurseries.

Keywords: AFLP; nematode; resistant germplasm; wheat.

Differential adaptation of *Meloidogyne incognita* and *M. javanica* to the host-parasite interaction on zucchini

Soledad Verdejo-Lucas¹, Manuel López-Gómez¹ and Miguel Talavera²

¹IFAPA, Centro La Mojonera, Camino de San Nicolas 1, La Mojonera, Almería, Spain

²IFAPA, Centro Camino de Purchil s/n, 18004 Granada, Spain

corresponding author: soledad.verdejo@juntadeandalucia.es

Cucurbits and Solanaceous crops are frequently rotated in double cropping systems under protected cultivation. Cucurbits such as zucchini show lesser ability to reproduce root-knot nematodes than cucumber and melon. Such differences in host suitability levels can be exploited for the sustainable management of the nematode. In this study, the infection frequency (egg masses/ juvenile inoculum), nematode maximum width, gall surface area, egg production, female fecundity (eggs/female) and reproduction rate (eggs per plant/ juvenile inoculum) were measured to gain insight into the mechanisms involved in the response of zucchini cv. Amalthee to *Meloidogyne incognita* in comparison to *M. javanica*. Seedlings grown in a growth chamber were inoculated with 400 juveniles of the respective nematodes, and four replicated plants were harvested at 3, 7, 11, 15, 18, 21, 25, 29 and 42 days-post inoculation (dpi). Both nematodes invaded the roots and developed into third- and fourth-stage juveniles from 7 to 11 dpi. The maximum width of *M. incognita* was smaller than that of *M. javanica* and significant differences were observed from 15 to 25 dpi. By contrast, *M. incognita* galls were larger than those of *M. javanica* from 18 to 25 dpi. The infection frequency was 11% for *M. incognita* and 42% for *M. javanica*. The reproduction rate was 5.6 for *M. incognita* and 21.4 for *M. javanica* at 42 dpi. The fecundity of *M. incognita* females was lower than that of *M. javanica* (165 and 472 eggs per female, respectively). The host-parasite interaction between *M. javanica* and zucchini cv. Amalthee was more successful than that with *M. incognita* in terms of nematode infection and reproduction. Late post-infection mechanisms prevented a large number of *M. incognita* from reaching the egg-laying female stage and those females that laid eggs showed reduced fecundity in comparison to *M. javanica*.

Keywords: *Cucurbita pepo*; resistance; reproduction; root-knot nematodes.

Reproductive ability of *Meloidogyne ethiopica* populations on tomato plant with *Mi* resistance gene

Gökhan Aydınli¹ and Sevilhan Mennan²

¹ Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

² Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

corresponding author: gokhanay@omu.edu.tr

Root-knot nematodes are distributed widely in glasshouses in the Middle Black Sea Region of Turkey. Recent extensive survey in this area has shown that *Meloidogyne ethiopica* is the one of the common root-knot nematode species. Using resistance plants to nematode control is considered as an alternative method to chemicals. Cultivation of tomato crops with *Mi* gene which confers resistance to *M. arenaria*, *M. incognita* and *M. javanica* is expanding in glasshouses of this region. In surveys, *M. ethiopica* were only found in weed plants when resistant tomato plants carrying *Mi* gene were grown in glasshouse. This result and previous data from different authors showed that plants with the *Mi* gene were also resistant to *M. ethiopica*. The aim of this study is to investigate the reproductive ability of *M. ethiopica* populations on a tomato plant bearing *Mi*. Pot experiments were conducted in the glasshouse under controlled conditions ($24 \pm 2^\circ\text{C}$). Seedlings were inoculated with 1000 eggs per plant and pots were arranged in a completely randomised block design with five replicate for 8 weeks. The experiment was repeated twice. The reproduction of 37 populations was evaluated on a resistant tomato cv. Alsancak and was compared with that on the susceptible tomato cv. Beril. Egg production in all populations of *M. ethiopica* on resistant tomato was lower than on the susceptible tomato. Reproduction index varied from 0 to 51.22%. Reproductive indices of four populations were greater than 10% and were classified as resistance-breaking populations of *M. ethiopica*. Of these populations, two resulted in a similar gall index, which did not differ significantly on resistant and susceptible tomato varieties. To our knowledge, this is the first report of populations of *M. ethiopica* that can reproduce on tomato plant with *Mi* gene. This study was supported by TÜBİTAK-TOVAG (111O793).

Keywords: *Meloidogyne ethiopica*; *Mi* gene; tomato; Turkey; resistance.

Susceptibility of Pa3-resistant potato varieties to an unusually virulent *Globodera pallida* populationBjörn Niere

Julius Kühn Institut for Plant Protection in Field Crops and Grassland, 38104 Braunschweig, Germany

corresponding author: bjorn.niere@julius-kuehn.de

The white potato cyst nematode (*Globodera pallida*) is a serious pest of potato and is extremely difficult to control due to the lack of resistant potato varieties for all production systems. This species has already become the dominating species in several regions. A field population of potato cyst nematodes that has overcome resistance of potato varieties to currently available Pa3-resistant starch potatoes has been detected recently in a starch potato growing area in Germany. At present the most likely explanation is that selection for virulence in this population has occurred. Selection for virulent populations of *G. pallida* has been experimentally demonstrated more than 30 years ago by several authors. Until now this has not been shown to have happened under field conditions. Details on the characteristics of this population and on resistance in commercially available potato cultivars against this unusually virulent population will be presented.

Keywords: Potato cyst nematodes.

Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype based diagnostics and reveals evidence of recent reticulate evolution

Toon Janssen¹, Gerrit Karssen^{1,2}, Myrtle Verhaeven¹, Daniel Coyne³ and Wim Bert¹

¹ Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

² National Plant Protection Organization, Wageningen Nematode Collection, P.O. Box 9102, 6700 HC Wageningen, The Netherlands

³ International Institute of Tropical Agriculture (IITA), c/o icipe, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya

corresponding author: toon.janssen@ugent.be

The polyphagous parthenogenetic root-knot nematodes of the genus *Meloidogyne* are considered to be the most significant nematode pest in sub-tropical and tropical agriculture. Despite the crucial need for correct diagnosis, identification of these pathogens remains problematic. The traditionally used diagnostic strategies, including morphometrics, host-range tests, biochemical and molecular techniques, now appear to be unreliable due to the recently-suggested hybrid origin of root-knot nematodes. In order to determine a suitable barcode region for these pathogens nine quickly-evolving mitochondrial coding genes were screened. Resulting haplotype networks revealed closely related lineages indicating a recent speciation, an anthropogenic-aided distribution through agricultural practices, and evidence for reticulate evolution within *M. arenaria*. Nonetheless, nucleotide polymorphisms harbour enough variation to distinguish these closely-related lineages. Furthermore, completeness of lineage sorting was verified by screening 80 populations from widespread geographical origins and variable hosts. Importantly, our results indicate that mitochondrial haplotypes are strongly linked and consistent with traditional esterase isozyme patterns, suggesting that different parthenogenetic lineages can be reliably identified using mitochondrial haplotypes. The study indicates that the barcode region *Nad5* can reliably identify the major lineages of tropical rootknot nematodes. (Scientific Reports, 6: 22591 DOI : 10.1038/srep22591, <http://www.nature.com/articles/srep22591>)

Keywords: Barcoding; haplotype network; hybridisation; isozyme electrophoresis; *Meloidogyne incognita* group.

Nematodes from mushrooms, a neglected part of nematode biodiversity

Dieter Slos and Wim Bert

Nematology Research Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium

corresponding author: dieterg.slos@ugent.be

Both fungi and nematodes are among the most diverse groups of living organisms but almost nothing is known about nematodes in fungi. The current study shows that mushrooms are a suitable habitat for several nematodes and several species appear to be new for science. These new species are morphologically and molecularly characterised and their life-traits are analysed. Several of these mushroom-associated nematodes appear to be transmitted by insects, either via phoresy or as an insect-parasitic life stage. Remarkably, some of these nematodes belong to the understudied Sphaerularioidea, which are very closely related to economically important plant-parasitic nematodes. These evolutionary key taxa collected from mushrooms may provide crucial and unique information in understanding the evolution of the mode and direction of plant-parasitism. This study provides the first comprehensive overview of nematode diversity inside fungi since Meyl (1954).

Keywords: insect-parasitic nematode; phoresy; phylogeny; systematics; taxonomy.

Phylogeny and diversity of the genus *Aphelenchoides*, a reverse taxonomy approach

Gerardo Alcides Sánchez Monge^{1,2}, Toon Janssen¹, Yiwu Fang^{1,3}, Marjolein Couvreur¹, Gerrit Karssen^{1,4} and Wim Bert¹

¹ Nematology Research Unit, Department of Biology, Ghent University, Ledeganckstraat 35, B-9000, Ghent, Belgium

² Escuela de Estudios Generales, Universidad de Costa Rica, San José 2060, Costa Rica

³ Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

⁴ National Plant Protection Organization, The Netherlands Food and Consumer Product Safety Authority, Geertjesweg 15, 6700HC Wageningen, The Netherlands

corresponding author: alcides.sanchez@ugent.be

The superfamily Aphelenchoidea comprises fungivorous and predatory species, as well as insect- and plant-parasites. Among the last group, species of *Bursaphelenchus* and *Aphelenchoides* pose an important threat on several plant species. Selected taxa of both genera have been characterised and the resulting molecular analyses support a monophyletic origin for *Bursaphelenchus*, while *Aphelenchoides* shows a less clear phylogenetic position. Furthermore, species of the genera *Ficophagus*, *Laimaphelenchus*, *Martininema* and *Schistonchus* are found within those clades of *Aphelenchoides*, highlighting a non-monophyletic origin and a big gap in the knowledge of the relationships within this genus. Thus, molecular-based phylogenetic relationships do not agree with morphology-based classification. In addition to this, molecular information is missing for most described species, and available sequences usually belong to non-identified species for which morphological vouchers are absent. In order to clarify the taxonomy and phylogeny of this genus, we collected and analysed several specimens of *Aphelenchoides* and related taxa, including both free-living and plant-parasitic taxa from soil, bark, fungi and host plants. Subsequently, a molecular framework was constructed based on three different molecular markers (18S rDNA, 28S rDNA D2D3 expansion region and COI mtDNA), on which morphological and biological features were plotted. Based on this integrative work molecular clades that are supported with morphological information can serve as a well-founded starting point for species descriptions, *i.e.*, the practice of reverse taxonomy. Our study also indicates that the COI fragment is a promising barcode region to identify plant-parasitic *Aphelenchoides* species.

Keywords: Barcoding; foliar nematodes; mtCOI; rDNA; taxonomy.

Third species of the rare genus *Enchodorus* (Dorylaimida, Nordiidae) from southern Iran

Majid Pedram and Ebrahim Pourjam

Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

corresponding author: mj_pedram@yahoo.com

Genus *Enchodorus*, one of the rarest dorylaim genera, currently contains two known species namely *E. dolichurus* and *E. neodolichurus*. Till date, molecular data have been available only for an Iranian population of the type species. *Enchodorus* sp., an unknown species, representing the third species of this genus, was recovered from the rhizosphere of mosses collected from Khouzestan province, southern Iran. Females of this population are characterised by having 1511.3-1792.5 μm long slender body, angular lip region having prominent papillae, 12-13 μm long odontostyle, simple guiding ring, vulva at 43.5-44.9 %, and 102-160 μm long elongate-conoid tail, ventrally bent at distal part and having a rounded tip. Males of the new species are abundant and functional, characterised by 1483.8-1576.5 μm long body, ventrally bent elongate conical tail, 40-43 μm long spicules and 5-6 ventral supplements ending at 45-59 μm distance from cloacal pair. Compared to the type species, the recovered population has basic difference in tail morphology (elongate conoid, uniformly attenuating toward distal end vs conical, tapering rapidly in first third), and compared to *E. neodolichurus*, the new species has basic differences in female tail length (tail=102-160 μm , $c' = 4.6-5.7$ vs 49-73 μm , $c' = 2.1-3.5$), and longer spicules (40-43 vs 31-34 μm). Molecular phylogenetic studies using partial sequences of 28S ribosomal RNA gene D2/D3 fragment, revealed sequences of this fragment have remarkable differences between the recently recovered population and *E. dolichurus*. In Bayesian tree reconstructed using the available sequences of Nordiidae members and several other dorylaim species/genera, the clade including *Enchodorus* sp. and *E. dolichurus* achieved a 0.81 Bayesian posterior probability. This clade formed a sister clade to the clade of *Heterodorus* sp. and *Rhysocolpus vinciguerrae*.

Keywords: 28S ribosomal gene D2/D3; bayesian; Khouzestan province; new species; taxonomy.

Characterisation of *Xiphinema americanum* group species (Nematoda: Dorylaimida) and their co-evolution with bacteria from the genus *Candidatus Xiphinematobacter*

Valeria Orlando¹, John J. Chitambar², Ke Dong², Vladimir N. Chizhov³, Dimitre Mollov⁴, Wim Bert¹ and Sergei A. Subbotin^{1,2,3}

¹ Department of Biology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium

² Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, USA

³ Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow 117071, Russia

⁴ National Germplasm Resources Laboratory, USDA, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

corresponding author: sergei.a.subbotin@gmail.com

The *Xiphinema americanum* group contains over two-dozen different species of nematode. They are economically important because they vector nepoviruses, which cause damage to several crops. Taxonomic differentiation among species of the *X. americanum* complex is problematic because many of the species share similar morphological characteristics and morphometric data. We collected nematode samples from the *X. americanum* group in different locations in the USA, Italy and Russia. These samples were examined for verrucomicrobial endosymbionts in addition to determining morphological data and genomic sequences of the nematode itself. Six valid species: *X. americanum*, *X. californicum*, *X. brevicolle*, *X. pachtaicum*, *X. rivesi* and *X. simile*, and five unidentified putative *Xiphinema* species were characterised by morphology and sequencing of the D2-D3 segments of 28S rRNA and the mitochondrial COI genes. More than two hundred new sequences of nematodes and bacteria were generated. Phylogenetic relationships of the *Xiphinema americanum* group species were reconstructed using Bayesian inference. The analysis of the D2-D3 of 28S rRNA gene sequences did not provide clear species delimitation of the samples studied, however, the mtDNA presented interspecific variations useful for demarcation among species. The species of *X. americanum* group harbour specific verrucomicrobial endosymbionts belonging to the genus *Candidatus Xiphinematobacter*. We provided evidence of several new *Candidatus* species based on 16S rRNA sequence analysis. Comparison of the bacteria (16S rRNA) and nematode (COI of mtDNA) phylogenies showed a high congruence in their topology, suggesting a high level of co-speciation between hosts and symbionts.

Keywords: Bacteria; co-evolution; dagger nematode; molecular systematics.

Phylogeny of Tylenchidae (Nematoda): establishing the root of plant-parasitism

Xue Qing and Wim Bert

Nematology Research Unit, Department of Biology, Ghent University, K. L.Ledeganckstraat 35,
9000 Ghent, Belgium

corresponding author: xueqing4083@gmail.com

Tylenchidae is one of most important soil inhabiting nematode group. Nematodes of the Tylenchidae may constitute 30% or more of the individuals in a soil sample and. On the evolutionary ladder of modern Tylenchomorpha, they represent the first rung in being both weak plant parasites and as a conservative group with many ancestral characters (e.g. weak feeding apparatus, undifferentiated non-muscular corpus, filiform tails, 4 cell rows in the uterus, etc.). Moreover, the food resources used by this group are important for ecology studies; however, this aspect remains poorly documented and highly uncertain and. Our study focused on this neglected group. Multi-gene sequences based phylogeny was combined with detailed morphology characters newly introduced by SEM and TEM. Remarkably, results show the most specious genus *Filenchus* should be split into several groups; the genus *Malenchus*, which was previously considered as a well defined genus characterised by coarsely annulated cuticle, is actually mixed with some of *Filenchus* species. A future ancestor state reconstruction with multiple methods shows a complex cuticle pattern and character evolution history within Tylenchidae.

Keywords: Phylogeny; systematic; taxonomy; *Tylenchomorpha*.

The genus *Ditylenchus* (Tylenchida: Anguinidae) in Canada

Qing Yu¹, Mario Tenuta² and Fengcheng Sun³

¹ Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, 960 Carling Avenue, Neatby building, Ottawa, K1A 0C6, Ontario, Canada

² Department of Soil Science, University of Manitoba, R3T 2N2, Winnipeg, Canada

³ Canadian Food Inspection Agency, Ottawa, Ontario, Canada.

corresponding author: Qing.yu@agr.gc.ca

Potato rot nematode, *Ditylenchus destructor*, and stem and bulb nematode, *D. dipsaci*, are two of the most serious pests in Canada, and they are quarantined pests which are strictly regulated. These nematodes had not been considered common in Canada, but recent discoveries are worrisome. *Ditylenchus destructor* was recently found in Ontario, the first discovery of the pest in continental Canada, and *D. dipsaci* is epidemic on garlic in Ontario, and spreading to neighbouring provinces. The discovery of the non-crop pest *D. weischeri* of creeping thistle in the Prairie Provinces rather than *D. dipsaci* has resolved phytosanitary concern of grain exports. This paper covers taxonomy, and biodiversity of the genus in Canada and recent research on species of economic important for Canada.

Keywords: Biodiversity; Canada; *Ditylenchus*; taxonomy.

Phylogeny and biogeography of the genus *Cephalenchus* (Tylenchomorpha, Nematoda): inferring species relationships from morphological and molecular data

Tiago Jose Pereira¹, Xue Qing², Kuei-Fu Chang¹, Manuel Mundo-Ocampo¹, Juvenil E. Cares³, Erik J. Ragsdale⁴, Chau N. Nguyen⁵ and James G. Baldwin¹

¹ Department of Nematology, University of California, Riverside (UCR), California, 92521, USA

² Research Group Nematology, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

³ Departamento de Fitopatologia, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900, Brasília, Distrito Federal, Brazil

⁴ Department of Biology, Indiana University, 915 E. 3rd Street, Bloomington, IN 47405, USA

⁵ Department of Nematology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Rd., Hanoi, Vietnam

corresponding author: tiagojp@ucr.edu

The phylogenetic position of *Cephalenchus* is enigmatic with respect to other tylench nematodes. To investigate the phylogeny of *Cephalenchus*, populations representing 11 nominal species were sampled worldwide for molecular and morphological characterisation. Morphological identification was based on light microscopy and scanning electron microscopy observations of multiple individuals and by following published diagnostic keys. Molecular analyses were based on three rRNA genes (*i.e.*, 18S, 28S, ITS) and using different alignment procedures (*i.e.*, full vs reduced alignments). Phylogenetic analyses of either concatenated or single genes always supported *Cephalenchus* as a monophyletic group. A sister relationship between *Cephalenchus* and *Eutylenchus excretorius* was recovered by most analyses, although branch support for this relationship varies depending on the dataset used. The position of *Cephalenchus* + *E. excretorius* within Tylenchomorpha nevertheless remains ambiguous, thus highlighting the importance of using additional genes as well as increasing taxon sampling. Placement of *Cephalenchus* + *E. excretorius* as sister of Tylenchinae or Boleodorinae could not be rejected on the basis of two rRNA genes (*i.e.*, 18S and 28S). Within *Cephalenchus*, amphid opening morphology shows congruence with molecular-based phylogenetic relationships, whereas the number of lines in the lateral field is likely to be convergent. Morphometric analyses clearly distinguished short tail species from medium-long tail species and scanning electron microscopy observations suggest that *Cephalenchus* species with a shorter tail also might be characterised by a laterally oriented amphid opening. Furthermore, all three rRNA genes support the non-monophyly of four morphologically defined *Cephalenchus* species. The known extent of *Cephalenchus* diversity is increased with the inclusion of two new species and the biogeography of the genus is discussed.

Keywords: Amphid morphology; *Cephalenchus*; geographic distribution; phylogeny; ribosomal rRNA.

New insights to understand the diversity and the phylogeny of the genus *Aporcelinus* Andrassy, 2009 (Dorylaimida: Aporcelaimidae)

Reyes Peña-Santiago¹, Duong Nguyen^{1,2,3}, Sergio Álvarez-Ortega¹ and Joaquín Abolafia¹

¹ Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n, 23071-Jaén, Spain

² Institute for Zoology, Department of Terrestrial Ecology, University of Cologne, ZùlpicherStraÙe 47 b, D-50674 Köln, Germany

³ Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Sciences and Technology, 18 Hoang Quoc Viet, Hanoi, Vietnam

corresponding author: rpena@ujaen.es

The representatives of the genus *Aporcelinus* are not characterised by an easily recognisable distinctive feature. They share, however, a peculiar combination of more or less usual characters in other dorylaims: lip region appreciably (but variably) differentiated, strong odontostyle with wide aperture occupying around one-half of its total length, pharyngo-intestinal junction bearing a distinct dorsal lobe, frequent presence of a dorsal cell mass at level of the anterior end of the intestine, caudal region conical and curved dorsad in both sexes, and inner cuticle layer especially distinct (refractive). This morphological pattern fits that found in some Aporcelaimidae (wide odontostyle aperture, inner cuticle layer refractive) but also resembles that observed in typical Qudsianematidae (conical tail), raising some controversy about its taxonomic position as, in addition, the mentioned families are not natural (monophyletic) taxa. The first available molecular data (LSU-D2D3) show a narrow relationship between *Aporcelinus* and *Aporcelaimellus*, both sharing the presence of refractive inner cuticle layer. Regarding the intrageneric diversity, the study of abundant material belonging to nearly ten species has revealed the existence of a remarkable variability of several characters, among others: the differentiation of the perioral area in the lip region (no delimitation, existence of a delimiting circular incisure, or presence of liplets), the morphology of the genital tract (uterus mono- to tripartite), presence of a more or less developed hyaline terminal part at tail, and tail tip very acute to finely rounded.

Keywords: Diagnostic features; intrageneric variability; morphology; taxonomy.

Integrative taxonomy and molecular phylogeny of “dagger” and “needle” nematodes (Fam. Longidoridae) infesting grapevine soils in Portugal

Carlos Gutiérrez-Gutiérrez¹, Maria Antonia Bravo², Margarida Teixeira Santos², Paulo Vieira¹, Manuel Mota^{1,3}

¹ NemaLab/ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas e Departamento de Biologia, Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal

² Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta do Marquês, 2780-159 Oeiras, Portugal

³ Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal

corresponding author: carlosg@uevora.pt

Longidorid nematodes are one of the most economically important parasitic nematode groups in grapevine worldwide. They are polyphagous root ectoparasites causing severe damage to plants by their direct feeding, and in addition some species can transmit plant viruses. Grapevine fanleaf virus (GFLV) is transmitted by *Xiphinema index*, and it is one of the main factors responsible for a progressive degeneration of grapevines, which occurs in most vineyards worldwide. Surveys are being conducted since 2015 on representative vineyards. An integrative taxonomy strategy based on the combination of morphometric and morphological characterisations with molecular analysis using ribosomal DNA segments (rDNA) (D2–D3 expansion regions of the 28S gene, and ITS1 region) and/or a segment of a mitochondrial DNA (mtDNA) (cytochrome c oxidase 1 subunit or COI), were used for identification of longidorids. Severe nematode infestations have been found in grapevine soils in the oldest vineyard regions, highlighting *X. index* by its phytopathological importance. Longidorid nematodes detected, in order of decreasing frequency of infestation were *X. pachtaicum*, *X. santos*, *X. index*, *X. dissimile*, *X. italiae* and *Longidorus* sp. Disease symptoms were observed on aboveground plant parts of the infected grapevines with *X. index*, displaying a yellow mosaic pattern in leaves characteristic of infections by GFLV.

Keywords: Longidorids; *Longidorus*; rDNA; virus vector; *Xiphinema*.

Using single nucleotide polymorphisms to evaluate intraspecific diversity and genetic determinants of virulence of the soybean cyst nematode, *Heterodera glycines*

Anne-Frédérique Gendron St-Marseille^{1,2,3}, Pierre-Yves Veronneau¹, Joël Lafond-Lapalme¹, Jacques Brodeur^{2,3} and Benjamin Mimee¹

¹ Centre de Recherche et de Développement de Saint-Jean-sur-Richelieu (Agriculture and Agri-Food Canada), 430, boul. Gouin Saint-Jean-sur-Richelieu, Québec, Canada

² Université de Montréal, C.P. 6128, succursale Centre-ville Montréal, Québec, Canada

³ Institut de Recherche en Biologie Végétale (IRBV), 4101 Sherbrooke Est Montréal, Québec, Canada

corresponding author: anne-frederique.gendron@outlook.com

The soybean cyst nematode (SCN), *Heterodera glycines*, is a major pest worldwide and causes severe economic losses in soybean production. Currently, the Hg Type test is the most accurate method to characterise SCN virulence by using its reproductive ability on resistant plant genotypes. This test is conducted under glasshouse conditions for 35 to 42 days and evaluates SCN cyst development on seven soybean lines with different resistance profiles and one susceptible genotype. Despite being effective, this method is time-consuming. Furthermore, it is based on population phenotypes and does not evaluate the composition of genetic diversity. An avirulent phenotype could include virulent genotypes that are likely to be selected in a field where a resistant soybean line is used. DNA-based diagnostics could be a good alternative to solve these problems. Mutations are a major source of genetic variation and single nucleotide polymorphisms (SNP), fixed in a population, accumulate with time. They can be used in complementary studies to define genotypes and potentially to highlight sequencing (GBS) offers a cost effective alternative for the simultaneous SNP discovery and population genotyping. This study goal was to characterise population genetics and ultimately link virulence to genetic markers. To do so, we have used GBS to characterise several SCN populations from two Canadian provinces and twelve U.S. states. Genetic links between the populations were analysed and based on the Fixation Index (F_{st}) we have found different origins and geographic affiliations between populations at a state/province level and at the field level. Moreover, thousands of SNP were identified and are currently used in association studies with different traits. Whole genome sequencing on four populations with different virulence profiles was also realised. Our efforts to establish links between allele frequency at specific loci and virulence on major soybean resistant genotypes will be presented.

Keywords: parasitism; resistance; SNP; soybean cyst nematode; virulence.

Transcriptomic and histological responses of the African rice (*Oryza glaberrima*) to *Meloidogyne graminicola* provide new insights into root-knot nematode resistance in monocots

Anne-Sophie Petitot¹, Tina Kyndt², Rana Haidar¹, Alexis Dereeper¹, Janice de Almeida Engler³, Godelieve Gheysen² and Diana Fernandez¹

¹ UMR IPME, Institut de Recherche pour le Développement (IRD), 34394 Montpellier, France

² Department of Molecular Biotechnology, Ghent University, 9000 Ghent, Belgium

³ UMR IBSV INRA, 06903 Sophia Antipolis Cedex, France

corresponding author: diana.fernandez@ird.fr

The root-knot nematode, *Meloidogyne graminicola*, is responsible for rice (*Oryza sativa*) production losses in Asia and Latin America. The resistant accession TOG5681 of African rice (*O. glaberrima*) is used to develop interspecific rice lines with improved resistance to several biotic and abiotic factors. The aim of this study was to assess the cytological and molecular mechanisms underlying nematode resistance in TOG5681. *M. graminicola* penetration and development in TOG5681 and susceptible plants (*O. sativa* 'Nipponbare') were compared by microscopic observation of infected roots and histological analysis of galls. In parallel, host molecular responses to *M. graminicola* were assessed by RNA-Seq analyses at 2, 4 and 8 days post infection (dpi). Differential expression between TOG5681 and Nipponbare of 875 candidate genes, including genes involved in plant basal immunity and resistance, or associated with *M. graminicola* QTL was analysed. Specific treatments with hormone inhibitors were conducted on TOG5681 to assess the impact of the jasmonic acid (JA) and salicylic acid (SA) pathway on nematode penetration and reproduction. In TOG5681, fewer *M. graminicola* juveniles penetrated and developed galls as compared to susceptible roots, and giant cells degenerated from 15 dpi. Contrasting transcriptomic changes were observed as soon as 2 dpi: genes involved in defence, phenylpropanoids and hormone pathways were strongly induced in TOG5681 but were poorly altered in Nipponbare. No specific hormonal pathway could be evidenced in rice-nematode incompatible interactions. Candidate genes involved in TOG5681 resistance to *M. graminicola* were identified based on their expression pattern and QTL position. This study provides a novel set of *O. glaberrima* genes potentially associated to nematode resistance and highlights the rice - *M. graminicola* pathosystem as a model to study plant-nematode incompatible interactions in monocotyledons.

Keywords: Differentially expressed genes; giant cell; hormones; QTL; RNA-Seq.

Transcriptome analysis of cowpea [*Vigna unguiculata* (L.) Walp] near isogenic lines with contrasting phenotypes upon *Meloidogyne incognita* infection.

Jansen Santos¹, Arsenio Ndeve¹, Bao-Lam Huynh¹, William C. Matthews¹, Maria Munoz-Amatriain², Steve Wanamaker², Timothy J. Close² and Philip A. Roberts¹

¹Department of Nematology, University of California, Riverside, CA 92521, USA

²Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

corresponding author: jansenps@ucr.edu

Cowpea is one of the most important food and forage legumes in drier regions of the tropics and subtropics. Cowpea is a susceptible host of root-knot nematodes and infection results in significant yield loss. Previous studies demonstrated that cowpea resistance to this pest is controlled by at least two dominant genes *Rk* and *Rk2*, localised in two major quantitative trait loci, *Rkn-1* and *Rkn-2*. The aim of the present work was to select candidate genes for root-knot nematode resistance based on transcriptome profiling of roots of two near-isogenic lines, CB46 NULL (susceptible) and CB46 *Rkn-1/Rkn-2* (resistant with both loci). Transcriptome analyses were performed 3 and 9 days after nematode inoculation and sequence reads were aligned to the common bean whole genome, resulting in 19,922 unique genes mapped to the reference genome. Comparison between resistant and susceptible near-isogenic lines 3 and 9 days after inoculation revealed up-regulation of 109 and 110 genes and down-regulation of 98 and 89 genes, respectively. Functional annotation showed that differentially expressed genes are mostly involved in metabolic and cellular processes, biological regulation and defence. Among the genes assigned to defence, 45 showed contrasting expression patterns between the near-isogenic lines, including nucleotide binding site–leucine-rich repeat genes and peroxidases. For the resistance conferred by *Rk* and *Rk2*, analysis was performed on differentially expressed genes specifically localised in the respective mapped regions. Together with RNA sequencing results, seven genes were chosen for validation using quantitative reverse transcription polymerase chain reaction. One of these genes in the *Rkn-1* region and two in the *Rkn-2* region were highly expressed in resistant plants and are members of the Toll/interleukin1 receptor-nucleotide binding site-leucine-rich repeat family of proteins. Current work is investigating the role of these genes in cowpea resistance to root-knot nematode.

Keywords: Candidate genes; resistance; RNA sequencing; root-knot nematode.

Genome-wide detection of copy number variations in avirulent versus virulent near-isogenic lines of the asexual root-knot nematode *Meloidogyne incognita*

Philippe Castagnone-Sereno, Karine Mulet, Martine Da Rocha, Etienne G.J. Danchin, Laetitia Perfus-Barbeoch and Pierre Abad

INRA, Université de Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

corresponding author: Philippe.Castagnone@sophia.inra.fr

Due to their parthenogenetic mode of reproduction, a theoretical lack of genetic plasticity is expected in asexual root-knot nematodes. However, these pests exhibit remarkable capacities of adaptation, *i.e.*, their ability to overcome plant resistance. Apart from classical genetic recombination, copy number variations are a form of genomic structural variation with potential adaptive impact widely distributed in eukaryotic genomes. The goal of this study was to investigate whether such variations may be involved in the ability of *Meloidogyne incognita* to overcome the tomato *Mi-1.2* resistance gene, which raises concern about the durability of resistance. Thanks to nematode clonal reproduction, experimental evolution gave rise to pairs of virulent and avirulent near-isogenic lines just differing in their capacity to reproduce or not on resistant tomatoes, respectively. Here, two pairs of such avirulent/virulent lines were analysed using a comparative genomic hybridisation assay. Based on the *M. incognita* Mi1V1 genome sequence, 4 × 180 K custom arrays were generated using a total of 173,539 oligonucleotide probes designed from the entire gene set (19,212 genes). Arrays were then hybridised with a mixture of genomic DNA from each line labelled with either a Cy5 or Cy3 fluorochrome. After washing, arrays were scanned and analysed to detect potential copy number variants. Overall, 1,405 probes (*i.e.*, 459 genes) showed significant differences between both pairs of avirulent and virulent lines. A custom selection pipeline finally identified a short list of 33 genes exhibiting significant copy number variations, including eight pioneer sequences. Quantitative PCR experiments were implemented further to assess the accuracy of these structural genomic variations. Together with the results of this validation step, the potential roles of the genes identified here will be discussed in connection with the implication of genomic structural variations in *M. incognita* (a)virulence.

Keywords: Comparative genomic hybridization; copy number variation; resistance; root-knot nematode; virulence.

Genome scans on experimentally evolved *Globodera pallida* populations to identify molecular basis of adaptation to GpaVvrn potato resistances

Delphine Eoche-Bosy¹, Fabrice Legeai^{1,2}, Anthony Bretaudeau^{1,2}, Mathieu Gauthier^{3,4}, Magali Esquibet¹, Sylvain Fournet¹, Eric Grenier¹ and Josselin Montarry¹

¹ UMR IGEPP, INRA, 35653 Le Rheu, France

² IRISA, INRIA, 35042 Rennes, France

³ UMR CBGP, INRA, IRD, Cirad, Montpellier SupAgro, 34988 Montferrier-sur-Lez, France

⁴ Institut de Biologie Computationnelle, 34095 Montpellier, France

corresponding author: eric.grenier@rennes.inra.fr

Globodera pallida is a major pest of potato crop for which a promising resistance factor, the QTL GpaVvrn, has been identified and has been introduced in several new resistant cultivars in Europe. However, a previous study having employed an experimental evolution protocol showed that *G. pallida* was able to overcome this resistance. The aim of the present study is to investigate the genomic regions involved in the resistance breakdown using a genome scan approach on the lineages resulting from the experimental evolution. A first low throughput genome scan was performed using 202 microsatellite markers distributed along the genome and three different tests of neutrality, based on genetic differentiation and heterozygosity. We identified eight outlier loci, several being found by different outlier detection methods and/or in two independent adapted lineages, indicative of genomic regions putatively involved in the resistance breakdown where several effectors of interest were identified. We also showed that the same adaptive genetic pathways seem to be involved in the overcoming of potato genotypes harboring the same resistant QTL but differing by their genetic background. These results have validated the feasibility of a genome scan approach on a biological material coming from a short experimental evolution and encouraged to target more precisely the genomic regions involved in the adaptation. A second high throughput genome scan was thus performed using a whole genome resequencing of pools of individuals belonging to some of the lineages coming from the experimental evolution. 1,6 million SNP are currently used for the genome scan performed with the BAYPASS program. Identified outlier loci will allow us to target more precisely the best candidate genes involved in the adaptation and will also provide a molecular tool to follow frequencies of alleles of virulence in wild *G. pallida* populations.

Keywords: Microsatellite; outlier; pool-seq; positive selection; virulence.

High-throughput identification of soil-borne nematodes for bulk community analyses: Selection of DNA barcode marker and similarity cut-off level

Mohammed Ahmed¹, Melanie Sapp², Thomas Prior², Gerrit Karssen³ and Matthew Back¹

¹ Harper Adams University, Newport, TF10 8NB, Shropshire, UK

² Fera, Sand Hutton, YO41 1LZ, North Yorkshire, UK

³ National Plant Protection Organization, Geertjesweg 15, 6706 EA, Wageningen, The Netherlands

corresponding author: mahmed@harper-adams.ac.uk

High throughput sequencing technologies have made it possible to identify bulk samples of nematodes in environmental samples. This technique, referred to as metabarcoding, has been evaluated and successfully used for analysing nematode community samples. Regions of the 18S rDNA have been favoured as being the most suitable barcode region for identifying bulk samples of phylogenetically diverse nematode taxa. The two most commonly used barcodes of this gene are the regions spanning the sites complementary to the primer sets NF1/18Sr2b and SSUFO4/SSUR22 of approximately 400 bp each. These two barcodes, as well as a region of the 28S rDNA were tested to determine which of these provides the best taxonomic coverage. Each barcode was amplified separately from triplicates of mock nematode communities of 23 phylogenetically diverse nematode genera spanning 18 families. The resultant amplicons were then pooled together and sequenced using the Illumina MiSeq platform. The two 18S rDNA barcodes provided better coverage than the 28S rDNA barcode. Of the two 18S rDNA, region amplified by the primer set NF1/18Sr2b gave better coverage, recovering all sampled taxa. SSUFO4/SSUR22, on the other hand, gave 95% coverage. Using the NF1/18Sr2b primers, a 95% similarity cut-off was sufficient for the recovery of all genera/species. At 97, 98 and 99% similarity cut-offs, primitive but phylogenetically close relatives of our sampled taxa were also found as matches for some of the reads. Quantitatively, sequence reads across replicates correlated well, but none of the replicates showed correlation with abundance of the sampled taxa. This study essentially identifies the right molecular tools and data analysis pipeline for identification of bulk nematodes samples using next generation sequencing technology. This will be used in an ongoing experiment aimed at understanding how soil cultivation and field trafficking regimes affect soil properties and nematodes communities in the soil.

Keywords: Metabarcoding; next generation sequencing.

A multiplex real time PCR assay based on microsatellite markers for the detection of cyst nematodes of importance in seed potato trade

Sylvie Gamel¹, Aude Letort¹, Didier Fouville², Laurent Folcher¹ and Eric Grenier²

¹ Anses, Plant Health Laboratory, Nematology Unit, Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France

² INRA, UMR1349 IGEPP (Institute of Genetics, Environment and Plant Protection), Domaine de la Motte au Vicomte, BP35327, 35653 Le Rheu, France

corresponding author: eric.grenier@rennes.inra.fr

The potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, are regulated in the European Union and in some other countries. The beet cyst nematode, *Heterodera schachtii*, is largely widespread in Europe and regulated in many countries outside the European Union. A reliable diagnostic protocol for the detection of these three nematode species is required considering the growing trade worldwide, including seed potato. This study aims at developing a specific and sensitive real time PCR method in order to detect *G. pallida*, *G. rostochiensis* and *H. schachtii*. The development of a multiplex real time PCR has been performed to screen a lot of soil samples within routine analysis framework. Thanks to the phylogenetic signal present in microsatellite flanking regions, we were able to show that some microsatellite primers represent very efficient species-specific markers. The specificity was assessed among a wide range of non-target species and among various populations of the target species. Thus a total of 31 species and populations of *Globodera* spp. (*G. pallida*, *G. rostochiensis*, *G. tabacum* (*solanacearum*, *tabacum* and *virginiae*), *G. ellingtonae* and *G. mexicana* from Europe and South America) and 20 species and populations of *Heterodera* spp. (*H. schachtii*, *H. betae*, *H. ciceri*, *H. daverly*, *H. glycines*, *H. trifolii*, *H. avenae*, *H. carotae*, *H. cruciferae* and *H. goettingiana*). The primers and probes designed allow the detection of all the target populations tested and no cross reaction was observed for the non-target species. The results of our study show that this multiplex real time PCR method is able to detect simultaneously 1 or 2 isolated juveniles of each species. Furthermore, this detection method is also able to detect DNA of these species, even in mixture, and in a total DNA solution obtained directly from a soil extract.

Keywords: Beet cyst nematode; diagnostic method; potato cyst nematodes; soil extract, TaqMan probes.

Barcoding and species delineation of marine nematodes from rocky intertidal seaweed habitats

Hong Zhou

College of Marine Life Science, Ocean University of China, 5 Yushan Road, 266003, Qingdao, China

corresponding author: hzmengyuan@163.com

Molecular barcoding has been widely accepted as a useful approach for species delineation and diagnostics and for a better assessment of nematode biodiversity in both marine and terrestrial environment. However, the standardised mtDNA fragment of COI-5P has not been successfully used for nematodes and other meiofaunal taxa because of its amplification inefficiency. Instead 18S rDNA fragments are frequently utilised for nematode species delineation. Seaweed habitats provide food resources, refuges and residential space for many benthic animal groups, among which nematodes are usually the most dominant. To compare the efficiency and accuracy for barcoding and species delineation using partial sequences of 18S rDNA and COI-5P and to evaluate their estimation of species richness against morphospecies of marine nematodes, we sampled nematode specimens at two intertidal rocky shore seaweed habitats in Chinese temperate coast. Our results showed that while both of the markers delineated the morphospecies well, the success rate of barcoding specimen and species is below 20% and 30% for COI-5P, but above 60% and 70% for 18S. A total of 25 species from 10 families of Chromadorida, Enoplida, and Monhysterida were successfully barcoded. It is suggested that even though 18S potentially underestimates meiofaunal cryptic diversity, its barcoding efficiency makes it a useful complementary marker to COI-5P and can be used in conjunction in marine nematode barcoding and biodiversity survey projects.

Keywords: 18S rDNA; COI-5P; intertidal seaweeds; molecular barcodes; phytal nematodes.

***Xiphinema americanum* – group: what have we learned so far?**

Stela S. Lazarova¹, Milka T. Elshishka¹, Mariana B. Groza², Shesh Kumari³ and Vlada K. Peneva¹

¹ Institute of Biodiversity and Ecosystem Research - BAS, 2, Y. Gagarin Street, 1113 Sofia, Bulgaria

² National Phytosanitary Laboratory, 11 Voluntari Blv., 077190 Voluntari, Ilfov, România

³ Division of Plant Health, Crop Research Institute, Drnovská 507, Ruzyně, 16106 Prague 6, Czech Republic

corresponding author: vpeneva@ecolab.bas.bg

Xiphinema americanum Cobb, 1913, the type species of genus *Xiphinema* was described more than a hundred years ago. Nowadays this name is used for designation of a well defined natural complex of species having high significance to agriculture caused by the ability of few species to transmit economically important plant viruses and thus being involved in the ecosystem disservices. Currently, the group comprises about 60 species, although a considerable taxonomic debate about the number of species exists. This presentation will draw upon selected findings from the scientific literature to illustrate the current state of research on *X. americanum* – group along with a short review of studies on these emblematic and intriguing plant parasites. Main morphological and biological characteristics are considered, in particular those having taxonomic and diagnostic values. Further, some molecular markers used for species identification are discussed. The major subgroups based both on morphological features and phylogenetic relationships are examined in regard to biogeographical patterns, plant associations, biological characteristics. Some prospective for the future studies are outlined. Acknowledgements: The authors are grateful to STACCATO, MZe–RO0414 and ANIDIV2 projects for supporting this presentation.

Keywords: Biogeography; molecular markers; morphology; phylogeny.

Molecular nematode diagnostics as routine laboratory practice

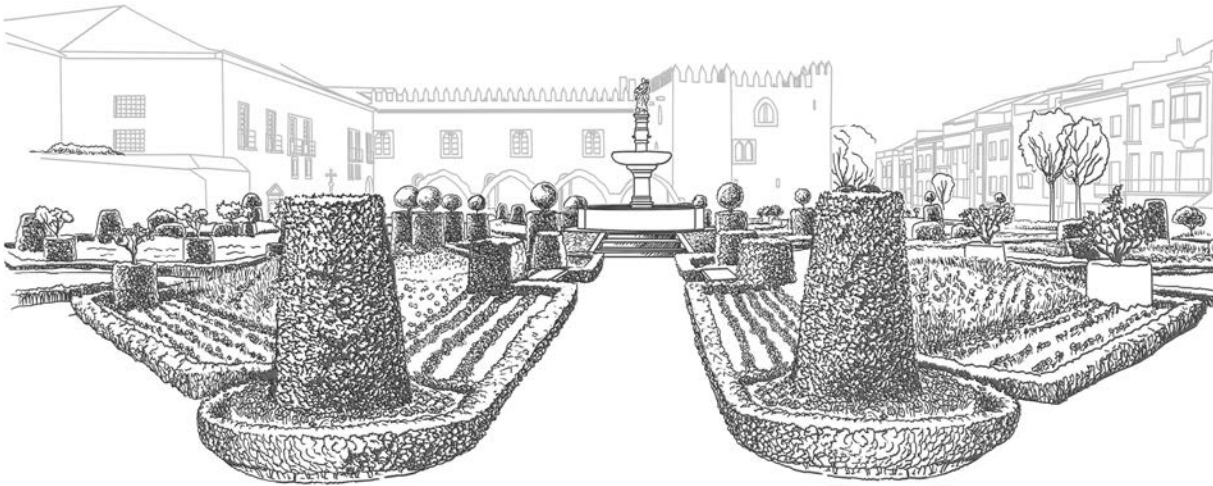
Renske Landeweert, Winfried Mulder, Pawel Deka, Margarida Oliveira, Marieke Beltman and Scott Griffiths

ClearDetections, Nieuwe Kanaal 7H, 6709 PA, Wageningen, The Netherlands

corresponding author: renske.landeweert@cleardetections.com

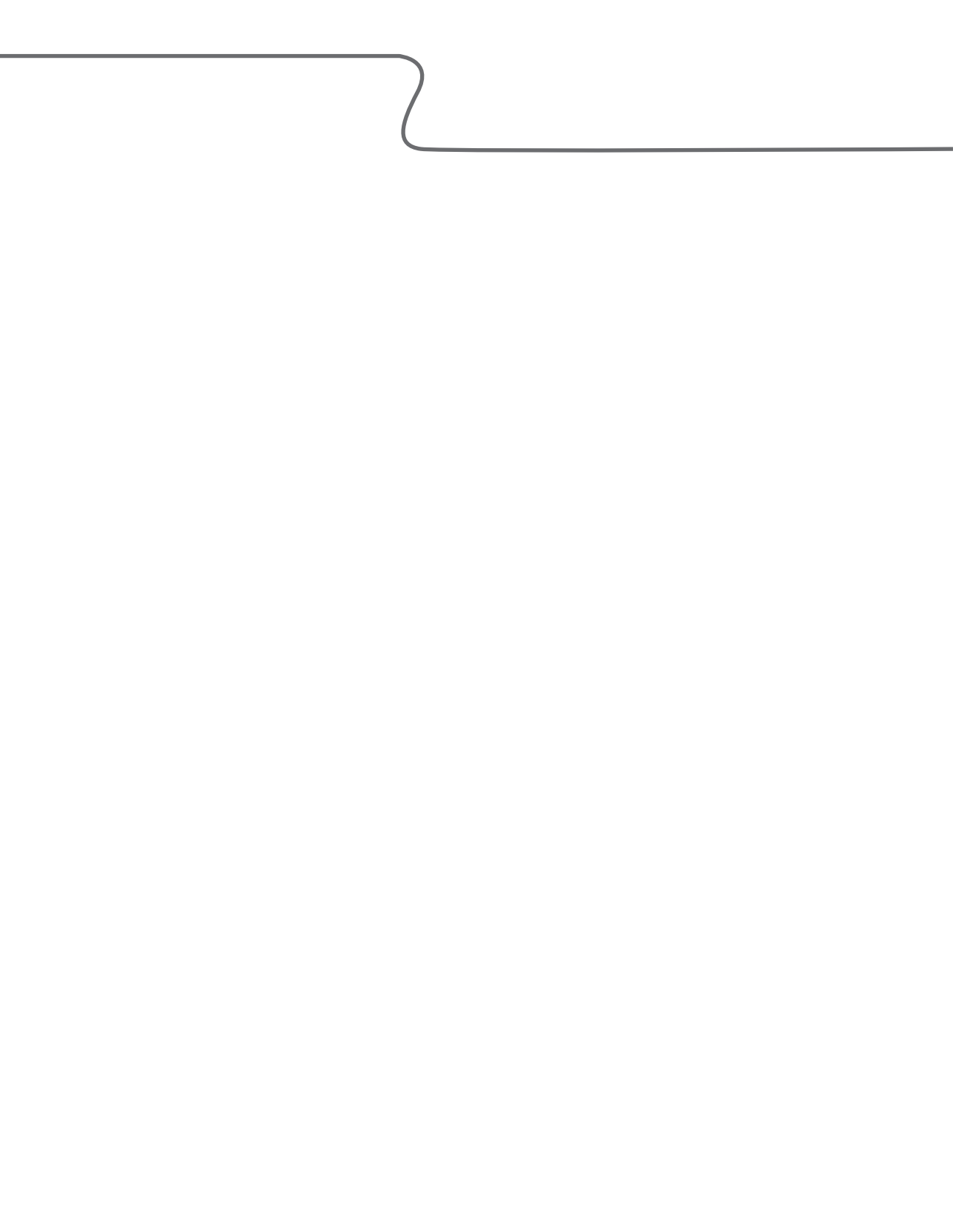
The molecular identification of plant pathogens is daily practice in many plant-health laboratories. Whilst bacterial, fungal and viral pathogens are typically detected directly from plant tissues or cultured isolates, microscopic analysis has remained the golden standard for insects and nematodes. Genetic analyses offer a number of advantages over traditional approaches, such as increased speed, sensitivity and the ability to discriminate between closely related species (specificity). Unlike techniques based on morphology, genetic tests are not affected by nematode life stage and sample size. Many challenges are encountered when starting a molecular laboratory service to diagnose plant pathogenic nematodes with DNA-based techniques. What will be tested and how often? If used occasionally to confirm microscopic analyses, nematode DNA analysis can be performed by a third party. If used to replace microscopic analyses completely, on-site PCR or Real-Time PCR are two excellent options. Real-Time PCR offers significant advantages over conventional PCR, such as the option to quantify the number of nematodes. Furthermore, Real-Time PCR does not require the use of toxic stains such as ethidium bromide. Another important consideration is the choice of gene or DNA sequence upon which a species-specific nematode assay can be based. There are many publicly available molecular assays for nematode identification, many of which are 'home brewed'. Whilst these can work well in academic or research environments, the implementation of diverse and technically complex analytical methods is typically unsuited to service laboratory environments. We will present the challenges a plant health laboratory encounters during the transition towards molecular techniques, including sample preparation and data analysis. Examples will be presented of routine applications of molecular techniques for the quantitative and species-specific detection of nematodes.

Keywords: Diagnostics; identification; Real-Time PCR.



Jardim de Santa Bárbara (1955)

POSTER ABSTRACTS



Variability of results in studies migration and multiplication of *Bursaphelenchus xylophilus* studies in pine stem cuttings

Adela Abelleira, Andrea Abelleira-Sanmartin, Alicia Prado, Yoel Castro, Pilar Piñón and Pedro Mansilla

Estación Fitopatológica Areeiro, Deputación Pontevedra, Subida a la Robleda s/n, 36153 Pontevedra, Spain

corresponding author: adela.abelleira@depo.es

Migration and distribution studies of *Bursaphelenchus xylophilus* in pine trees are used to evaluate the susceptibility or resistance of pines, and are designed with different inoculum densities, tree age and genotype, experimental length and environmental factors. This study was designed without varying these conditions but alternating inoculation dates to check that it is possible to obtain a different result. To evaluate the ability of migration and multiplication we used *B. xylophilus* (two isolates) and one isolate of *B. mucronatus kolymensis* in three species of pine: *Pinus pinaster*, *P. radiata* and *P. pinea*. Maintaining the same conditions (nematodes isolates and density, pine species, temperature, incubation times and extraction method), two assays were done; in the first the pine species were changed at each inoculation date and in the second the nematode isolates were alternated at each inoculation date. In the first assay, the three isolates of nematodes were inoculated in the same day in one pine species and repeated weekly with the rest of the species of pine. In the second repetition, one nematode isolate was inoculated in the three species of pine and repeated weekly with the rest of the nematodes in these three pine species. We wanted to know if this change, but in the same conditions, caused variability in the results. Results showed that these types of studies under laboratory conditions provide significantly different outcomes according to Tukey's honestly significant difference and Student's t-test, so these studies may influence the grade of susceptibility and resistance in pine species due to the variability in data obtained.

Keywords: Distribution; pine susceptibility; pinewood nematode.

Migration and multiplication of *Bursaphelenchus xylophilus* and *B. mucronatus kolymensis* in five pines species

Adela Abelleira, Andrea Abelleira-Sanmartin, Alicia Prado, Yoel Castro, Pilar Piñón and Pedro Mansilla

Estación Fitopatológica Areeiro, Deputación Pontevedra, Subida a la Robleda s/n, 36153 Pontevedra, Spain

corresponding author: adela.abelleira@depo.es

Bursaphelenchus xylophilus is a pathogenic nematode associated with pine wilt disease. A closely related species, *B. mucronatus*, has a highly similar morphology to *B. xylophilus*, although was classified as being non-pathogenic to pine trees. The objective of this study was evaluating under controlled conditions the ability of migration and multiplication, key factors in the development of pine wilt disease, of both species of nematodes in different species of pine. The nematodes species were reared in *Botrytis cinerea*. Two isolates of *B. xylophilus* (isolated in different years) and one of *B. mucronatus kolymensis* were inoculated in five species of pines: *Pinus pinaster*, *P. pinea*, *P. radiata*, *P. sylvestris* and *P. canariensis* (only used in multiplication study). To evaluate the ability of migration and multiplication 20 cm long 1-year-old stem cuttings of 5-year-old pines were used. In the migration study, cuttings were inoculated with 1000 nematodes, half of the samples incubated for 1 day and the others for 5 days and divided in eight segments (2.5 cm). In multiplication study, cuttings were inoculated with 200 nematodes and incubated for 7 days. After incubation, nematodes were extracted and counted. In the migration study, the mean numbers of nematodes found in the cuttings were, in ascending order, in *P. pinea*, *P. sylvestris*, *P. pinaster* and *P. radiata*, being considerably higher with *B. xylophilus* than *B. mucronatus*. Moreover, the most recent *B. xylophilus* isolate has the highest mean number of nematodes found. In *P. pinea* neither nematode species managed to advance beyond 5 cm. In the last segments of *P. sylvestris* a few nematodes of only one isolate of *B. xylophilus* and of *B. m. kolymensis* were found. In *P. pinaster* and *P. radiata*, *B. xylophilus* isolates were found in the last segments; however, *B. m. kolymensis* stopped at 12.5 cm. In the other study, *B. xylophilus* multiplied only in *P. pinaster* and *P. radiata*, but *B. m. kolymensis* did not multiply in any pine.

Keywords: Distribution; pathogenicity; pinewood nematode; population increase; stem cuttings.

Differential expression of a cysteine protease in *Bursaphelenchus xylophilus* and *B. mucronatus*

Joana M.S. Cardoso¹, Luís Fonseca¹, Carla M.N. Maleita², Conceição Egas³ and Isabel Abrantes¹

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

³ CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Rua Larga, 3004-504 Coimbra, Portugal

corresponding author: joanasa_cardoso@hotmail.com

Cysteine proteases (CP) are known to play various essential roles during host-parasite interactions associated with several parasites pathogenicity. To gain further insights into the possible roles of these cathepsins in the pathogenicity of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, a cysteine protease transcript from *B. xylophilus* and an homologous sequence in *B. mucronatus* transcriptome were selected. Sequence analyses were performed and revealed that selected *B. xylophilus* (BxCP3) and *B. mucronatus* (BmCP3) cathepsins belong to the Peptidase C1A family with the cysteine, histidine and asparagine active sites in the catalytic triad, a Cathepsin propeptide inhibitor domain (I29) and a predicted signal peptide. According to Gene Ontology term prediction, the analysed proteins are involved on proteolysis, with cysteine-type peptidase activity. Differences in the transcripts level were determined by reverse transcription quantitative real time PCR (RT-qPCR) and BxCP3 transcript was found overexpressed in *B. xylophilus* compared to BmCP3 transcript in *B. mucronatus*. *In situ* hybridisation was also performed to find out the spatial expression of selected proteins. In *B. xylophilus*, BxCP3 transcript was detected in the post-vulvar region of the female reproductive system. On the other hand, in *B. mucronatus* no hybridisation signal was detected in any developmental stage, which is in accordance to the low levels of BmCP3 transcript detected by RT-qPCR. Although the data suggest its involvement in the nematodes reproduction capacity inside the host trees, further studies must be performed to shed light on the role of this cysteine protease in PWN pathogenicity.

Keywords: Cathepsins; *in situ* hybridisation; pathogenicity; peptidases; pinewood nematode.

Assay of feeding RNAi efficiency of prolyl 4-hydroxylase coding genes in *Bursaphelenchus xylophilus*

Xinyue Cheng¹, Meng Wang¹ and Bingyan Xie²

¹ College of Life Sciences, Beijing Normal University, Beijing 100875, China

² Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China

corresponding author: chengxy@bnu.edu.cn

Prolyl 4-hydroxylase (P4H) is a key enzyme in collagen synthesis, which catalyses the formation of 4-hydroxyproline that is essential to the proper three-dimensional folding of newly synthesised procollagen chains in collagen and related proteins. P4H in nematodes composes of two alpha subunits (PHY-1 and PHY-2) and a single β subunit to form dimers or as a tetramer. In this study, genes encoding the two alpha and the beta subunits of P4Hs (*Bx-phy-1*, *Bx-phy-2* and *Bx-pdi-2*) were obtained in the pinewood nematode *Bursaphelenchus xylophilus*, which is an important invasive species that causes a disastrous forest disease in its introduced regions. Homologous sequences of the three genes were inserted into a silencing vector (pDH-RH), which contains a transcriptional unit for a hairpin loop structure. Double-stranded (ds)RNAs with sequences homologous to the target genes were expressed in strains of transformed filamentous fungus *Fusarium oxysporum*, via *Agrobacterium tumefaciens*-mediated transformation technology. Feeding *B. xylophilus* on the positive fungal transformants, mRNA expression levels of the target genes were obviously down-regulated in the nematode, with more than 90% knockdown of *Bx-phy-1* and *Bx-phy-2* after 20 days feeding, but only 48% knockdown of *Bx-pdi-2* after 40 days feeding. Recovery experiments showed that, after feeding RNAi nematodes to the wild-type *F. oxysporum* for three generations, mRNA expression levels was recovered to about 40% of the control for *Bx-phy-1*, but nearly equal to the control for *Bx-phy-2*, and significantly higher than the control for *Bx-pdi-2*. A `small` phenotype was observed for RNAi *Bx-dpy-18* and *Bx-pdi-2*, and a `weak dumpy` phenotype for RNAi *Bx-phy-2*. Moreover, we found that *Bx-pdi-2* could compensate for *Bx-phy-1* or *Bx-phy-2*, but RNAi *Bx-pdi-2* could lead to off-targets effects on *Bx-phy-1* and *Bx-phy-2*. Our results indicate that different coding genes of a proteins have different RNAi effects and efficiencies.

Keywords: *Bursaphelenchus xylophilus*; feeding RNAi; prolyl 4-hydroxylase.

Molecular detection of *Bursaphelenchus tusciae* in Tunisia associated with *Hylurgus ligniperda*

Manel Mejri¹, Luís Fonseca², Joana M.S. Cardoso², Mohamed L. Ben Jamâa¹ and Isabel Abrantes²

¹ INRGREF - National Research Institute of Rural Engineering, Water and Forests, Carthage University, HediKarray Street, 2049 Ariana, Tunisia

² CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: isabel.abrantes@uc.pt

After the detection of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of the pine wilt disease in Europe, Tunisia, as part of the Mediterranean area, has listed *B. xylophilus* and its insect vectors, *Monochamus* spp., as quarantine pests. In Tunisia, pine forests are mainly threatened by *Tomicus destruens*, *Orthotomicus erosus*, *Pityogenes calcaratus* and secondarily by *Hylurgus ligniperda* and *M. galloprovincialis*. A survey to assess the presence of PWN and other *Bursaphelenchus* species associated with bark and wood boring insects in Tunisia was carried out in 2013. Insects were collected using multi funnel flight traps and nematodes extracted by crushing the insects and leaving them in water for 12 h at room temperature. Nematodes similar to *Bursaphelenchus* dauer juveniles were collected from *H. ligniperda* insects and species identification was performed molecularly by rDNA internal transcribed spacer (ITS) regions amplification, restriction fragment length polymorphism and sequencing. PCR amplification of ITS rDNA regions yielded a single product of 920 bp and restriction patterns, obtained after *Hinf*I, *Alu*I, *Hae*III, *Msp*I and *Rsa*I endonucleases digestion, identified these nematodes as belonging to the species *B. tusciae*. Phylogenetic analysis revealed that Tunisian *B. tusciae* clusters together with Italian and Spanish *B. tusciae* isolates forming a separate phylogenetic group close to *B. hildegardae* and *B. eggersi*. Since *H. ligniperda* is a significant maritime pine scolytid pest in Tunisia and is widely distributed, this study is an important contribution to the knowledge of *Bursaphelenchus* species associated with this bark beetle. Moreover, this study demonstrates the value of the molecular methodologies as helpful tools for the identification of *Bursaphelenchus* species.

Keywords: Insect vectors; ITS-RFLP; morphology, phylogenetic analysis.

Are pine species constitutive and inducible defences determining the susceptibility to the pinewood nematode?

Carla S. Pimentel¹, Elsa V. Gonçalves², Paulo N. Firmino¹, Teresa Calvão³, Luís Fonseca⁴, Isabel Abrantes⁴, Otilia Correia² and Cristina Máguas²

¹ CEF - Forest Research Centre, School of Agriculture, University of Lisbon, 1349-017, Lisbon, Portugal

² cE3c - Centre for Ecology, Evolution and Environmental Changes, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal

³ Department of Environmental Sciences and Engineering, Faculty of Science and Technology, New University of Lisbon, Campus de Caparica, 2825-516 Caparica, Portugal

⁴ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: luisbidarra@gmail.com

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, originated from North America, is a major invasive pine pest on Eurasia. It was first detected in Portugal, in 1999, associated with *Pinus pinaster*, a very susceptible species to PWN. In southern Portugal, *P. pinaster* coexists with *P. pinea* that was demonstrated to be less susceptible. Field studies and glasshouse assays with *P. pinaster*, *P. pinea* and *P. radiata* were performed to assess whether the differences in constitutive and inducible defences (total phenolics and condensed tannins contents) determine the pine species susceptibility to PWN. Host co-evolution with the pathogen was also evaluated including the study of the North American *P. radiata*. In the field, *P. pinaster* mortality was positively related with PWN abundance and with total phenolics and condensed tannins contents. *Pinus pinea* presented higher levels of defences than *P. radiata* and *P. pinaster*. In the glasshouse assays, under controlled conditions, constitutive investment in defences and the potential inducibility of those defences by PWN inoculation (2000 nematodes) were analysed in 2 year-old seedlings. Slower growing *P. pinea* revealed higher levels of constitutive defences than fast growing *P. pinaster* and *P. radiata*. However, North American *P. radiata* seedlings presented an inducible response to PWN infection, effective on slowing the progression of the disease, suggesting a co-evolution with the pathogen. These studies highlight the importance of data integration on pines life history traits, related with growth rate, and production of constitutive and inducible defences, into predictive models for this invasive forest pest.

Keywords: *Bursaphelenchus xylophilus*; condensed tannins; *Pinus* spp.; total phenols.

Efficacy of *Eclipta prostrata* extract and terthiophene against the pinewood nematode, *Bursaphelenchus xylophilus*, in laboratory and field tests

Jin Hee Shin¹, OhGyeong Kwon², Chae Min Lee³, Sang Myeong Lee⁴, Young Hwa Choi⁵, Jin Ho Kim⁵ and Dong Woon Lee¹

¹ Department of Ecological Science, Kyungpook National University, Sangju, 37224, Republic of Korea

² Syngenta Korea Ltd. K.P.O Box 1676, Seoul, 03160, Republic of Korea

³ Korea Forestry Promotion Institute, Gangseo-gu, Seoul, 07570, Republic of Korea

⁴ SM Bio. Co. Ltd. Jinju, 52828, Republic of Korea

⁵ School of Ecology and Environmental System, Kyungpook National University, Sangju, 37224, Republic of Korea.

corresponding author: ychoi@knu.ac.kr

Pinewood nematode (PWN), *Bursaphelenchus xylophilus* is one of the most serious forest pest in Korea. PWN in Korea was firstly recorded in 1988 and spread widely to the whole country. Control of PWN in Korea is done in two ways: 1) trunk injection is one of the effective methods to protect the live pine tree from nematode infection; and 2) fumigation is the main elimination method for PWN infected pine tree. The objective of this research was screening of environmentally friendly effective nematicidal materials from 88 Vietnam plant material and 21 bioactive material (α -terpinene, α -pinene, β -thujaplicin, cinnamaldehyde, eugenol, emodin, geraniol, limonone, methyl palmitate, matrine, myrcene, methyl gallate, nicotine, quassin, resveratrol, rotenone, thymol, thujaplicin, terthiophene, tuberstemone, γ -terpinene) isolated from plants for trunk injection agents against PWN. Of the plant extracts, *Eclipta prostrata* extract was highly effective against PWN, and terthiophene had the best nematicidal activity overall. Foliar spray of terthiophene on Japanese black pine (*Pinus thunbergii*) tree reduced proliferation of inoculated of PWN in 30 days after treatment, but was no more effective after 30 days. *Eclipta prostrata* extract and terthiophene can be used as nematicides when additional nematicidal efficacy tests against other plant-parasitic nematodes have been done.

Keywords: Environmental friendly control; pine tree; plant extract; trunk injection.

Main components of essential oils in the control of the pinewood nematode

Pedro M. Barbosa¹, Ana M. Rodrigues², Jorge M.S. Faria², José G. Barroso², Luis G. Pedro², A. Cristina Figueiredo², Manuel M. Mota^{1,3}

¹ NemaLab, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal.

² Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal (CBV), Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal.

³ Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal.

corresponding author: mmota@uevora.pt

The pine wilt disease is associated to the pinewood nematode, *Bursaphelenchus xylophilus*. This phytoparasitic nematode is extremely difficult to eradicate and control procedures are extremely costly besides being hazardous to the environment and having only a preventive action. Today, it is commonly accepted that essential oils are a valuable tool for disease control, offering new fields of research. The joint efforts of NemaLab (Universidade de Évora) and Centro de Biotecnologia Vegetal (CESAM Lisboa) in the evaluation of the nematotoxic activity of essential oils obtained from the Portuguese flora, lead to the identification of *Ruta graveolens*, *Satureja montana* and *Thymbra capitata* nematotoxic essential oils, able to induce $\geq 96\%$ mortality after 24 h exposure. Essential oil composition was analysed by gas chromatography and gas chromatography coupled to mass spectrometry. Bioassays, following the standard methodology, were performed with the essential oils' main components, namely oxygen-containing compounds, trans-anethole, carvacrol, citral, eugenol, geraniol, linalool, thymol, 2-undecanone, and the monoterpene hydrocarbons, p cymene, limonene and γ -terpinene. From oxygen-containing compounds, only linalool was unable to achieve 100% mortality at 2mg ml⁻¹ after 24 h exposure, whereas with the monoterpene hydrocarbon molecules the mortality was <10%. The nematotoxic activity of other essential oil components is being evaluated in an ongoing project. Acknowledgments: This study was partially funded by FCT, under PhD grant SFRH/BD/43738/2008 and research contract.

Keywords: *Bursaphelenchus xylophilus*; hydrocarbon molecules; *in vitro* bioassays; nematotoxics; oxygen-containing molecules.

Natural control of nematodes with organic compost on carrot crop

Luísa I. Coelho¹, Francisco A. Bueno-Pallero¹, Mário M. Reis^{1,2} and Maria A. Gonçalves^{1,2}

¹ Universidade do Algarve, FCT, Campus de Gambelas, 8005-114 Faro, Portugal

² MeditBio, Centre for Mediterranean Bioresources and Food, Universidade do Algarve, Campus de Gambelas – Edifício 8, 8005-139 Faro, Portugal

corresponding author: lcoelho6@gmail.com

Compost application to the soil, in addition to its effects as fertiliser, has the capacity to suppress certain soil diseases and pests, thus reducing the use of pesticides, contributing to the sustainability of agriculture. The aim of this work was to study the effect of compost application to the soil on the natural control of phytopathogenic nematodes. Compost (Organical®) was applied to a sandy soil, at the rate of 0; 12.5; 25, 50 and 100 t ha⁻¹ and incorporated at 10 to 15 cm depth. Carrot (*Daucus carota* L.) 'Nanes 5' was seeded and, following emergence, plant density was adjusted in order to obtain a crop density of 6.25 plants m⁻². At the end of growing period, carrot roots were observed and the damage caused by nematodes was ranked. At the same time soil samples were collected from the rizosphere. A decrease of the root damage caused by the nematodes was observed, directly related with the increasing rate of compost. By contrast, the number of beneficial nematodes increased with the compost rate. Among beneficial nematodes, predator and entomopathogenic species were identified, belonging to the genera *Diplogaster*, *Heterorhabditis*, *Steinernema* and *Rhabditidia*. These genera dominated in the soil samples with higher compost rate. Phytopathogenic nematodes, mainly present in the treatments with less compost, belonged to the genera *Longidorus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus* and *Rhabditis*. Results suggest that compost can reduce the population of phytopathogenic nematodes in the soil, without the use of pesticides, while increasing beneficial nematodes populations.

Keywords: Biocontrol; entomopathogenic; predators; soil; sustainable agriculture.

Comparative 16S targeted metagenomic characterisation of endogenous bacterial microbiota of root-knot nematode *Meloidogyne javanica* females in relation with infection by the endospore forming firmicute bacteria *Pasteuria penetrans*

Carlos Condemarín¹, David Lindo², Savina Gutiérrez², Juan Quimi³, Carolina Barreto⁴, César Túllume⁴, Paulo Duarte⁴, Gustavo León⁴ and Eric Mialhe³

¹Biotecoop, calle Filipinas 214, Tumbes, Perú

²Universidad Nacional de Tumbes, Av. Universitaria s/n, Tumbes, Perú

³Inca' Biotec SAC, calle Filipinas 212, Tumbes, Perú

⁴Ecosac Agrícola SAC, Car. Chapaira s/n, Chapaira, Piura, Perú

corresponding author: ccondemarin@gmail.com

The root-knot nematode, *Meloidogyne javanica*, has been recently identified molecularly in Peruvian vineyards. The endospore forming firmicute bacteria *Pasteuria penetrans* has been observed frequently as bacterial endospores externally fixed on second-stage juveniles (J2) and has been detected at an extremely low relative abundance in some vineyard soil microbiota by metagenomic analysis. *Pasteuria* is considered as non-cultivable bacteria and it is assumed that endospores are exclusively produced inside nematode females infected at the J2 stage, suggesting that some female endogenous factors are indispensable for *Pasteuria* multiplication and endospore formation, possibly some bacteria from female endogenous native microbiota. Endogenous bacterial microbiotas of and non-infected nematode females have been comparatively characterised by 16S targeted metagenomics, with 56% and 76% unclassified sequences respectively. In *Pasteuria*-infected microbiota, 112 bacterial species and 65 genera were identified, the most abundant being *Pseudomonas* (10.4%), *Paenibacillus* (7.0%), *Variovorax* (6.7%), *Acidovorax* (2.9%), *Pasteuria* (2.7%), *Sphingomonas* (2.6%), *Actinomadura* (1.2%), *Bacillus* (1.1%), *Brevundimonas* (1.1%), *Cytophaga* (0.8%), *Marinobacter* (0.7%), *Methylobacillus* (0.6%). In non-infected microbiota, 131 bacterial species and 85 genera were identified, the most abundant being *Pseudomonas* (16.7%), *Flavobacterium* (5.3%), *Methylobacillus* (3.4%), *Chitinophaga* (1.1%), *Acidovorax* (1.1%), *Aeromonas* (0.3%), *Serratia* (0.3%), *Cytophaga* (0.3%), *Chryseobacterium* (0.2%), *Burkholderia* (0.2%), *Spirochaeta* (0.1%), *Selenomonas* (0.1%). Such important differences in endogenous microbiota of *Pasteuria*-infected and non-infected females could be related to *Pasteuria* multiplication and endospore formation with specific contribution of bacteria from female native microbiota.

Keywords: Microbiome; nematode; NGS; *Pasteuria*.

Nematicidal effects of essential oils from *Thymus serpyllum*, *T. vulgaris* and pure thymol on stem and bulb nematode (*Ditylenchus dipsaci*)

Ondřej Douša¹ and Miloslav Zouhar²

¹ Crop Research Institute, Division of Plant Health, Drnovská 507, 161 06 Prague 6 – Ruzyně, Czech Republic

² Czech University of Life Sciences, Faculty of Agrobiological Sciences, Department of Plant Protection, Kamýcká 129, 165 21 Prague 6 – Suchbátka, Czech Republic

corresponding author: douša@vurv.cz

Stem and bulb nematode (*Ditylenchus dipsaci*) recently became a problem in the Czech Republic. This is especially a consequence of the renaissance of growing garlic in this territory. High demand for seed material sometimes leads to planting of garlic cloves infested with *D. dipsaci* and subsequent damage. Moreover *D. dipsaci* is listed as a quarantine pest by Czech as well as European authorities and its presence in seed material is not acceptable. As there are no registered substances for chemical treatment of garlic against this pest, the aim of the research was to evaluate nematicidal effects of essential oils from *Thymus serpyllum*, *T. vulgaris* and pure thymol on mortality of *D. dipsaci* under *in vitro* conditions. Results show overall good efficiency of the all tested chemicals on *D. dipsaci* mortality with the best effect of the oil from *T. vulgaris*. Subsequent research will focus mainly on formulation of this oil for practical utilisation and detailed study of its phototoxic properties. The research was supported by the Czech Ministry of Agriculture, project number QJ1310226.

Keywords: *Allium sativum*; nematode management; plant essences.

**Alternative methods for management of sugarbeet nematode (*Heterodera schachtii*)
– results from 1st year of trials**

Ondřej Douša¹, Miloslav Zouhar², Jaromír Chochola³ and Pavel Fridrich⁴

¹ Crop Research Institute, Division of Plant Health, Drnovská 507, 161 06 Prague 6 – Ruzyně, Czech Republic

² Czech University of Life Sciences, Faculty of Agrobiological Sciences, Department of Plant Protection, Kamýcká 129, 165 21 Prague 6 – Suchbátka, Czech Republic

³ Sugar Beet Institute, 294 46 Semčice 69, Czech Republic

⁴ Moravskoslezské Cukrovary, a.s., Cukrovarská 657, Hrušovany nad Jevišovkou 671 67, Czech Republic

corresponding author: douda@vurv.cz

Sugar beet production constitutes important and traditional segment of agriculture business in the Czech Republic. Among pests of sugar beet, *Heterodera schachtii* still presents a problem. Even if resistant cultivars are widely available research of alternative methods of sugar beet protection against this pest should be investigated. Monogenically based resistance of current sugar beet cultivars could be overcome in the future so designing of functional antiresistant strategy with direct protection measures would be great benefit for sugar beet producers. As a part of research project aiming development of alternative methods for management of *H. schachtii*) the influence of application of plant essences, nematophagous fungi and plant stimulants were tested. Even if statistically significant differences were not observed when treated and untreated variants were compared, certain trends showed at least partial function of the tested methods, which will be utilized in the next research. The study was supported by the Technology Agency of the Czech Republic, project number TA04021117.

Keywords: *Beta vulgaris* ssp. v. convar. *vulgaris* var. *altissima*; nematophagous fungi; plant essence; plant stimulant.

An investigation into plant-parasitic nematode and endophytic bacterial interactions

Aoife Egan and Thomae Kakouli-Duarte

Molecular Ecology and Nematode Research Group, enviroCORE, Dargan Centre, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

corresponding author: aoife.egan@itcarlow.ie

Plant-parasitic nematodes cause billions of dollars annually in global crop damage, and their control relies heavily on chemical pesticides. Endophytic bacteria colonise the internal structures of host plants, indirectly promote plant growth and induce plant defence. The work presented investigates the susceptibility of plant-parasitic nematodes to endophytic bacteria and the secondary metabolites they produce. This work is currently ongoing. *Meloidogyne javanica* and *Globodera pallida* were tested along with *Caenorhabditis elegans* and *Pristionchus pacificus*, which were used as controls. The nematodes were subjected to several strains of endophytic *Pseudomonas* isolates, from the enviroCORE collection, and different concentrations of the antibiotic they produce (2, 4-diacetylphloroglucanol). Two approaches were adopted to evaluate the sensitivity of the nematodes: (1) The effect of endophytic bacteria and 2, 4-diacetylphloroglucanol on nematode hatch and juvenile mortality in *M. javanica* and *G. pallida* was investigated. The average percentage hatch for *M. javanica* was 9% and 16% for *G. pallida*, compared to 69% and 66% in the controls, respectively. The results also indicate that up to 100% juvenile mortality occurred in *M. javanica* and *G. pallida* in the presence of endophytic bacterial metabolites. (2) The effect of endophytic bacteria on nematode development in tomato and potato plants was studied. The plants were inoculated with endophytic bacteria and 2, 4-diacetylphloroglucanol and infected with *M. javanica* and *G. pallida*. Observations will take place at 14, 28 and 45 days after nematode infection in plants. The results of this study will enhance understanding of these types of interactions, with a view towards developing efficient strategies for the biological control of plant-parasitic nematodes.

Keywords: Biocontrol; development; infectivity; mortality; susceptibility.

Management of cyst nematode of rice (*Heterodera sacchari*) with agricultural waste

Oluwatoyin A. Fabiyi¹, Gabriel A. Olatunji², Oluremi S. Osunlola³ and Ajeka. K. Umar¹

¹ Faculty of Agriculture, Department of Crop Protection, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

² Faculty of Physical Sciences, Department of Industrial Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

³ College of Agriculture, Department of Crop Production, Kwara State University, Malete, p.m.b, 1530, Kwara State, Nigeria

corresponding author: fabiyitoyinike@hotmail.com

Pests bring about significant decrease in rice production, which translates into huge financial loss in export trade. Plant-parasitic nematodes are economically important pests of rice. Export trade restrictions are often imposed due to the presence of quarantine nematode pests. Among the important nematode species that attack rice, the cyst nematode is on the regulatory pest lists of several countries. Different management strategies have been employed in the control of plant-parasitic nematodes of rice with various degrees of success. The use of synthetic nematicides, though an established internationally recognised effective approach is negated by the toxicity and adverse effects on humans and the environment. This has warranted worldwide intensive research for safer alternatives. Agricultural wastes have been identified as an excellent renewable source for the production of bio-pesticides. Corn cobs (CNCB), Rice husks (RCEH) and sorghum husks (SGMH) were air dried, milled and hydrolysed with 30% sulphuric acid at 2000°C to produce furfural, a bio-pesticide that was tested as an alternative to synthetic nematicide carbofuran in the management of rice cyst nematode (*Heterodera sacchari*). The yield of furfural from 50 g of each agricultural waste material was determined and this was used to evaluate the amount of furfural in 100, 150 and 200 g agro-wastes as soil admixes in the experiments. Results showed that the sorghum husk (SGMH) at 200 g was significantly ($P=0.05$) more effective than all other treatments. Agricultural wastes, especially sorghum husks, can serve as an alternative to synthetic nematicides. Furfural is quickly broken down by soil microorganisms under aerobic conditions; hence, it can be regarded as non-toxic to the environment.

Keywords: Bio-pesticide; extraction; furfural; hydrolysate; pollution.

Assessment of several plant extract products against *Meloidogyne javanica* on tomato plants

Magda Galeano, Jesús Moreno and Jose E. Belda

Koppert España, S.L. R&D Department, C/ Cobre, 22-24, Polígono Industrial Ciudad del Transporte de Poniente, 04745 La Mojonera, Almería, Spain

corresponding author: mgaleano@koppert.es

For the assessment of the nematicide or nematostatic effects of two different plant extract products on *Meloidogyne javanica*, we carried out two type of trials: firstly, an *in vitro* assay and then a semi-field pot trial at the Research Koppert facilities in Vicar (Almería, Spain). The laboratory bioassay demonstrated that both products produced high mortality on second-stage juveniles of *Meloidogyne javanica*. Semi-field trial in pots consisted of five treatments: 1) inoculated control, 2) chemical standard, 3) plant extract (thyme) + humic acids, 4) plant extract (cinnamon) 5) non-treated control. Each treatment was replicate ten times in an unit formed by 1 plant per pot. Experimental products were applied four times, one application 3 days before nematode inoculation, the second application 48 h after inoculation, the third application after 2 weeks and the fourth application after 4 weeks, always at a rate of 0.1% of the a.s. At the end of the crop (more than 8 weeks after the nematode inoculation), the damage by the plant-parasitic nematodes to roots was evaluated by assessing the gall index, the free juvenile nematodes in soil, and the multiplication of the nematodes by assessing the eggs per g of roots. Although treatment 3 reduced the damage on the treated plants compared with the inoculated control by a similar level to the chemical standard, the phytotoxicity of the product to small plants disqualify it as a candidate for nematode control, and we can only advise the use of the tested product in the treatment 4, as it reduces the damage on roots significantly compared with the inoculated control and shows very low gall index values.

Keywords: *in planta*; *in vitro*; *Meloidogyne*; plant extracts; tomato.

Assessment of the Natugro™ system to control *Meloidogyne* spp. in protected tomato crops in the south of Spain

Magda Galeano, Jesus Moreno, María Victoria Rodriguez, Julian Rodriguez, Luis Santiago, Alicia Ruiz and Jose Eduardo Belda

Koppert España, S.L. R&D Department, C/ Cobre, 22-24, Polígono Industrial Ciudad del Transporte de Poniente, 04745 La Mojonera, Almería, Spain

corresponding author: mgaleano@koppert.es

Two field trials were carried out in commercial glasshouses in Almería (Spain) suffering historical big infestations with root-knot plant-parasitic nematodes (*Meloidogyne* spp.) Both trials were performed in long cycle tomato crops (planting dates July-September and finishing in June) under IPM strategy. One of them was located in La Paula, in the East of Almeria province, cultivating a local fresh tomato (Raf type, cv. Dumas) in 833 m² into one of the four sectors of 2.500 m² in which the glasshouse was divided. The second trial was located in La Cañada, very close to Almeria city, cultivating too a fresh tomato (cv. Ramyle) in 486 m² included in a typical Almeria glasshouse of 11.600 m². The trials were focused to the reduction of root knot plant-parasitic nematodes using a strategy of crop management (NatuGro™ System), testing the effect of several Koppert NatuGro products and the bionematicide product KC0413 in different combinations, under local glasshouse conditions. The effects were assessed on the following parameters: free juvenile population in soil, root damage and reproduction of the nematode. Both trials consisted of four treatments: 1) Untreated control, 2) KC0413 alone, 3) Natugro + TH Promotor, and 4) Natugro + KC0413, with three replicates per treatment. All the applications of the products to the soil were performed according the technical protocol of the NatuGro System. After the assessments on the two trials results, we can conclude that the NatuGro System including TH Promotor (Trial 3) is a good option to manage the nematode population in soil and the level of damage in the roots. The NatuGro System, as a strategy to improve the biodiversity of the organisms into the soil, in this case also the free-living soil nematode species, is a good option to reduce the level of the damage in plants from the plant-parasitic *Meloidogyne* populations.

Keywords: *Meloidogyne*; Natugro System; protected crop; South of Spain; tomato.

Effect of application of the nematode parasitising fungus *Clonostachys rosea* on nematode communities and plant health

Mudassir Iqbal¹, Maria Viketoff², Mukesh Dubey¹, Dan F. Jensen¹ and Magnus Karlsson¹

¹ Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-75007, Uppsala, Sweden

² Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, SE-75007, Uppsala, Sweden

corresponding author: mudassir.iqbal@slu.se

Plant diseases caused by plant pathogenic nematodes are serious constraints to sustainable crop production due to high yield losses, the persistent nature of the nematodes and the lack of efficient control methods. *Clonostachys rosea* is a nematode parasitising fungus that also can control various different fungal plant pathogens. In this study, we have tested the nematode antagonistic potential of *C. rosea* (2.5×10^5 spores g^{-1} soil) on nematode communities in a naturally infested soil planted with wheat and carrot under controlled conditions (relative humidity: 70%, temperature: 15°C, light and darkness: 12/12 h) and relating it to plant health. Our results show that the numbers of several genera of plant-pathogenic nematodes (such as *Pratylenchus*, *Tylenchorhynchus* and *Helicotylenchus*) extracted from soil and roots significantly decreased when *C. rosea* was applied, while genera of non-pathogenic nematodes (bacterivores, fungivores, and omnivores) were not affected. In addition, fresh shoot weight and shoot length of wheat plants significantly increased with the strongest impact on shoot length, whilst in carrot plants we only observed a significant effect on average dry weight of shoot. The statistical analysis suggests a direct effect of *C. rosea* on plant-pathogenic nematode communities and plant health. This study clearly demonstrated that application of *C. rosea* leads to control of plant-pathogenic nematodes and at the same time improves the growth of plants. The antagonistic potential of *C. rosea* can be used to control plant-pathogenic nematodes, which may contribute to reduced applications of chemicals in an integrated pest management perspective.

Keywords: Biological control; integrated pest management; plant growth parameters; plant pathogenic nematodes.

Extrinsic factors governing the spore encumbrance of an indigenous population of *Pasteuria* hyperparasitising root-knot nematodes, *Meloidogyne* speciesAnju Kamra¹, Swapnil Pawar¹, Keith G Davies² and Sharad Mohan¹¹ Division of Nematology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India² Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

corresponding author: sharad@iari.res.in

Isolates of indigenous populations of gram-positive endospore-forming bacterial hyperparasite *Pasteuria* spp. infecting root-knot nematodes were made from arid, semi-arid, humid and sub humid ecosystems of India. A semi-arid population was successfully multiplied on cowpea (*Vigna unguiculata*), cv. Pusa Komal under soil-less conditions in growth pouches maintained at 25°C for 32±2 days. The recovery of *Pasteuria* infected females varied from 38.4 to 91.3% per plant. A nematode inoculum of 15 second-stage juveniles (J2) per root tip with 15 spores per J2 resulted in significantly higher number of infected females than inoculum levels of 5 or 10 J2 per root tip with 10 spores J2⁻¹. Host-specificity assays with four species of root-knot showed a greater affinity of endospore attachment to *Meloidogyne incognita* (6.25 spores J2⁻¹), followed by *M. javanica* (3.90), *M. hapla* (1.10) and *M. graminicola* (0.90) at a spore density of 6.9 × 10⁵ spores ml⁻¹. Endospore attachment was not observed on infective juveniles of *Heterodera cajani*, *H. avenae* or *Rotylenchulus reniformis*, nor to the developmental stages of *M. incognita* (third- and fourth-stage juveniles and males). A pre-exposure of spores to high temperatures (40, 45 and 50 °C) resulted in a progressive decline in spore attachment on *M. incognita* J2 with an increase in exposure period; resulting in 81.5% decline on exposure at 50°C for 10 min. Preincubation of the *Pasteuria* endospores with *Bacillus cereus*, *B. subtilis* and *B. pumilus*, followed by spore attachment assays with *M. incognita* J2, exhibited a decline in attachment of 97.2, 94.6 and 96.5%, respectively, over the untreated controls.

Keywords: Growth pouches; *Meloidogyne* spp.; *Pasteuria*; temperature.

Potential of BioAct for biological control of nematodes and yield enhancement in high value crops

Sebastian Kiewnick

Agroscope, National Competence Center for Nematology, 8820 Waedenswil, Switzerland

corresponding author: sebastian.kiewnick@agroscope.admin.ch

For many years, research on biological control of nematodes has focused on a small group of organisms that seemed suitable for mass production and commercial applications. *Purpureocillium lilacinum* strain 251 (PL251, syn. *Paecilomyces lilacinus*) is one of the few commercialised strains registered for nematode control as BioAct. One of the main restrictions to foster the use of biological nematicides was the shelf life of the formulated product. Therefore, for the strain PL251, different formulation types from water dispersible granules, to water dispersible powder and recently a liquid dispersible concentrate have been developed. As a change of formulation can affect the biological efficacy, a thorough testing of the biological efficacy was required. However, over the past 14 years, the overall efficacy of PL251 was never affected, but differences in the biological activity were observable. Although shelf life and handling were improved over the years, acceptance by farmers has not evolved in the same way. One reason might be that biocontrol efficacy mostly affects root damage, but yield is not always improved as expected. One main field of use is the control of root-knot nematode species (*Meloidogyne* spp.) damaging tomato or other high value crops. Over the past years, a number of semi commercial glasshouse trials have been conducted to establish a correlation between the reduction of root damage and fruit yield. In these trials, tomato fruit yield increased between 11 and 22%. It was further demonstrated, that, depending on the setup of the experiment, significant reduction of root damage and reproduction of root-knot nematodes not always allowed for higher yields. The main reasons were the short cultivation time and plant responses to a lower nematode invasion early on in the growing season. Therefore, efficacy of biological nematicides, such as PL251, must be seen in the context of plant responses to nematode damage at different plant developmental stages.

Keywords: Biocontrol fungus; formulation; *Meloidogyne*; root-knot nematodes.

Alteration in endospore attachment of *Heterodera cajani* specific *Pasteuria* to different age groups of *H. cajani* second-stage juveniles exposed to host and non-host root exudatesKommu Kiran Kumar¹, Keith G. Davies² and Sharad Mohan¹¹ Division of Nematology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India² Department of Biological and Environmental Sciences, University of Hertfordshire, Harfield, AL10 9AB, UKcorresponding author: sharad@iari.res.in

Plant root exudates and rhizosphere microbes, including nematodes, are involved in highly specific chemical communication that we know little about. Freshly hatched second-stage juveniles (J2) of the pigeon-pea cyst nematode and those left in water for 7 and 14 days were separately exposed for 24 h to the root exudates of their host plant (cowpea) and non-host plants (tomato and potato). These J2 were then used for performing spore attachment tests with the *Pasteuria* isolate from *H. cajani*. Exposure to the root exudates of tomato resulted in a significant decrease in attachment by 57.7, 65.1 and 77.6% in fresh, 7 and 14 days old J2, respectively, over the water control. By contrast, when fresh and 7 day-old J2 were incubated in root exudates from potato, which is also a non-host for *H. cajani*, the attachment of endospores was found to be on par with cowpea root exudates and the water control. However, the 14 day old J2, when incubated in cowpea and potato root exudates, showed a significant increase in attachment by 73.8 and 90.6% over the water control. Interestingly root exudates from potato were able to stimulate cuticular changes in the J2 that promoted Hcp endospore attachment. This population of Hcp, originally isolated from *H. cajani* exhibits cross-generic attachment and life cycle completion on the potato cyst nematode *Globodera* spp. In a more time-sensitive assay, incubation of fresh J2 in tomato root exudates for 1 h reduced the attachment significantly by 28.3%, which further decreased to 52% after 2 h incubation over the water control. Endospore attachment performed with J2 of *H. cajani* retrieved from cowpea roots after 24 and 48 h of penetration showed a drastic reduction in attachment by 83.4 and 98.3%, respectively, over non-inoculated control. These results show that the nematode cuticle is highly dynamic and that the host plant has unpredictable consequences regarding *Pasteuria* endospore attachment and therefore biocontrol outcomes.

Keywords: *Heterodera cajani*; cowpea; tomato; potato.

Effects of biostimulants on *Meloidogyne hapla* in carrot

Ivana Majić¹, Ankica Sarajlić¹, Branimir Nježić² and Gabriella Kanizai Šarić¹

¹ Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek, Kralja Petra Svačića 1d, Osijek, Croatia

² Faculty of Agriculture, University of Banja Luka, Bulevar vojvode P. Bojovića 1a, Banja Luka, Bosnia and Hercegovina

corresponding author: imajic@pfos.hr

The current restrictions of nematicides in carrot and need for development of environmentally safe products have contributed to increased research of biocontrol agents. A gap in knowledge still exists regarding multitrophic interactions of soil microorganisms. The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is a economically important pest of carrot (*Daucus carota* L.). In Croatia, *M. hapla* is causing severe yield losses on carrot grown in glasshouses and in the field. Our research was conducted in a glasshouse during winter (spring crop) where we assessed the potential of commercially available plant biostimulants of microbial origin, fermented plant extracts, humic substances and stone powder to protect carrot yield against *M. hapla*. Initial population of *M. hapla* was on average 320 second-stage juveniles (J2), and final population at harvest ranged from 22.50 to 100 J2 in 100 ml of soil. The lowest, statistically significant nematode populations were detected in treatment with combination of beneficial soil microorganisms and stone powder. Combination of beneficial soil microorganisms and humic substances caused significantly greater root mass under moderately high nematode pressure, indicating improved plant tolerance. There were no significant effects of other treatments on total plant mass and index of galling. Population dynamics of *M. hapla* on average declined during the vegetation period in all treatments because of low soil temperatures, and marketable carrot yield was satisfactory. Beneficial soil organisms did not suppress population of *M. hapla*. However, promising results were obtained when combined with stone powder and humic substances. Further research is needed in conditions optimal for nematode development.

Keywords: Beneficial microorganisms; carrot yield; humic substances; plant extracts; root-knot nematodes; stone powder.

Characterisation of the putative antigenic determinants on *Pasteuria* endospore surface using *Bacillus thuringiensis* as a comparative tool

Arohi Srivastava¹, Sharad Mohan² and Keith G Davies¹

¹Department of Biological and Environmental Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK

²Division of Nematology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India

corresponding author: a.srivastava@herts.ac.uk

Biological control agents are emerging as potential environmentally benign means of combating phytonematodes across the world. Some species of *Pasteuria* are endospore forming bacterial hyperparasites of nematodes associated with nematode suppressive soils. The prospective use of these bacteria in nematode management is well acknowledged by the scientific community. However, the mechanism by which *Pasteuria* endospores attach to the nematode cuticle and infect their nematode hosts is yet to be understood. Previously the involvement of parasporal collagen-like fibres present on the exosporium of *Pasteuria* endospores have been suggested as specificity determinants. Collagen-like fibres similar to *Pasteuria* parasporal fibres are known to exist and are well characterised on the exosporium of other closely related animal pathogenic *Bacillus* spp. Using comparative studies we explore the surface architecture and antigenic properties of *Pasteuria* endospores. Protein extracts from endospores of *Bacillus thuringiensis* and *Pasteuria* were compared using Western blot analysis. Antibodies raised to whole *Pasteuria* endospores and to synthetic peptides of *Pasteuria* collagen-like proteins showed cross-reactivity to endospore extracts of *B. thuringiensis*. Further antigenic similarities in their exosporium have been confirmed by immunofluorescence microscopic studies where endospores from both the genera were labelled equally with *Pasteuria*-specific antibodies. To understand further the determinants involved in the *Pasteuria*-nematode attachment process, *Pasteuria* endospores were treated with collagenase, β -N acetylglucosaminidase and anti-collagen antibodies and *in vitro* attachment assays were performed. We observed a marked decrease in the number of endospores attached to the nematode juveniles.

Keywords: *Bacillus* endospores; collagen-like proteins; enzyme digestion; immuno-characterisation; phytonematodes.

Assessment of *in vitro* activity of different *Bacillus firmus* strains against the root-knot nematode *Meloidogyne incognita*

Nik Susič¹, Saša Širca¹, Maja Rupnik², Gregor Urek³ and Barbara Gerič Stare³

¹ Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

² Institute of Public Health Maribor, Centre for Microbiology, Prvomajjska 1, SI- 2000 Maribor, Slovenia

³ Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

corresponding author: nik.susic@kis.si

Root-knot nematodes of the genus *Meloidogyne* are the most damaging of all plant-parasitic nematodes in terms of yield loss and because of their broad range of host plants. As many nematicides used in the past have been banned because of environmental concerns, development of alternative control strategies for root-knot nematodes is urgently needed. Application of biological control agents that act as antagonists to plant pathogens and pests can be effective alternatives and recently have been increasingly used. In our study we assessed *in vitro* activity of different *Bacillus firmus* strains against *Meloidogyne incognita*. *Bacillus firmus* strain I-1582 was isolated from the Votivo® formulation containing the bacteria. Two additional *B. firmus* strains were isolated from garden soil, where peas were previously grown, in Slovenia. Bacterial identity was confirmed by sequence analysis as *B. firmus*. *M. incognita* eggs were exposed *in vitro* to bacteria cultured in LB medium, culture filtrates and washed bacteria cells in buffer. The number of hatched juveniles and motility of juveniles was recorded and compared to control treatments in sterile water, buffer and sterile fresh LB medium. Inhibitory effect on nematode hatching and motility was significant and highest in the treatment where bacterial culture was added to nematode eggs. Extracellular metabolites in culture filtrate as well as direct contact of washed bacteria cells with nematode eggs also showed inhibition of hatching and effect on juvenile motility. Differences were observed in activity of different tested *B. firmus* strains.

Keywords: Bacteria; biological control; culture filtrate; mortality; paralysis.

Microbial attachment to endoparasitic phytonematodes in soil

Olivera Topalović¹, Johannes Hallmann² and Holger Heuer¹

¹ Julius Kühn Institut, Messeweg 11-12, 38104 Braunschweig, Germany

² Julius Kühn-Institut, Toppeideweg 88, 48161 Münster, Germany

corresponding author: olivera.topalovic.best@gmail.com

Evidence is mounting for a potential of naturally soil-occurring microorganisms to suppress infection of host plants by plant-parasitic nematodes, and a role of microbe-induced systemic resistance of the plant in this suppression. Recent studies of our group have shown the attachment of specific bacteria and fungi to the cuticle of *Meloidogyne hapla* in soil. Attached microbial species varied between soils with different potential to suppress invasion and reproduction rate of the nematode on tomato plants. We hypothesised that microbes attached to second-stage juveniles were responsible for induced systemic resistance in plants, rather than directly antagonising nematodes. Consistent with that, the objectives of our project are to identify the microbiome attached to second-stage juveniles of *M. hapla* in potentially suppressive soils and to investigate the specificity of attachment between different populations and nematode species by culture-dependent methods and next generation sequencing. Furthermore, we want to confirm the induction of plant systemic resistance by nematode-attached microbes and to unravel which defence genes are responding to invading nematodes with or without attached microbes from soils that differ in suppressive potential. In respect to this, we have already tested ten soils for their suppressiveness and found a significantly reduced penetration and reproduction of *M. hapla* for some of them. We established an experimental system for assessing soil suppressiveness by baiting of surface-sterilised second-stage juveniles in soil suspensions and applying them to tomato plants grown in a sterile environment, and protocols for RNA extraction from roots and q-RT-PCR to analyse the expression of defence genes. We aim to provide a deep understanding of the role of nematode-attached microbes in nematodes suppression and plant protection, which will be the basis for including a soil microbiome management in integrated control strategies.

Keywords: Cuticle; juveniles; plant defence; root-knot nematode; suppressiveness.

Methods to measure the oxygen consumption rates of *Meloidogyne incognita* and *M. javanica* exposed to Soil Bio-Muti, a soil derived nematicidal solution

Willie J. van Aardt

Noordwes Universiteit, Potchefstroom kampus, Private Bag 6001, Potchefstroom 2520, South Africa

corresponding author: willie.vanaardt@nwu.ac.za

Techniques are presented on how to use the polarographic oxygen sensor (POS) and fibre optic oxygen sensor (FOS) to measure accurately the oxygen consumption rate (OCR) of *Meloidogyne incognita* and *M. javanica*, two plant-parasitic nematodes. Different stirring speeds from 50 rpm up to 1000 rpm did not change the mean OCR of $26 \pm 1.7 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ after 20 min of stirring. Population batches from the same cultured populations of second-stage juveniles (J2) of *M. incognita*, hatched within 24 h, during a 5 month period of measurement in sterile water have OCR values of $80.7 \pm 34.0 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ for *M. incognita* and $65.9 \pm 24.7 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ for *M. javanica* using the FOS sensor. The OCR measurements for *M. incognita* with the FOS sensor were significantly higher ($359.6 \pm 73.4 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ compared to the POS sensor ($178.0 \pm 39.8 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$). For *M. javanica*, the OCR values obtained by the FOS analyser (measuring 100 individuals each) and the POS analyser (measuring 2000 individuals each) are, respectively, $126 \pm 18.2 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ and $124.4 \pm 47.7 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$. The OCR is reduced by 63.6% when exposed to 2.5% Soil Bio-Muti to less than $66.8 \mu\text{mol O}_2 \text{ h}^{-1} \text{ g}^{-1}$ as determined by the POS analyser using 48 h-old *M. incognita* J2. For *M. javanica*, OCR is reduced by 27.6% to $79.5 \pm 19.6 \mu\text{moles O}_2 \text{ h}^{-1} \text{ g}^{-1}$ as measured by the FOS sensor.

Keywords: Fibre and polarographic oxygen sensors; *Meloidogyne incognita*; oxygen consumption rate.

Combined sustainable strategies for rootknot nematodes management in protected crops – the COCOoN Project

Maria Clara Vieira dos Santos¹, Isabel Luci Conceição¹, Isabel Abrantes¹, Rosane H.C. Curtis², Isabel Mourão³, Luísa Moura³, L. Miguel Brito³ and Sofia R. Costa^{4,5}

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² Bionemax UK Ltd, Rothamsted Centre for Research and Enterprise, Harpenden, AL5 2JQ, Hertfordshire, UK

³ Mountain Research Centre (CIMO)/Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Refóios, 4990-706 Ponte de Lima, Portugal

⁴ Mountain Research Center (CIMO), Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

⁵ CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

corresponding author: mcvs@sapo.pt

The COCOoN (COMbined CONTROL of Nematodes) Project aims to develop a management strategy against root-knot nematodes (RKN), *Meloidogyne* spp., based on the combined use of a biological control agent with the activation of natural plant defence mechanisms and partially resistant rootstock cultivars. *Pochonia chlamydosporia* is an ubiquitous facultative egg parasite of cyst nematodes and RKN. The efficacy of some isolates against RKN has been demonstrated in pots, microplots and field assays. However, the fungus is not able to prevent the initial infection of roots by nematode juveniles. Therefore, its biocontrol efficacy should be enhanced by integration with other control measures that prevent initial infection. Plant defence mechanisms can be triggered by the application of defence activators and, when integrated into good agricultural practices, productivity and disease resistance can be improved. The performance of plant defence activators is often variable and may not provide the same level of disease control as nematicides. Systemic defence mechanisms may also play an important role in plant defence as a result of grafting. Vegetable grafting is an emerging technique to tackle a wide range of soil-borne pathogens. The three nematode control methods can in theory be made to work synergistically, and their combination applied in nursery plants as part of the production line. The clarification on the molecular mechanisms behind the improved nematode control obtained under laboratory conditions will help to develop sustainable and environmentally friendly management strategies in protected crops. Supported by *Fundação para a Ciência e a Tecnologia* (FCT) and COMPETE2020, POCI-01-0145-FEDER-016611 (PTDC/AGR-PRO/3438/2014).

Keywords: Grafting; *Meloidogyne*, plant defence activators; *Pochonia chlamydosporia*.

Probing plant-nematode interactions *ex planta*: Asking old questions with new tools

Deborah Cox, Leonie Wilson, Ashley Cathcart and Johnathan Dalzell

Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL Northern Ireland, UK

corresponding author: d.cox@qub.ac.uk

Plants have developed sophisticated defence mechanisms against invading pathogens, and in turn pathogens have fine-tuned recognition and parasitism of a host. Plant roots naturally shed a chemical cocktail into their immediate surroundings termed plant root exudate which interacting organisms perceive as a complement to other environmental signals. Plant root exudate generally serves as an attractant for plant-parasitic nematodes. While rich sugar content is a common attribute of exudate, the abundances of each type of sugar therein is inconsistent and minor components differ considerably both within and between species, particularly during development and stress. This essentially gives unique signatures to be de-coded and transformed into a response by nematodes. It is unclear at present which elements of this heterogeneous mix mediate behavioural changes in plant-parasitic nematodes; however, it is hypothesised that specific exudate profiles attract specific parasites. Here we present data on the use of two RNA interference approaches to probe the involvement of root-expressed ABC and sugar transporters of *Solanum lycopersicum* in regulating parasitic behaviour. These data will underpin efforts to identify differentially secreted exudate components and relevant receptors and signalling pathways in nematodes. Specifically, we demonstrate that tomato sugar transporters STP1 and STP2 are involved in exudation of monosaccharides in seedlings. Their knockdown triggers a reduction in glucose and fructose exudation which impacts key behaviours of the promiscuous *Meloidogyne incognita*, but not the Solanacea-specific *Globodera pallida*. Identifying the signalling mechanisms and compounds responsible for this chemical cross-talk between plant and nematode will facilitate a step change in our understanding of these interactions, and will facilitate improved use of cultivars with particular exudate profiles, or the manipulation of signalling by transgenic approaches.

Keywords: Exo-RNAi; exudate; host-parasite interactions; VIGS.

Support of CNPq to nematologists through PQ fellowshipVânia Moreira de Freitas

Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, Distrito Federal, Brazil

corresponding author: vania.Freitas@cnpq.br

CNPq is a Agency that foments scientific investigation in different knowledge areas and geographical regions, financing projects and fellowships. The Agronomy program (AG), belonging to the Directorate of Agricultural Sciences (DABS), has an assessor committee (AC) comprising renowned researchers in Agronomy. When CNPq publishes a public call, the proposals are analysed for scientific merit by AC. However, the final decision is made by DABS. This summary aimed to analyse data about PQ (Productivity and Research) fellowships over the last four years in Brazil. 9977 proposals were submitted in PQ Call 2012, 444 in AG program, 227 were approved with only four in nematology (two in the south and two in the Southeast of Brazil). 11043 were submitted in the PQ call 2013, 545 in AG program, 330 were approved, eight in nematology (four in the Midwest, two in the South, one in the Northeast and one in the Southeast). 10068 were submitted in the PQ call 2014, 404 in AG program, 170 approved, two in nematology (one in the Midwest and one Southeast). 10269 were submitted in the PQ call 2015, 418 in AG program, 253 were approved, seven in nematology (four in the Southeast, one in the Northeast, one in the Midwest and one in the South). There was no change between the general demand (other programs) and demand in agronomy over the years, but in absolute numbers, there was a decrease in the general number of proposals, number of proposals in agronomy and approved proposals in agronomy in 2014, but this situation did not persist in 2015. This situation is confirmed by noting the proportion of proposals approved in Agronomy in relation to total demand for this program in 2014, which was lower than the other years, 30% in 2014 and 35-40% in other years. The Nematologists who won PQ fellowship on the AG program was low in those years, ranging about 1%-3%. Nematologists from North region not won PQ fellowship in the last four years. These data help to understand nematology science in Brazil.

Keywords: Brazil; CNPq; fellowship PQ.

Food leaving in *Caenorhabditis elegans*: a simple model to define the genetic determinants of neural networks that control social interaction

Lindy Holden-Dye, Emily Feist, Adam Hudson, Fernando Calahorro, James Dillon and Vincent O'Connor

Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK

corresponding author: voconno@soton.ac.uk

Caenorhabditis elegans shows a range of environmentally cued behaviours. On defined lawns of OP50 *Escherichia coli* the worms reside on the bacterial patch and engage pharyngeal pumping to allow filter feeding. With increasing time on the OP50 adult worms show an increasing tendency to leave the food patch. The probability of adopting food leaving arises from changes in resource availability *i.e.*, feeding reduces food, triggering the worms to wander in search of an enriched sources (Milward *et al.* 2011, PNAS 108. 20672). Under conditions of our experiments the progressive increase in food leaving is not driven by food depletion. By contrast, leaving events correlate with the slow but steady increase in progeny that arise from eggs laid by the adult worms used to measure food leaving behaviour. Pre-loading plates with progeny prior to initiating food leaving measurements shows an immediate dose dependent enhancement of the basal food leaving seen on progeny naive plates. This suggests that food leaving is triggered by progeny, a conclusion reinforced by the block of food leaving in worms treated with 5'flurodeoxyuridine to prevent egg-laying. This suggests that an offspring dependent cue triggers food leaving behaviour. We have identified that distinct mutants deficient in neuromodulation and implicated in autism in higher animals are fundamental to the regulation of this 'social' behaviour. The robust quantification that can be indexed in the assay will allow for a chemical biological and genetic investigation of social interaction behaviours in the worm with an ability to highlight mechanisms important in human dysfunction.

Keywords: Behaviour; chemical cues; nematode.

Surface lipids and proteins of the potato cyst nematode *Globodera rostochiensis*

James A. Price^{1,2}, John T. Jones^{1,2} and Terry K. Smith²

¹ The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland

² BSRC, University of St. Andrews, North Haugh, St. Andrews, KY16 9ST, Scotland

corresponding author: jp203@st-andrews.ac.uk

Potato cyst nematodes (PCN) are estimated to cause over £50 million worth of crop losses in the U.K. each year. Hatching of PCN is stimulated by hatching factors released by roots of the host species. These exudates are thought to alter permeability of the eggshell in a calcium-dependent manner. Although a role for calcium-binding proteins in this process has been suggested, nothing is known about the proteins present in the eggshell of PCN. Similarly, little is known about the proteins or lipids present on the cuticle surface of PCN juveniles, although it has been shown that the infective juveniles are able to alter their surface composition to avoid damage from host defence mechanisms. This project will identify the proteins and lipids found on the eggshell surface of potato cyst nematodes and detect the changes occurring after exposure to host root exudates. The surface proteome and lipidome of the infective juvenile will also be investigated and changes to these surface components will be compared throughout the infection of the host. We have developed methods that allow labelling of proteins present on the surface of eggshells and the cuticle surface of PCN. These proteins are currently being affinity purified and analysed using mass spectrometry, this information will then be compared to the *G. rostochiensis* genome to allow identification of proteins.

Keywords: Eggshell; juveniles; lipidome; proteome; surfaces.

Probing neuropeptide-like protein function in plant-parasitic nematodes

Matthew Sturrock, Neil Warnock, Leonie Wilson, Aaron Maule and Johnathan Dalzell

Queen's University Belfast, 97 Lisburn Road, Belfast, Northern Ireland, UK

corresponding author: msturrock01@qub.ac.uk

Plant-parasitic nematodes (PPN) impose significant economic losses on global agriculture, threatening food security. EU Legislation imposing the withdrawal of numerous chemical nematicides due to environmental toxicity will increase the burden on crop production across the EU, unless novel control strategies can be developed. Localisation of neuropeptide-like protein genes in the model nematode *Caenorhabditis elegans* suggest pleiotropic roles in sensory perception, feeding, and development. Mapping neuropeptide-like protein genes across economically important PPN by *in situ* hybridisation will provide information on the expression patterns of these genes and will give insight in to the function and conservation of neuropeptide-like protein genes between species. Intriguingly, we have localised neuropeptide-like protein genes to the gonadal primordium and anterior neurons of plant-parasitic nematode second-stage juveniles for the first time, indicating potential pleiotropic roles in development, and sensory perception. RNA interference has proven invaluable in facilitating functional studies of nematode genes, improving our knowledge of basic biology, and informing control strategies. Data presented will outline *in vitro* RNA interference optimisation procedures across species. The ability to target nematode genes involved in sensory perception and development through *in planta* RNAi could facilitate next generation control approaches.

Keywords: *In situ* hybridisation; plant-parasitic nematode; RNA interference.

Brassicaceous cover crops interfere with mobility and host finding ability of *Globodera rostochiensis* juveniles

Yirina Valdes Vazquez^{1,2}, Nicole Viaene^{2,3} and Maurice Moens^{2,4}

¹ Universidade Federal de Pernambuco, Centro Acadêmico de Vitoria, Rua Alto do Reservatório s/n, Bela Vista, CEP- 55608-680, Vitoria de Santo Antão-PE, Brazil

² Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium

³ Ghent University, Laboratory of Nematology, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

⁴ Ghent University, Laboratory for Agrozoology, Coupure links 653, 9000 Ghent, Belgium

corresponding author: yirina80@yahoo.es

For many decades, European farmers have faced difficulties for the management of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. These quarantine species cause severe yield reduction in potato, as their biological characteristics allow them to survive in the soil for several years. In addition, there is a need for environmentally desirable strategies for nematode control, as chemical nematicides have been restricted in several countries. Crop rotation practices and soil amendments with plants from the Brassicaceae family received much attention due to their satisfactory biofumigant effect on soil-borne pathogens caused by a series of compounds (e.g., isothiocyanates) released at the breakdown of their tissues. This study examined the effects of root diffusates and extracts from brassicaceous plants on the movement and sensory response of *G. rostochiensis* second-stage juveniles (J2) towards the host. Root diffusates and extracts from roots and the above-ground parts of plant tissues were obtained from yellow mustard (*Sinapis alba*), rapeseed (*Brassica napus*) and fodder radish (*Raphanus sativus*). The effect on the movement of J2 was tested by direct exposure and in sand columns. The effect on the sensory perception was evaluated in water-agar and pluronic-gel plates with a tomato seedling as the attracting host. The movement of J2 was significantly reduced by direct exposure to extracts made from roots and from above-ground tissues, and so was the migration through sand columns. Juveniles pre-exposed to the diffusates or extracts of green manures could not readily detect the presence of a host in the agar or pluronic-gel plates, moving away from it during the first 8 h of the experiment. These results indicate that breakdown of brassicaceous plant tissues can impair the mobility and sensory perception of *G. rostochiensis* juveniles, making these crops interesting for integrated soil management programs in potato fields.

Keywords: Green manures; isothiocyanate; potato cyst nematode; sensory perception.

Developing neuropeptides as transgenic nematicides

Neil Warnock¹, Leonie Wilson¹, Cheryl Patten², Colin Fleming³, Aaron Maule¹ and Johnathan Dalzell¹

¹ Queens University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

³ Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast BT9 5PX, Northern Ireland, UK

² Department of Biology, University of New Brunswick, PO Box 4400, Fredericton, NB E3B 5A3, Canada

corresponding author: n.warnock@qub.ac.uk

Plant-parasitic nematodes (PPN) seriously threaten global food security. It is estimated that they are responsible for a 12.3% reduction in agricultural productivity worldwide which equates to \$120 billion annually. Conventionally, an integrated approach to PPN management has relied heavily on various nematicides. As environmental concerns rise over the systemic effects of sustained nematicide use, withdrawal has left a significant shortcoming in our ability to manage this problem and highlights the need for novel and robust control methods. It has been discovered that nematodes can assimilate exogenous peptides through retrograde transport along the chemosensory amphid neurons. These peptides accumulate within cells of the central nerve ring and can elicit physiological effects when released to interact with receptors on adjoining cells. We are harnessing bioactive neuropeptides from the neuropeptide-like protein (NLP) family of plant-parasitic nematodes as novel nematicides. We have identified numerous discrete neuropeptides that negatively impact chemosensation, stylet thrusting and infectivity of the root knot nematode *Meloidogyne incognita*, and of the potato cyst nematode *Globodera pallida*. Transgenic secretions of these peptides from the rhizobacterium, *Bacillus subtilis*, and the terrestrial microalga *Chlamydomonas reinhardtii* reduce plant infection levels by up to 90% when compared with controls.

Keywords: Nematicides; neuropeptides; plant-parasitic nematodes (PPN).

Let the environment decide: Sex determination in the plant-parasitic cyst nematode *Heterodera schachtii*

Muhammad Shahzad Anjam¹, Christiane Matera¹, Miroslaw Sobczak², Florian M. W. Grundler¹ and Shahid Siddique¹

¹ Rheinische Friedrich-Wilhelms-Universität Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, 53115 Bonn, Germany

² Department of Botany, Warsaw University of Life Sciences, PL-02787 Warsaw, Poland

corresponding author: shahzad.anjam@uni-bonn.de

Cyst nematodes are biotrophs that infect roots and cause physiological and structural modifications of host cells leading to the formation of syncytial nurse cells in the roots. They are sexually dimorphic but the differentiation into male or female occurs only after feeding has started. The effect of environment on sex determination of cyst nematode has been under investigation since 1920. As male to female sex ratio is highly variable with culturing conditions and cultivar of the host plants, it is generally assumed that environmental conditions play a decisive role in sex determination. Under favourable conditions, more females develop, whereas males develop under adverse conditions. However, it is not clear, whether this phenomenon is due to epigenetic sex determination or differential mortality of females or males. Comparative transcriptome analysis of male-associated syncytia (MAS) and female-associated syncytia (FAS) at early feeding stages will help to unravel host factors influencing male or female development. In the present studies, we developed and validated a method to predict the sex of the beet cyst nematode, *Heterodera schachtii*, during the early stages of their life cycle in the host plant *Arabidopsis*. Root segments containing putative MAS or FAS were collected and syncytial cells were isolated by laser microdissection. Total RNA was extracted and amplified to perform a transcriptome analysis via microarrays. The subsequent data analysis revealed that genes belonging to categories of defence responses, nutrient deficiency and starvation were preferentially upregulated in MAS as compared to FAS. Based on these data, we hypothesise that in those syncytia where there is a strong plant response of turning on defence genes and limiting nutrient availability, these signals may result in juveniles developing as males. Juveniles that are able to overcome plant defence responses most likely will become females. To validate this hypothesis, we generated a number of promoter:reporter and loss-of-function mutant lines for candidate genes. In this way, we will elucidate a 100-year-old biological problem.

Keywords: Female; microarray; nutrient; sex; syncytia.

Transcriptome analysis reveals the role of Rboh-mediated ROS in modifying auxin homeostasis to facilitate nematode parasitism in *Arabidopsis*

Christiane Matera, Shamim Hasan, Divykriti Chopra, Florian M. W. Grundler and Shahid Siddique

Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, KarloerbertKreiten-Straße 13, D-53115 Bonn, Germany

corresponding author: s7mdhasa@uni-bonn.de

Plasma membrane bound NADPH (reduced form of nicotine amide adenine dinucleotide phosphate) oxidase, known as respiratory burst oxidase homologue (RBOHs) in plants produce reactive oxygen species (ROS) upon nematode infection. The cyst nematode *Heterodera schachtii* induces an elaborate feeding site in the roots of host plants from where it withdraws all required nutrients. Our previous work suggested that mutation in *Arabidopsis* Rboh homologues RbohD and RbohF (rbohD/F) has a strong negative effect on susceptibility to this sedentary endoparasite. In the present work, a comparative transcriptome expression analysis was performed between Col-0 and rbohD/F plants at 10 h after nematode inoculation (hai). Our data analysis showed that out of 24,000 genes, 6079 were differentially regulated between Col-0 and rbohD/F infected root segments (FDR<5%), whereby 3157 showed increased and 2922 decreased transcripts. The most striking feature of this transcriptome analysis is a strong decrease in transcript abundance for a number of genes involved in indole metabolite pathways including auxin biosynthesis, transport and signaling genes in rbohD/F compared with Col-0. Subsequent investigation of these genes involved in auxin homeostasis reveals their role in regulating ROS mediated susceptibility in plants upon nematode infection.

Keywords: *Arabidopsis*; *Heterodera schachtii*; hormone; rbohD/F; transcriptomics.

Hormone-based responses triggered by ozonated water and *Meloidogyne incognita* in tomato plantsMaria Isabella Prigigallo, Maria Teresa Melillo and Pasqua Veronica

Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Unit of Bari, 70126 Bari, Italy

corresponding author: pasqua.veronico@ipsp.cnr.it

Ozonated water can be considered an effective alternative control strategy to nematode infection since its application as a soil drench to tomato roots does not negatively affect plant growth and induces a reduction in tomato susceptibility to *Meloidogyne incognita*. The aim of this study was to examine the role of O₃wat on tomato-*M. incognita* interaction with particular emphasis to the expression of genes involved in plant defence responses. Transcripts of defence related genes *PR1*, *PR5*, commonly used as molecular markers for SA-dependent SAR signalling, and transcripts of genes *AOS*, *LoxD*, *Pin1* involved in the jasmonic acid pathway were analysed. After a four day-treatment, O₃wat-treated and non-treated plants were inoculated with second-stage juveniles of *M. incognita*. Transcript levels were examined in non-treated and O₃wat-treated roots at the end of the treatment and after 2, 4 and 7 dpi in non-treated and O₃wat-treated galls. Ozone treatment promptly enhanced transcription of *PR1* and *LoxD*, induced down-regulation of *PR5* and did not affect *AOS* and *Pin1* gene expression. Upon nematode infection the genes were differentially modulated during the time course both in untreated and in O₃wat-treated galls. *PR1* was up-regulated compared to non-treated and non-infected roots. In particular, transcript levels were higher in response to combined O₃wat and nematode stresses at 4 and 7 dpi. *LoxD* acting upstream in JA biosynthesis pathway, showed a moderate up-regulation only in 2-dpi non-treated galls, whereas it was down-regulated in 4- and 7-dpi non-treated and O₃wat-treated galls. These results indicate that nematodes are able to trigger changes in hormone biosynthetic and signalling pathways in strict dependence on their parasitism stage. The positive effect of the O₃wat application in the control of nematode infection is likely related to the modulation of the balance between SA and JA signalling and biosynthetic pathways. Study funded by FCRP.

Keywords: Gene expression; plant resistance; root-knot nematodes.

The use of real-time PCR-HRM analysis for the detection and discrimination of virulent and avirulent populations of the quarantine nematode *Bursaphelenchus xylophilus*

Anna Filipiak

Institute of Plant Protection – National Research Institute, Department of Biological Pest Control,
Władysława Węgorka 20, 60-318 Poznań, Poland

corresponding author: a.filipiak@iorpib.poznan.pl

The quarantine nematode *Bursaphelenchus xylophilus* is the causal agent of pine wilt disease in Asia (Japan, China, Korea, Taiwan) and Europe (Portugal and Spain). Among populations of *B. xylophilus* a great variation in the virulence level was reported. Morphological discrimination of different virulence nematodes is impossible; therefore, a molecular biological techniques are necessary to investigate the population structure of different virulence nematodes within a single pine. High Resolution Melting Analysis (PCR-HRM) was developed for fast, high-throughput post-PCR analysis of genetic mutations or variance in nucleic acid sequences. It enables researchers rapidly to detect and categorise genetic mutations, identify new genetic variants without sequencing, or determine the genetic variation in a population prior to sequencing. PCR-HRM technique was also used to detect and distinguish of virulent and avirulent populations of *B. xylophilus*. In the research reported here PCR-HRM was conducted on the rDNA of pathogenic (China, Ka4, S10, T4) and non-pathogenic populations (C14-5 and OKD-1) of *B. xylophilus*, with the use of specifically designed primers. The melting profiles were clearly distinctive for virulent and avirulent populations of *B. xylophilus*, providing single melting peaks. The normalised DNA melting curves obtained in the real-time PCR-HRM analysis differed from each other in denaturation temperature, as evidenced by a substantial shift of these curves in relation to each other. This indicates differences between the examined populations in the composition of nucleotides within the amplified region of their genomes. The conducted study revealed that PCR-HRM allowed for easy distinguishing of virulent and avirulent populations of *B. xylophilus*. This technique is very sensitive and enables detection of differences even in single nucleotides. PCR-HRM technique is also much cheaper when compared to commonly used PCR-RFLP.

Keywords: *Bursaphelenchus xylophilus*; PCR-HRM; quarantine pest detection; virulence.

Nematicidal activity of naphthoquinones against the pinewood nematode

Luís Fonseca¹, Carla M.N. Maleita², Ivânia Esteves¹, Mara E. M. Braga², Isabel Abrantes¹ and Hermínio C. de Sousa²

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

corresponding author: luisbidarra@gmail.com

Human activities and global world trade of non-treated pinewood products are generally considered as the main causes for the worldwide dissemination of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Environmental-friendly strategies for PWN control are nowadays a primary concern. Phytochemicals can be used for this purpose since they can be used as nematicides themselves or can serve as model-compounds for the development of new eco-friendly synthetic derivatives with enhanced activity. Most phytochemicals are safer for the environment and human health than synthetic nematicides, and a large number of secondary metabolites from plants have been tested as efficient nematicides. Naphthoquinones, an important subgroup from the quinone family and a group of reactive phenolic compounds, are bioactive compounds arousing great interest due to their broad-range of biological properties. They are cytotoxic with antibacterial, antifungal, insecticidal, herbicidal and also nematicidal activity. The main objective of this study was to assess the *in vitro* nematicidal activity of the 1,4-naphthoquinone and of two of its derivatives (juglone and plumbagin) on PWN dispersal third juvenile stages (JIII) and adults obtained from infected *Pinus pinaster* wood. Twenty nematodes were placed in 1 ml of each compound concentration (500, 250 and 150 ppm) and mortality monitored during 72 h. Each treatment consisted of five replicates and tap water and Triton X-100 5000 ppm were used as controls. In 24 h, the three pure compounds caused 100% JIII mortality, at 500 ppm. 100% adult mortality was achieved with juglone and 1,4-naphthoquinone, at 500 ppm. The results revealed that these phytochemicals have potential to be used as natural nematicides in the control of PWN and research is currently being carried out in order to develop aqueous phytochemical-formulations to be applied directly in infected wood and wood products.

Keywords: 1,4-naphthoquinone; *Bursaphelenchus xylophilus*; juglone; plumbagin; wood.

Optimising conditions for trapping *Monochamus galloprovincialis* beetles using pheromone traps in the Czech Republic

Vladimír Gaar¹, Václav Čermák¹ and Jiří Foit²

¹ Central Institute for Supervising and Testing in Agriculture, Division of Diagnostics, Šlechtitelů 773/23, 779 00 Olomouc, Czech Republic

² Mendel University in Brno, Department of Forest Protection and Game Management, Zemědělská 3, 613 00 Brno, Czech Republic

corresponding author: vladimir.gaar@ukzuz.cz

To contain the pinewood nematode successfully, it is necessary to monitor its vector. Monitoring to determine the effectiveness of trapping the long-horn beetle *Monochamus galloprovincialis* was conducted at four locations in the Czech Republic (Brno - Soběšice; Bzenec, Jičín – Podkost; Bělá pod Bezdězem) in 2015. The effectiveness of height of trap placement (at 2 m height and in the canopy at ca 25 m height), as well as various pheromones, were compared (Galloprotect pack; Gallopro Pinowit). The type of trap used in the experiment was selected based on previous testing of different kinds of traps (Teflon uncoated multi-funnel traps; black and transparent cross-vane panel traps). The testing was conducted in areas for which an occurrence of the vectors had been verified. The results were unambiguous. Based on verification by the Kruskal-Wallis one-way analysis of variance, the best results were achieved with Teflon coated traps (CROSSTRAP® DRY WITH COLLECTOR Econex®), placed in the canopy of pine trees, and equipped with the Spanish pheromone (Galloprotect pack). The total number of beetles trapped was 849, the majority of which (580 beetles) were trapped using the most efficient combination.

Keywords: Czech Republic; *Monochamus galloprovincialis*; pinewood nematode.

Survey of pinewood nematode from the insect *Monochamus galloprovincialis* captured in the national monitoring traps in Portugal

Maria L. Inácio¹, Filomena Nóbrega¹, Luís Bonifácio¹, Pedro Naves¹, José M. Rodrigues² and Edmundo R. Sousa¹

¹ INIAV, Instituto de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras, Portugal

² ICNF, Instituto da Conservação da Natureza e das Florestas, Avenida da República, 16, 1050-191 Lisboa, Portugal

corresponding author: lurdes.inacio@iniav.pt

Bursaphelenchus xylophilus, the pinewood nematode (PWN), is the causal agent of the pine wilt disease (PWD), annually killing thousands of pines in Portugal since its introduction in 1999. The pine sawyer *Monochamus galloprovincialis* is its sole vector in Europe. A national trapping programme of the insect vector is yearly implemented during its flight period, to prevent the disease spread by reducing its populations and to survey the insects' PWN infection rate. This strategy employs the multi-funnel trap, selected as one of the most effective in capturing the flying *Monochamus* beetles, and which are baited with specific attractants for this insect genus. Captured insects are collected periodically, and each beetle is taken to the laboratory to undergo an individual nematode extraction, aiming to detect the PWN. In this work we present the results of three years of national surveying (2012-2014), comprising thousands of insects' analyses, and relating the number of insects caught in the traps and their infestation rates with the evolution and spatial spread of this disease in the field, in order to characterise and understand the present situation of PWD-infestation in Portugal.

Keywords: *Bursaphelenchus xylophilus*; insect trapping; insect vector; pine wilt disease.

Monitoring strategy of pinewood nematode in France focused on its vector

Corinne Sarniguet¹, Anne-Marie Chappé¹, Jean-Luc Flot², Emmanuel Kersaudy³ and Laurent Folcher¹

¹ Anses, French Agency for Food, Environmental and Occupational Health and Safety/Plant Health Laboratory – Nematology Unit – Domaine de la Motte au Vicomte, BP 35327, 35653 Le Rheu Cedex, France

² Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt – Département de la Santé des Forêts – 251, rue de Vaugirard, 75732 Paris Cedex 15, France

³ Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt Aquitaine-Limousin-Poitou-Charente – Pôle Santé des Forêts – 51, rue Kieser, CS 31387, 33077 Bordeaux Cedex, France

corresponding author: corinne.sarniguet@anses.fr

The pinewood nematode, *Bursaphelenchus xylophilus*, is a quarantine pest according to the European directive 2000/29/EC. Its introduction in France would have huge economic and environmental consequences for the pine forests (2.5 million ha of susceptible coniferous forest out of 16.7 million ha total French forest). Following the first report of this nematode in Portugal in 1999, the French State has developed a monitoring strategy, since the beginning of the 2000s, to allow regular surveillance of pine trees, timber and wood packaging. Since 2013, this strategy has been improved by the use of a trapping network of insect vectors (*Monochamus* spp.) throughout France for the early detection of the nematode. The nematode screening in the insect vector body involved the development of a specific method using real-time genomic amplifications, in addition to the official analysis methods already implemented for *B. xylophilus* detection in wood samples. The specific surveillance of the vector enabled collection of around 24 000 *Monochamus* spp. widespread in all involved French regions. None of them were carrying the pinewood nematode. However, according to the considered area, between 20 and 100% of *Monochamus* spp. analysed carried a closely related non-pathogenic species, *B. mucronatus* (results of the 2013 campaign only). As already demonstrated by previous works, *B. mucronatus* is not an obstacle to the establishment of *B. xylophilus* which presents a high level of competitiveness. Considering the wide distribution of *Monochamus* spp., significant and continuous forest of conifers and climate conditions, France offers favourable conditions for the development, establishment and spread of PWN. This reinforces the importance of the monitoring system set up which has to contribute to the early detection of the nematode.

Keywords: *Bursaphelenchus xylophilus*; *Monochamus* spp.; pine forest; surveillance; trapping.

Multitrophic interactions among entomopathogenic nematodes, entomopathogenic fungi and nematophagous fungi: who kills whom?

Francisco A. Bueno-Pallero^{1,2}, Rubén Blanco-Pérez^{1,2}, Lidia Dionisio¹ and Raquel Campos-Herrera²

¹ Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

² MeditBio, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

corresponding author: fapallero@ualg.pt

In agroecosystems, it is critical to understand how biological control agents interact with both the pest itself and other organisms in the rhizosphere community. Entomopathogens are a diverse group of organisms that impart top-down effects on insect populations, which is exploited as a pest management strategy. Little is known about how the interspecies interactions among beneficial soil organisms can impact insect control. Furthermore, it is not known how the presence of antagonists might counteract the well-known benefit of regulating insect populations. We hypothesise that simultaneous application of two species of entomopathogens (nematode, EPN or fungus, EPF) might support higher control of insect, whereas the addition of an antagonist/natural enemies (nematophagous fungi, NF) will unbalance the relationship. Herein, we evaluated the multitrophic interaction among the NF *Arthrobotrys musiformis*, *A. oligospora* and *Purpureocillium lilacinum* and two entomopathogens: *Beauveria bassiana* (EPF), and *Steinernema* sp. (EPN), both isolated in Algarve (Portugal). Firstly, we confirmed the antagonist interaction between fungi type (EPF and NF) by the direct opposition method in Potato Dextrose Agar (PDA). The EPF *B. bassiana* showed a similar growth rate to the NF *P. lilacinum*, both significantly lower than those of the trapping NF. However, in all cases, the EPF inhibited >40% the NF growth. Using *Galleria mellonella* larvae, we found entomopathogenic abilities for the three NF species, but mainly when injected rather than when immersed in the conidial suspension. The establishment of the CL50 of each of the organisms (NF, EPF and EPN) is being employed in ongoing experiments to assess the outcomes when one, two or three types of organisms co-occur simultaneously with regard to biological control of insect pests. The results of this study will provide the foundation for further evaluation under field conditions of niche plasticity of entomopatogens in complex soil systems.

Keywords: *Arthrobotrys musiformis*; *Arthrobotrys oligospora*; *Beauveria bassiana*; *Purpureocillium lilacinum*; rhizosphere.

Effect of compost on the nematode diversity in the soil at different depths

Luísa Coelho¹, Francisco A. Bueno-Pallero¹, Mário M. Reis^{1,2} and Maria A. Gonçalves^{1,2}

¹ Universidade do Algarve, FCT, Campus de Gambelas, 8005-114 Faro, Portugal

² MeditBio, Centre for Mediterranean Bioresources and Food, Universidade do Algarve, Campus de Gambelas – Edifício 8, 8005-139 Faro, Portugal

corresponding author: lcoelho6@gmail.com

The application of organic composts affects different properties of the soils, namely its chemical, physical and biological characteristics. The aim of this work was to study the effect of compost application on nematode populations, on four different areas of soil profile, related to root system and depth. Compost (Organical®) was applied on a sandy soil, at the rates of 0, 12.5, 25, 50 and 100 ton ha⁻¹, and incorporated in the soil at 10 to 15 cm depth. A carrot crop (*Daucus carota* L.) was planted at a density of 6.25 plants m⁻². Following harvest, samples were collected in four zones: near the roots (up to 20 cm distance) and at three soil depths: from 0 to 15 cm, at 30 cm and at 50 cm. Nematodes species were quantified and identified at the three different soil depths. In total, 25 nematodes genera were identified and distributed into three trophic groups: bacteriophage/saprophyte (BSN), phytophagous/phytoparasitic (PPN) and predators (PN). It was observed that the nematode genera were distributed according to soil depth. The first nematode group, BSN, which are considered to be beneficial nematodes, was mainly at 50 cm depth, at the surface and at the root zone. The largest populations of plant -arasitic nematodes (PPN) were located near the surface and at 50 cm depth. Predator nematodes were mainly near the surface, in the root zone and at 50 cm depth. The lowest populations of all nematode species were observed at 30 cm depth. Compost application rate also influenced nematode populations. Above 25 ton ha⁻¹ of compost applied to the soil, the PPN group predominated at 50 cm depth, while predator species dominate near the surface. This work showed that the dynamics of nematode populations in the soil is affected by soil depth and compost dose.

Keywords: Bacteriophage/saprophyte nematodes; carrot; nematode population; plant-parasitic nematodes; predatory nematodes.

Cyst extraction of >140.000 soil samples a year can be done by one personJan Luimes

Dutch General Inspection Service (NAK), Randweg 14, 8304 AS Emmeloord, The Netherlands

corresponding author: jluiimes@nak.nl

As per European regulations, seed potatoes may only be planted on fields free of potato cyst nematodes. Consequently, each year more than 140.000 soil samples coming from 45.000 ha are tested by the Dutch General Inspection Service (NAK). It is done under authority of The National Plant Protection Organisation (NPPO; government). Up to now automated cyst extraction of soil samples was performed by a Fenwick-based "carrousel", able to rinse 700 samples a day. Although this machine has served over 35 years, improvements on reliability, efficiency and ergonomics were identified. For this reason, a completely new and fully automated extraction machine was developed by the Dutch General Inspection Service in cooperation with an engineering company. Functionally, a new method of sieving was developed, yielding a cleaner sample making it easier to analyse. Furthermore, as multiple steps are now automated, the entire rinsing process can be operated by only one person. The machine will be up and running by the end of July 2016. All process steps in the new machine can be adjusted independently. This leads to numerous possibilities, making it possible to adjust setting of the machine parameters based on soil type or any other parameter. The validation results and results of various process parameters will be presented during the congress.

Keywords: Efficient potato cyst nematodes testing; quick; reliable.

Analysis of plant attractant of root-knot nematode, *Meloidogyne incognita*

Morihiro Oota¹, Takanori Ida², Hayato Ishikawa¹, Masatsugu Hashiguchi³, Ryo Akashi³, Takashi Ishida¹ and Shinichiro Sawa¹

¹ Kumamoto University, 2-40-1 Kurokami, Chuo-ku, Kumamoto-shi, 860-8555 Japan

² University of Miyazaki, Kihara 5200, Kiyotake, Miyazaki-shi, 889-1692 Japan

³ University of Miyazaki, Faculty of Agriculture, 1-1 Gakuenkiibanadai nishi, Miyazaki, 889-2192, Japan

corresponding author: 126s6006.kumadai@gmail.com

Root-knot nematodes (*Meloidogyne incognita*) are plant-parasitic nematodes that infect many crops such as sweet potato and tomato. The world-wide agricultural damage caused by *M. incognita* is estimated to be over billions of dollars per year. It has been suggested that *M. incognita* recognises plant roots of the appropriate hosts through an unknown attractant. In order to identify this plant root attractant, we used the Super Root (SR) culture system derived from *Lotus corniculatus* as a model. SR can be maintained by using liquid culture medium without any phytohormones, and it can grow continuously without above-ground organs. Interestingly, SR culture supernatant was shown to contain *M. incognita* attraction activity, suggesting *L. corniculatus* roots indeed secrete *M. incognita* attractants. Chromatographic analyses suggest this attractant is hydrophobic, basic, and is over 5000 Da. Together with the results of chemical screening, here I discuss about our recent progress to identify *M. incognita* attractant.

Keywords: Attractant; *Meloidogyne incognita*; super root.

Developing automated methods for analysis and sorting of *Globodera pallida* cysts, eggs and juveniles using the COPAS FP1000 large particle flow cytometer

Francis Smet¹, Mikalai Malinouski¹, Jenny Rowley² and Louise-Marie Dandurand²

¹ Union Biometrica, 84 October Hill Rd, Holliston, MA 01746, USA

² Department of Plant, Soil and Entomological Sciences, University of Idaho, 82844-2339 Moscow, USA

corresponding author: fsmet@unionbio.com

Potato cyst nematodes, such as *Globodera pallida*, are economically important plant parasites and threaten global food security; *G. pallida* is also a valuable model organism to study the biology of cyst nematodes. Handling large quantities of *G. pallida* for screening purposes is a labour intensive process. Here we report the development of an automated method for the purification of *G. pallida* cysts from soil samples using a COPAS FP 1000 large-particle flow cytometer. COPAS FP 1000 flow cytometers enable analysis of particles with diameters of 40-700 μm in a continuously flowing stream at a rate up to 300 objects s^{-1} . It measures several parameters: size (TOF, Time of Flight), optical density (EXT, Extinction), internal complexity (SSC, side scatter) and the intensity of fluorescent markers (three simultaneously). Once analysed, objects are sorted according to user selectable criteria and then may be dispensed into stationary bulk receptacles or multi-well microtitre plates for high-throughput screening.

Keywords: *Globodera pallida*; large particle flow cytometry; potato cyst nematodes.

Screening of chickpea genotypes collected from Turkey against the root-lesion nematodes, *Pratylenchus neglectus* and *P. thornei*

Tohid Behmand¹, Ece Börteçine Kasapoğlu¹, Abdullah Kahraman², Jens Berger³ and İbrahim Halil Elekcioglu¹

¹Cukurova University, Faculty of Agriculture, Department of Plant Protection, 01360, Balcalı, Adana, Turkey

²Harran University, Faculty of Agriculture, Department of Crop Science, Şanlıurfa, Turkey

³CSIRO Agriculture Flagship, Centre for Environment and Life Sciences, Australia

corresponding author: tohid.behmand63@gmail.com

The root-lesion nematodes, *Pratylenchus* spp. (RLN) are an economically important pest described as one of the major limiting factors in chickpea production. RLN produce severe lesions on chickpea roots, and can be managed effectively by the use of resistant cultivars. A major strategy to develop resistance to RLN in chickpea is to assess and exploit their natural variation. Chickpea is constrained by limited genetic and adaptive diversity. Widening this diversity is the focus of an international collaboration that is collecting, phenotyping and introgressing wild *Cicer* into chickpea involving institutes in Turkey, Australia, the USA, Canada, Ethiopia and India. This projects aims to widen the genetic and adaptive diversity of chickpea by collection, characterisation, evaluation and exploitation of wider *Cicer* genetic resources. Our contribution in this study is to find RLN resistance by screening more than 1000 genotypes against *Pratylenchus neglectus* and *P. thornei*. To optimise the screening study, nine chickpea varieties have been inoculated with *P. neglectus* and *P.thornei* to find best initial nematode density and harvest time. The results of growth room experiments indicated that there were significant differences between the resistant and susceptible cultivars, using sandy growth medium (70:29:1 sand, field soil and organic matter), small container (30 mm diameter × 160 mm length) inoculation density with 300 individuals per plant, 20 week growing period and bottom perlite irrigation system.

Keywords: Chickpea; *Pratylenchus*; resistance; screening methods.

***Meloidogyne hispanica* - *Pratylenchus neglectus* interactions in potato**

Ivânia Esteves¹, Carla M.N. Maleita² and Isabel Abrantes¹

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

corresponding author: iesteves@uc.pt

Root-knot nematodes (RKN), *Meloidogyne* spp., and root-lesion nematodes (RLN), *Pratylenchus* spp., are widespread and economically important parasites of several cultivated plants, including potato. Concomitant infections of RKN and RLN have been reported, which reinforce the need for a careful monitoring of these nematodes in potato crops. The aims of this study were to determine the effect of the interaction between *M. hispanica* and *P. neglectus* on nematode reproduction and to evaluate the host suitability of nine potato cultivars to *P. neglectus*. Potato cv. Agria plants grown in pots (1 dm³) were inoculated with 250, 500, 750 and 1000 nematodes of *M. hispanica* or *P. neglectus*, or a combination of both. For host status studies, potato cultivars were inoculated with 2000 *P. neglectus* per plant. After 60 days, *M. hispanica* and *P. neglectus* were recovered from infected roots using a sodium hypochlorite 0.52% solution and the Baermann funnel method, respectively, and from soil using an adaptation of the Tray method. Final nematode densities were determined and the reproduction factor (Rf) calculated. Potato cv. Agria was an excellent host for *M. hispanica*, but a poor host for *P. neglectus*. Concomitant inoculation of the two species resulted in higher numbers of *P. neglectus* compared with single inoculation. Moreover, the reproduction of *M. hispanica* was influenced by the presence of *P. neglectus*, as it decreased with the increasing inoculum of *P. neglectus*, indicating RKN-RLN competition. All potato cultivars were considered as poor hosts to *P. neglectus* with Rf values varying from 0.1 (cvs Colomba, Désirée, and Stemser) to 0.9 (cv. Kennebec). Considering the results and potential impact of RKN and RLN on potato fields, further studies are needed to understand the interaction among *M. hispanica* and *P. neglectus*, due to the prevalence of these species associated with potato.

Keywords: Pathogenicity; root-knot nematodes; root-lesion nematodes; *Solanum tuberosum*.

A proteomic view on plant-nematode interaction

Muhammad Ilyas¹, Elisabeth Escobar¹, Zoran Radakovic¹, Samer Habash¹, Syed Jehangir Shah¹, Badou Mendy¹, Alexander Graf², Florian M. W. Grundler¹ and Shahid Siddique¹

¹ INRES – Molecular Phytomedicine, Rheinische Friedrich-Wilhelms-University of Bonn, Germany
Karlrobert-Kreiten-Straße 13, 53115 Bonn, Germany

² Plant Proteomics, Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany

corresponding author: ilyas@mpipz.mpg.de

The plant-parasitic cyst nematode, *Heterodera schachtii*, infects several crop species, including the model plant *Arabidopsis thaliana*. The parasite induces the formation of a syncytial nurse cell system in the host plants' roots, which serve as the sole source of nutrients for these nematodes. Nematodes secrete a cocktail of effector molecules to modify host cells' functions for syncytium formation. Although, the characterisation of effector functions is currently the subject of intense investigation, the localisation of effectors inside host cells is rarely observed. Here we performed proteomic profiling of syncytia in *Arabidopsis* roots and nematodes at different stages of development using a quadrupole-orbitrap mass spectrometer. Our data analysis showed that proteins related to metabolic process and energy production are particularly enriched in the syncytium as compared to control roots. Importantly, our study also identified a repertoire of candidate nematode effectors within the proteome of the syncytium. Among identified candidate effectors were a few effectors that have been previously shown to play a role in parasitism, thus validating our approach. Further characterisation of identified effector proteins is underway and will provide new insights into the nematode parasitism process.

Keywords: Effectors; nematodes; proteome; syncytium.

Barley, wheat and oat resistance to the cereal cyst nematode *Heterodera latipons* in Turkey

Mustafa İmren¹, Nagihan Duman¹, Halil Toktay², Abdelfattah Dababat³ and İbrahim Halil Elekcioglu⁴

¹ Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Gököy, Bolu, Turkey

² Ayhan Sahenk Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, Nigde, Turkey

³ International Maize and Wheat Improvement Center (CIMMYT), Emek, Ankara, Turkey

⁴ Cukurova University, Faculty of Agriculture, Department of Plant Protection, Balcalı, Adana, Turkey

corresponding author: mustafaimren@ibu.edu.tr

The cereal cyst nematode, *Heterodera latipons* is widely distributed in cereal fields that reduces cereal yields in Eastern Mediterranean region of Turkey. Control measures including rotation, fallow, and tillage practices are applied to control cereal cyst nematodes. The introduction of host resistance is one of the most effective, environmentally friendly and economical method to suppress nematode populations under the economic threshold. This study was carried out to determine the resistance of barley, wheat and oat cultivars against *H. latipons* collected from Gaziantep and Kilis provinces in Eastern region of Turkey. Results revealed that the majority of the barley varieties were completely and/or partially resistance to *H. latipons*. The barley varieties Varde, Emir, Morocco, Bajo Aragon, Martin 403-2 and Harlan 43 were resistant according to Pf/Pi values below one. The barley varieties Herta with 51 new cysts per plant and Ortolan with 50 cysts per plant were identified as best hosts. The barley varieties Harlan 43, Morocco and Emir were resistant based on their Pf/Pi values below one; however, both varieties produced more than nine new cysts. The oat varieties Sun II, Silva and Mk. H. 72-646 were completely and/or partially resistance to *H. latipons*. Only the wheat variety Psathias and Loro × Koga were resistant to *H. latipons* (Pf/Pi < 1.0). Four varieties (Capa, Loros, Iskamish K-2 and Aus10894) were susceptible to *H. latipons* (Pf/Pi > 1.0). This is the preliminary report determining the resistance to *H. latipons* in Turkey. Therefore, detailed and comprehensive studies are indispensable to check their pathogenicity against different *H. latipons* populations. This study was funded by Scientific and Technological Research Council of Turkey (TUBITAK) for providing fund under the project no 115R006.

Keywords: Cereal; nematode; resistance; susceptible.

Effect of plant age and inoculation density over the final population of *Heterodera schachtii* on Chinese cabbage

Faisal Kabir¹, Mungi Jeong², Jeongeun Kim¹, and DongWoon Lee^{1,2}

¹ Department of Ecological Science, Kyungpook National University, Sangju, Gyeongsangbukdo 37224, Republic of Korea

² School of Environment and Tourism, Kyungpook National University, Sangju, Gyeongsangbukdo 37224, Republic of Korea

corresponding author: faisalmfk@yahoo.com

Recently the sugarbeet cyst nematode, *Heterodera schachtii*, has been found severely infesting the highland Chinese cabbage field of Korea. Two experiments were carried out to find out the effect of plant age and inoculation density of *H. schachtii* on Chinese cabbage. In the first experiment, different age Chinese cabbages (seedlings, 20, 30 and 40 days) were inoculated with 0, 1, 2 and 4 juveniles (g soil)⁻¹ to find out the effect of plant age. Thirty days after inoculation, 40 day-old plants that were inoculated with high inoculation density, reached the maximum number of final population. However, 60 days after inoculation, the final population number also ranked as maximum in 40 day-old plants that had been treated with a low inoculation density. To examine this further, 40 day-old plants were inoculated with different inoculation densities of eggs and juveniles separately to find out the effect of inoculation density. Plants inoculated with 4 juveniles (g soil)⁻¹, showed significant differences in the number of brown cysts, females and eggs. Similar results were also observed in plants inoculated with 4 eggs (g soil)⁻¹. We summarise that, at high inoculation density, comparatively older plants are much more affected by the attack of *H. schachtii*; however, many other factors also influence the final population.

Keywords: Cyst nematode; egg; female cyst; hatching; juvenile.

Dynamics of *Mi-1*-gene expression during compatible and incompatible tomato – root-knot nematode interactions

Victoria V. Lavrova¹, Elizaveta M. Matveeva¹, Svetlana V. Zinovieva² and Zhanna V. Udalova²

¹Institute Biology, Karelian Research Centre of Russian Academy of Sciences, 185910 Petrozavodsk, Russia

²Centre of Parasitology, A. N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, 119071 Moscow, Russia

corresponding author: VVLavrova@mail.ru

The *Mi* locus of tomato confers resistance to root-knot nematodes, potato aphids and white flies. The region of the genome to which *Mi-1* is localised carries two highly homologous genes – *Mi-1.1* and *Mi-1.2*. These genes belong to the NBS-LRR class of resistance genes. *Mi-1.1* and *Mi-1.2* genes expression in roots of resistant and susceptible tomato plants during *Meloidogyne incognita* invasion was studied. Results showed that *Mi-1.1* and *Mi-1.2* genes are expressed at low level in roots of resistant and susceptible plants. However, dynamics of gene expression depending on resistance/susceptibility of plants during *M. incognita* invasion were different. Nematode infestation of resistant plants was accompanied by increased *Mi-1.1* gene expression by 18th day and *Mi-1.2* gene expression by 4th day. Expression of *Mi-1.1* and *Mi-1.2* genes in roots of susceptible plants remained at lower levels during nematode invasion. Differences established in dynamics of *Mi-1.1* and *Mi-1.2* gene expression between resistant and susceptible plants during nematode invasion may be one of the reasons why tomato resistance to *M. incognita* is associated only with the *Mi-1.2* gene. Salicylic acid (SA) is a plant defence stimulator and a central player in *Mi-1* gene-mediated resistance responses. The effect of exogenous SA on expression of *Mi-1.1* and *Mi-1.2* genes in tomato roots under the pest attack was also investigated. It was shown that SA-treated resistant plants had the same increased level of *Mi-1.1* and *Mi-1.2* gene expression during invasion. It should be noted that SA-treatment stimulated expression of *Mi-1.1* gene already by 4th day. In susceptible plants SA-treatment contributed to increase in expression of *Mi-1.1* and *Mi-1.2* genes during the nematode invasion. These results indicate the possibility of involving of SA in *M. incognita* management programmes. The study was supported by RFBR (№15-04-04625).

Keywords: *Meloidogyne incognita*; resistance genes; resistance and susceptible plants; salicylic acid; tomato.

Changes in *Meloidogyne incognita* feeding sites in tomato roots associated with *Rizophagus intraradices* colonisation and mild water stress

Maria Teresa Melillo¹, [Pasqua Veronico](#)¹, Isabella Pentimone¹, Laura Rosso¹, Mariantonietta Colagiero¹, Francesca De Luca¹, Elena Fanelli¹, Raffaella Balestrini² and Aurelio Ciancio¹

¹ Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Unit of Bari, 70126, Italy

² IPSP, CNR, Unit of Turin, 10125, Italy

corresponding author: pasqua.veronico@ipsp.cnr.it

Biocontrol effects of arbuscular mycorrhizal fungi against nematodes have been reported in various plants. The literature data suggest that mycorrhizal symbiosis affects plant-water relationships as well. Moreover, it is well established that water deficit and infection with plant-parasitic nematodes represent two environmental stresses with interacting effects under field conditions. Few data are available on the effect of combined mycorrhizal symbiosis and water stress on the development of nematode feeding sites. We studied the impact of the arbuscular mycorrhizal fungus *Rizophagus intraradices* on *Meloidogyne incognita*-tomato (cv. San Marzano nano) interaction, with or without water stress. Plants colonised or not with *R. intraradices* and maintained in growth chamber at 25°C, were subjected to mild water stress and subsequently infected with second-stage juveniles of *M. incognita*. Galls hand-dissected at 7 and 14 days were processed for light microscopy observations. *R. intraradices* colonisation was evaluated on roots from the plants subjected to the different treatments, after staining with cotton blue. The analysis performed on cross sections of galls i) with or without the fungus; ii) under water stress; and iii) with fungus and water stress, showed changes in the morphology of galls and nematode feeding sites, affecting density of cytoplasm and nuclei. The symbiosis with *R. intraradices* and water stress hampered development and structure of giant cells showing an effect on the modulation of host plant metabolism. NGS-based analysis of the transcriptome of galls is under study to unravel the molecular pathways involved in this multiple interaction. Research partially funded by CNR, Progetto Premiale Aqua.

Keywords: Arbuscular mycorrhizal fungi; giant cells; morphological analysis; root-knot nematodes.

Study of the behaviour of varieties of cucurbits and solanaceous crops towards a population of *Meloidogyne incognita* under glasshouse conditions in Algeria

Hammache Miloud¹, Doumandji Salaheddine¹, Sabri Karima² and Aissa Mokabli³

¹ Ecole Nationale Supérieure Agronomique, Hassan Badi, EL Harrach, 16200, Algeria

² Institut d'Agronomie, Université Blida 1 BP 270 Blida, 09000, Algeria

³ University Djilali Bounaama of Khemis Miliana, Ain Defla, Algeria

corresponding author: m.hammache@ensa.dz;

Resistance tests were performed on varieties of cucurbit (cucumber and melon variety Marketer and Charantais) and Solanaceae (tomato variety Neptune and Narita) in glasshouse pots with sterilised soil. The inoculum of *Meloidogyne incognita* was 3000 second-stage juveniles per pot for all transplants. The vigour index, the index of Wales and the final population were used to evaluate the degree of the resistance from the different varieties. The saved settings to compare their degree of resistance to root-knot nematodes are the strength index, the index of Wales and the final population. Results indicate that cucurbits are more sensitive to root-knot nematodes than the solanaceous crops. Melon (variety Charantais) exhibits a vigour index of 2.5 in inoculated plants and 3.1 for non-inoculated plants. Tomato varieties exhibits strength index of 4 and 3.9 for the varieties Narita and Neptune, respectively, with an average index of 3.9 in the controls. The strength index showed a slight decrease for an infested group if we compare it with another group that is considered not infested. Melon variety Charantais gives us an index strength 2.5 in inoculated plants and 3.1 for non-inoculated. The tomato varieties yielded indices strength 4 (Narita) and 3.9 (Neptune) with an average index of 3.9 in controls. Gall rating indexes (IG.) recorded on the four varieties were 0.5 for Marketer, 2.5 for Charantais, 0.5 for Neptune and 0.6 for Narita. The final population obtained at the end of the crop cycle was 62 for Charantais, 20 for Marketer, 60 for Narita and 49 for Neptune. The final population obtained at the end of the crop cycle is 62 (Charantais) and 20 (Marketer). For solanacius varieties, the values are 60 (Narita) and 49 (Neptune). In this case, the varieties tested were all found to multiply *M. incognita* in controlled conditions.

Keywords: *Cucurbita*; *Meloidogyne*; nematodes; resistance; tomatoes.

WEE1: a key cell cycle regulator involved in plant – root-knot nematode interaction

Danila C. do Nascimento¹, José Dijair A. de S. Junior², Gilbert Engler¹ and Janice de Almeida-Engler¹

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 Laboratório de Interação Molecular Planta-Praga, Embrapa Recursos Genéticos e Biotecnologia, PqEB, Av. W5 Norte final, Brasília/DF, 70770-900, Brazil

corresponding author: danila.cabraln@gmail.com

Plant-parasitic nematodes are among the most devastating plant pathogens. Root-knot nematodes (RKN; *Meloidogyne* spp.) are capable of infecting more than 3.400 host species and are responsible for half of the overall nematode damage in crops. RKN infect plant roots and trigger the formation of specialised feeding sites named “galls” by substantial reprogramming of root cell development. They usurp and modulate the plant cell cycle machinery for their benefit. Studies have shown that the plant mitotic cycle and the endocycle, are both essential targets for a successful susceptible interaction between the host plant and RKN. Key cell cycle genes, are important components to allow the induction and maintenance of the nematode feeding site development. Giant cells undergo acytokinetic mitosis and DNA endoreduplication and are bounded by actively dividing neighbouring cells. The increased ploidy levels in nuclei of giant-feeding cells most likely maintain their high metabolic activity needed for nematode growth and reproduction. Up to now, certain core cell cycle genes have been shown to be crucial for nematode feeding site development and the relevance of several others remain to be investigated. Among them, WEE1 belonging to a family of protein kinases and involved in terminal phosphorylation and inactivation of cyclin-dependent kinase 1-bound cyclin B resulting in G2 cell cycle arrest in response to DNA damage in *Arabidopsis*. Our functional analysis using tailored microscopy approaches permitted us to gain insight into the possible role of this key cell cycle regulator throughout RKN feeding site development.

Keywords: *Arabidopsis thaliana*; DNA-repair; endoreduplication; galls; mitosis.

Root developmental processes during gall formation

Rocío Olmo¹, Javier Cabrera¹, Fernando E. Díaz-Manzano¹, Alejandra Garcia¹, Marta Barcala¹, Isabel Martínez¹, Mari Fe Andrés², Ana Cláudia Silva¹, Carmen Fenoll¹ and Carolina Escobar¹

¹ Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha, Avenida de Carlos III s/n, 45071, Toledo, Spain

² Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain

corresponding author: carolina.escobar@uclm.es

Plant endoparasitic cause a high impact on agronomic systems. Among the most harmful are root-knot nematodes (RKN; *Meloidogyne* spp.). Root-knot nematodes induce a group of characteristic cells, called giant cells, from vascular cell precursors that nurture the nematode. Giant cells are induced by nematode effectors in the vascular cylinder and undergo repeated mitosis with partial cytokinesis. Additionally, a gall is formed by divisions and hypertrophy of several tissues within the root. We obtained the transcriptomes of early-developing giant cells (at 3 days post inoculation) induced by *M. javanica* in *Arabidopsis* using laser capture microdissection. The transcriptomes of undifferentiated root cell types, as the quiescent centre and genes characteristic of lateral root initial cells, shared some characteristics with that of giant cell and gall transcriptomes. Two genes encoding heat-shock proteins and a heat-shock transcription factor (HSF) were also up-regulated in giant cells. Interestingly, the *HSFB4*, namely SCHIZORIZA, is not related to the heat-stress responses, but was among the common genes with the quiescent centre cells transcriptome. We also showed that RKN may at least partially 'hijack' plant transduction pathways leading to the formation of lateral roots with a common transducer, LBD16. The LBD is a gene family with several members having roles during lateral root formation. Hence, we studied the activation patterns of the promoters of several LBDs during gall development, as well as their role during gall formation with loss of function lines in *Arabidopsis*. In conclusion, genes characteristics of root developmental processes play crucial roles during gall development.

Keywords: Giant cells; quiescent center; root development; root-knot nematodes; transcriptomes.

Grapevine Fanleaf virus: identification, distribution and specific transmission by vector *Xiphinema* spp. in Tekirdag District of Turkey

Lerzan Ozturk¹, Gurkan Guvenc Avci¹ and Ibrahim Halil Elekcioğlu²

¹ Viticulture Research Station, Tekirdag, 59100 Suleymanpasa, Tekirdag, Turkey

² Plant Protection Department, Faculty of Agriculture, Cukurova University, 01330 Balcali, Saricam, Adana, Turkey

corresponding author: lerzanzoturk@hotmail.com

Grapevine fanleaf virus (GFLV) is one of the most severe viral diseases in vineyards worldwide causing economic losses by affecting fruit quality and reducing the yield of grape production by up to 80%. This disease occurs worldwide and it is considered as the most important viral pathogen of grapevine in Europe. Infected grapevines show progressive decline, malformation, low yields and low fruit quality. This nepovirus is transmitted by two longidorid nematodes *Xiphinema index* and *X. italiae*. A survey was carried out in vineyards of Tekirdag district of Thrace region to determine the distribution of Fanleaf nepovirus, the occurrence of possible nematode vectors and the role of nematode in virus transmission. On this purpose randomly selected commercial vineyards were sampled between 2012-2014 and a total of 173 samples were collected from grapevines exhibiting virus like symptoms of GFLV to confirm the infection through Double antibody sandwich-enzyme linked immunosorbent (DAS-ELISA) assay. Soil samples were also taken from rhizosphere of infected plants for the evaluation of the presence of vector longidorid individuals. Out of 173 tested plant samples, 63 samples representing 36% gave positive reactions with values ranged between 0.189 and 4.500. Grapevine fanleaf virus (GFLV) infected samples showed typical symptoms including malformation, abnormal branching, short internodes with zigzag growth, leaf deformation and yellowing. Poor fruit setting and irregular berry formation was other symptoms observed on grapevines. Two *Xiphinema* species including *X. index* and *X. italiae* were extracted from soils near GFLV infected grapevines with frequencies of 60% and 3%, respectively. Transmission of two Grapevine fanleaf virus isolates of Tekirdag by *X. index* was tested in pots with Cabernet Sauvignon grape seedlings as bait plants. Experiments were conducted with 20 infected females per pot and virus was detected in roots of newly infected plants.

Keywords: Cabernet Sauvignon; nematode; nepovirus; vineyard; *Xiphinema index*.

Histological characterization of *Lilium longiflorum* infection by *Pratylenchus penetrans*, using bright-field and transmission electron microscopy

Paulo Vieira^{1,2}, Joseph Mowery³, James Kilcrease³, Jonathan. D. Eisenback¹ and Kathryn Kamo²

¹ Virginia Tech, Department of Plant Pathology, Physiology, and Weed Science, Blacksburg, USA

² USDA ARS, Floral and Nursery Plants Research Unit, BARC, USA

³ USDA ARS, Electron and Confocal Microscopy Unit, BARC, USA

corresponding author: pvieira.us@gmail.com

Lilium longiflorum cv. Nellie White, commonly known as Easter lily, is an important floral crop with an annual wholesale value of over \$20 million in the U.S. The root-lesion nematode (RLN), *Pratylenchus penetrans*, is a major pest of lily due to the significant root damage it causes. In this study we investigated the cytological aspects of this plant-nematode interaction using bright-field and transmission electron microscopy. Phenotypic reactions of roots inoculated with *P. penetrans* were evaluated under *in vitro* conditions from 0 to 60 days after nematode infection. Symptom development progressed from initial randomly distributed discrete necrotic areas to advanced necrosis along entire roots of each inoculated plant. The induction and severity of symptoms could be correlated with the number of nematodes (all developmental stages) found parasitising roots. A major feature characterising this susceptible-host response to nematode infection was the formation of necrosis, browning, and tissue death involving both root epidermis and cortical cells. Breaking down of consecutive cell walls resulted in loss of cell turgor pressure, lack of cytoplasm integrity, followed by cell death along the route of intracellular nematode migration. Endodermal cells collapsed, forming a potential physical barrier, and consequently blocking the progression of RLN to parenchymal root tissues. This study presents the first detailed pattern of *P. penetrans* infection of Easter lily, a very important flower crop.

Keywords: Lesions; lily; root-lesion nematode.

Molecular survey of *Xiphinema pachtaicum* according to 28S rDNA from Iran

Hadi Panahi¹ and Ebrahim Shokoohi²

¹ Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

² Unit for Environmental Sciences and Management, Potchefstroom, North West University, South Africa

corresponding author: Eshokoohi@gmail.com

Xiphinema is one of the causes of yield losses in higher plants. During a survey on the rizosphere of orange in Kerman, Iran, an isolate of *X. pachticum* was recovered and studied. Morphological study of this population showed high similarity with those studied previously. Nblast results of the Iranian isolate revealed 99% identity with the other populations of *X. pachtaicum*. Phylogenetic analysis using neighbour joining method placed *X. pachtaicum* and *X. incertum* in a group. In addition, genetic distance using Maximum Composite Likelihood showed that the Iranian isolate has the lowest genetic distance with the Bulgarian isolate (0.0000; KU250155) and the highest genetic distance with the Spanish isolate (0.0068; HM921393). The results indicate that *X. pachtaicum* forms a well-supported clade with bootstrap value of 90.

Keywords: 28S rDNA; Iran; *Xiphinema pachtaicum*.

The Iberian peninsula as intersection of three biogeographical patterns of disjunct distribution for the nematofauna of xeric environments

Joaquín Abolafia and Reyes Peña-Santiago

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

corresponding author: abolafia@ujaen.es

The xeric environments, characterised by the water shortage, are in general inhospitable places for life. Nonetheless, nematodes, especially the members of the order Rhabditida, have adapted to the dry soils of these habitats and dwell them. The study of the nematode fauna associated to xeric areas in southern Iberian Peninsula, with dune or salty soils, reveals that some species are specific of this kind of environments. Surprisingly, these species, often showing a reduced range, are also found in similar habitats from widely separated territories, hence displaying a remarkable disjunct distribution. Three recurrent disjunctive patterns apparently occur with Iberian cephalobids (Cephalobidae). First, four species (*Chiloplacus insularis*, *Heterocephalobellus magnificus*, *Paracrobeles psammophilus* and *Stegelleta ophioglossa*) have also been recorded from Sicilia (Italy), Samos (Greece) and/or Iran, fitting the western Mediterranean-Central Asia disjunction. Second, two species (*Acrobeles bushmanicus* and *Chiloplacus magnus*) are previously known to occur in Namibia, agreeing with the Europe-South Africa disjunction. And third, one species (*Cervidellus alutus*), also recorded in Senegal, represents an example of the disjunction observed among territories situated at the North and at the South of the Sahara desert. These results fit those obtained for other kinds of living beings, for instance plants and beetles, and confirm that nematodes may share biogeographical patterns with other organisms.

Keywords: Biogeography; cephalobids; disjunction; distribution patterns.

**An update on the biodiversity, occurrence, and distribution of the Family Longidoridae
In Portugal**

Carlos Gutiérrez-Gutiérrez¹, Maria Antonia Bravo², Margarida Teixeira Santos², Paulo Vieira¹ and Manuel Mota^{1,3}

¹ NemaLab/ICAAM, Instituto de Ciências Agrárias e Ambientais Mediterrânicas and Dept. de Biologia, Universidade de Évora, Herdade da Mitra, 7002-554 Évora, Portugal

² Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta do Marquês, 2780-159 Oeiras, Portugal

³ Departamento de Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal

corresponding author: carlosg@uevora.pt

The genera *Xiphinema*, *Longidorus* and *Paralongidorus* (Nematoda: Longidoridae) are ectoparasitic nematodes considered to be important economical pests because of their activity as vectors of important plant nepovirus, with some species included in the list of quarantine organisms in many European countries. Knowledge of the biodiversity and occurrence of nematode group species is a prerequisite for the establishment of sound management strategies and control measures. According to data collected from databases and published literature, an update on the distribution and occurrence of *Paralongidorus* spp., *Longidorus* spp. and *Xiphinema* spp. from Portugal (including the Madeira and Azores archipelagos) is herein proposed. To date a total of 55 species (15 *Longidorus*, 1 *Paralongidorus* and 39 *Xiphinema*) have been recorded from the Portuguese territories; 55 species in Continental Portugal, while 8 and 9 species in the archipelagos of the Azores and Madeira, respectively. The biodiversity of species in Portugal is probably one of the highest in the world, although it appears to be higher in “dagger” (*Xiphinema*) than in “needle” (*Longidorus* and *Paralongidorus*) nematodes.

Keywords: Azores islands; *Longidorus*; Madeira islands; *Paralongidorus*; *Xiphinema*.

Two-dimensional proteome analysis for the diapause and non-diapause cereal cyst nematode *Heterodera avenae*Aisu Mo, Zhuo Q. Qiu and Hai Y. Wu

Agricultural College of Guangxi University, Nanning 530004, China

corresponding author: wuhy@gxu.edu.cn

Cereal cyst nematode (CCN; *Heterodera avenae*) is a pest of graminaceous crops worldwide, and has been one of the major constraints on wheat and barley production. In China, damage of *H. avenae* was reported in 16 provinces. *H. avenae* finished one generation at end of the winter wheat growing season in Shandong, and juveniles in the recently formed cysts cannot hatch even though conditions are favourable. In order to understand this phenomenon and reason, the proteomics of CCN were analysed by the method of two-dimensional gel electrophoresis (2-DE) and MALDI-TOF-TOF. The total proteins of each diapause cysts and non-diapause cysts were extracted and separated by two-dimensional electrophoresis, the pattern of 2-DE was analysed by Image Scanner III photo system, and the proteins were analysed by mass spectrometry. 409 and 412 protein spots were detected in the diapause cysts and non-diapause cysts of *H. avenae*; 401 were common protein of diapause and non-diapause cysts, 19 differential proteins, and 10 proteins were identified by mass spectrometry. There were more proteins expressed in non-diapause cysts than in diapause cysts of *H. avenae*, which indicated that non-diapause cysts have more protein to regulate its development. Those proteins are mainly associated with signal transduction, energy production and cell proliferation during the development process. The proteome information could be of great help for better understanding diapause mechanism of CCN. This research was sponsored by the Special Fund for Agro-scientific Research in the Public Interest (201503114).

Keywords: Diapause; electrophoresis; protein.

Distribution and diversity of nematodes in the rhizosphere of willow trees along the Mooiriver (Potchefstroom, South Africa)

Ebrahim Shokoohi, Milad Rashidifard, Hendrika Fourie and Gerhard Du Preez

Unit for Environmental Sciences and Management, Potchefstroom, North West University, South Africa

corresponding author: Ebrahim.Shokoohi@nwu.ac.za

Thirty rhizosphere samples were taken from willow trees (*Salix* sp.) along the Mooiriver (Potchefstroom, South Africa). For the ten sites, the abundance and diversity of non-parasitic and plant-parasitic nematodes were studied. Species diversity was analysed using four diversity indices, viz. H' (Shannon), SR (Richness), E (Evenness) and S (Simpson). A remarkably high diversity of 26 nematode genera, belonging to 20 families was recorded. Among the plant-parasitic nematodes, *Helicotylenchus*, *Pratylenchus* and *Paratylenchus* had the highest density. The population levels and diversity of nematodes sampled varied substantially across the sampling sites according to the indices. For H', the lowest value was 0.52 and the highest 2.47, while the E value ranged from a low 0.12 to a high 2.6. However, no significant correlation was evident between soil EC and nematode density levels. However, soil pH showed a significant correlation ($P < 0.05$) for *Aphelenchoides* ($r = -0.0740$), Cephalobidae ($r = -0.884$), Dorylaimidae ($r = -0.783$) and *Longidorus* ($r = -0.637$). Furthermore, significant correlations existed between ($r = 0.763$; $P < 0.05$) between percentage (2.90) organic carbon (% C) and *Propanagrolaimus*. A significant, positive correlation ($r = 0.953$; $P < 0.05$) was observed between *Prismatolaimus* and *Diphtherophora* in terms of their population density.

Keywords: Biodiversity; Mooiriver; nematode; South Africa.

Efficacy of copper phosphonate on root-knot nematode and cucumber *Fusarium* wilt fungus

Gharam AbuJaleel and Luma Al Banna

Department of Plant Protection, School of Agriculture, University of Jordan, 11942 Amman, Jordan

corresponding author: gharamabujaleel@yahoo.com

The efficacy of copper phosphonate on the root-knot nematode, *Meloidogyne javanica*, and on the vascular wilt fungus, *Fusarium oxysporum* f. sp. *cucumerinum* was evaluated *in vitro* and *in vivo*. A series of laboratory experiments were conducted to study the effect of copper phosphonate at different concentrations on both hatching and mortality of the second-stage juveniles of *M. javanica* and on the growth of the fungus. Results showed that copper phosphonate at 0.01, 0.02 and 0.04% caused a 100% kill and delayed the hatching of second-stage juveniles after 7 days. The effect continued even after transferring the egg masses to fresh water. The secondstage juveniles exposed to copper phosphonate exhibited degenerated intestine and presence of many vacuoles along the intestinal region. The copper phosphonate at 0.02, 0.04 and 0.1% inhibited the growth of the wilt fungus in potato dextrose broth. The field experiment was performed to investigate the effect of the copper phosphonate at a rate of 0.02 % applied as 4 days pre-planting drenching or as soaking cucumber seedlings on the root-knot nematode and the wilt fungus on cucumber. Results showed that drenching with copper phosphonate did not suppress galling of cucumbers roots. On the other hand, soaking cucumber seedlings with copper phosphonate suppressed root galling in nematode treated plots. Both applications, drenching and soaking, suppressed the wilt fungus. Higher yield and foliage weight was obtained from plots infested with fungus but drenched with copper phosphonate. More field experiments should be conducted to optimise methods of applications of copper phosphonate to suppress both pathogens.

Keywords: Drenching; galling; hatching; mortality; soaking.

Nematicidal effects of the endophytic fungus *Guignardia* sp.YCC4

Maria Fe Andres¹, Carmen Elisa Díaz², Patricia Bolaños² and Azucena González-Coloma¹

¹ ICA - Instituto de Ciencias Agrarias, CSIC, Serrano 115-bis, 28006 Madrid, Spain

² IPNA – Instituto de Productos Naturales y Agrobiológica, CSIC. Avda. Astrofísico F. Sánchez, 3, 38206 Tenerife, Spain

corresponding author: mfayeves@hotmail.com; mafay@ica.csic.es

Root-knot nematodes (*Meloidogyne* spp.) are major threats to agriculture worldwide. In the last decades, environmental and human health concerns have steadily reduced the availability of efficient commercial nematicides. Therefore, environmentally friendly substances for effective nematode control are needed to be developed. Endophytes fungi reside inside healthy plant tissues without causing any detectable disease symptoms to the host. These fungi produce biologically active natural products that may be involved in the host-endophyte relationship. Therefore, these microorganisms represent a biotechnological source of bioactive metabolites. As part of our ongoing study of the endophyte biodiversity of the Canarian Laurel Forest, we have obtained an isolate of endophytic fungus *Guignardia* sp. from *Persea indica*, one of the dominant Lauraceae tree species reported as a potential source of insect-control agents. This *Guignardia* sp. endophytic isolate (YCC4) was fermented in liquid medium for 20 days. The organic extract was obtained by liquid extraction of culture medium with ethyl acetate. The organic extract ($1 \mu\text{g } \mu\text{l}^{-1}$) showed strong nematicidal *in vitro* activity against root-knot nematode *M. javanica* (infective juvenile mortality and long-term hatching inhibition effects). Based on bioassay-guided fractionation, three metabolites inducing high nematicidal effects were obtained. Several *in vivo* tests of organic extract from isolate YCC4 on tomato plants showed strong effects, suppressing the infectivity of juveniles and reducing the reproduction index of the nematode population. In conclusion, this study demonstrates that secondary metabolites from endophytic fungi are promising sources of new nematicide products.

Keywords: Endophyte fungi; *Guignardia* sp.; nematicidal activity; root-knot nematodes.

Screening of nematicidal activity of three plant extracts on *Meloidogyne arenaria* in vitro

Gökhan Aydın¹, Şeyda Şimşek² and Sevilhan Mennan³

¹ Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

² Bozok University, Agricultural Faculty, Plant Protection Department, 66900 Yozgat, Turkey

³ Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

corresponding author: gokhanay@omu.edu.tr

Some plants are a potential source of compounds with nematicidal activity and chemicals produced by plants generally do not have an adverse impact on the environment or humans compared to synthetic nematicide. Therefore, there is a need to search for naturally occurring compounds in plants for nematode control in organic agriculture. In this study, different concentration (1, 2 and 4%) of plant methanol extracts from *Achillea millefolium*, *Bifora radians* and *Peganum harmala* were evaluated for their effects on second-stage juveniles (J2) and eggs of *Meloidogyne arenaria* in vitro. Eggs or J2 in water were transferred to aqueous extracts and incubated at 24°C. Percentages of immobile J2 and hatched J2 were calculated 2 days and 7 days later, respectively. In J2 experiment, extracts were replaced with water after incubation, and percentage of immobile nematodes were calculated again 24 h later. Distilled water was used as a control. The experiment was conducted twice with four replicates per trial. All concentrations of extracts of *A. millefolium* showed the strongest activity on mobility of J2 and immobilised more than 75% of J2 at low concentration after transfer to water. Extracts of *P. harmala* had the least effect on J2 of *M. arenaria*, and large proportion of J2 exposed to high concentration of this extract recovered after transfer to water. Extracts of *A. millefolium* and *P. harmala* at high concentration (4%) inhibited hatching. Among the extracts tested in this study, *A. millefolium* showed the weakest hatching inhibition at low concentration.

Keywords: *Meloidogyne arenaria*; nematicidal activity; plant extract.

Biosurfactants control plant-parasitic nematodes

Sandra Bredenbruch, Koichi Matsuoka, Reenu Gurung, Karina Mellage, Florian Grundler and Sylvia Schleker

University of Bonn, Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, 53115 Bonn, Germany

corresponding author: sylvia.schleker@uni-bonn.de

Plant-associated microbes and their products can control plant pathogens either by direct antagonism or indirectly by triggering plant defence responses. One group of microbial molecules with known anti-bacterial, anti-fungal and anti-viral activity are biosurfactants. Biosurfactants are organic detergents and amphiphilic surface active molecules. Here we show that low concentrations of purified rhamnolipids, which are biosurfactants naturally produced by *Pseudomonas* and other soil bacteria, are effective against the free-living nematode, *Caenorhabditis elegans*, and the plant-parasitic nematode, *Heterodera schachtii*. They cause increased mortality, a reduced infection of *Arabidopsis thaliana* and a lower reproduction rate. Due to these findings we set up a strategy to identify new microbial biosurfactants and biosurfactant-producing bacteria with anti-nematode activity. An identification pipeline including specific culture conditions to enrich biosurfactant-producing bacteria from soil, metagenomic library cloning and screening is applied. 16S rRNA profiling of the enrichment culture's bacterial community compared to the bacterial microbiome of the soil inoculum validated the chosen culture conditions as only genera with known biosurfactant producing species were enriched. Further, a considerable amount of bacteria isolated during the enrichment procedure show anti-nematode activity, thus, confirming the potential of the chosen strategy.

Keywords: Bacteria; *Heterodera schachtii*; rhamnolipids.

Toxicity of deltamethrin on *Ditylenchus destructor* and the effects on its locomotion and feeding behaviours

Zhong Ding¹, Jianxi Liu¹, Deliang Peng² and Wenkun Huang²

¹College of Plant Protection, Hunan Agricultural University, 410128 Changsha, China

²Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100193 Beijing, China

corresponding author: dingzh@hunau.net

Effects of deltamethrin on the mortality, locomotion and feeding behaviours of *Ditylenchus destructor* were investigated by dipping method, sand column method and incubating fluorescent Cy3 method, using profenofos, carbofuran and abamectin as control. The results showed that deltamethrin had moderate nematicidal activity against *D. destructor*, with LC50 value of 459.8 mg l⁻¹, while the LC50 values of profenofos, carbofuran and abamectin to *D. destructor* were 159.9 mg l⁻¹, 331.9 mg l⁻¹ and 257.3 mg l⁻¹, respectively. Deltamethrin also showed better inhibitive activity on the locomotion of *D. destructor*, with IC50 value of 3.1 mg l⁻¹, which was higher than that of profenofos (8.3 mg l⁻¹) and carbofuran (16.1 mg l⁻¹), but lower than that of abamectin (0.8 mg l⁻¹). In the case of feeding, the minimal concentration of profenofos that stimulated more than 90% nematode intake of Cy3 was 0.6 mg l⁻¹, and that of carbofuran was 40 mg l⁻¹. The minimal concentration of profenofos that inhibited all of the nematode intake of Cy3 was 360 mg l⁻¹, and that of carbofuran was 300 mg l⁻¹. Deltamethrin and abamectin could not stimulate the feeding of *D. destructor*. After exposure to 10 mg l⁻¹ profenofos for 2 h, abamectin and deltamethrin could inhibit all of nematodes intake Cy3 at 20 and 200 mg/L, respectively. The results indicated that deltamethrin have the potential in the control of *D. destructor*.

Keywords: Deltamethrin; *Ditylenchus destructor*; toxic behaviour.

Effects of some indigenous plant extracts on hatching and juvenile mortality of *Meloidogyne javanica* (Tylenchida: Heteroderidae) infecting pepper under glasshouse-pot studies

İlker Kepenekci¹ and Hayriye Didem Sağlam²

¹ Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, 60250 Tokat, Turkey

² Department of Plant Protection, Faculty of Agriculture, Ahi Evran University, 40200 Kirsehir, Turkey

corresponding author: saglamhds@gmail.com

Root-knot nematodes (*Meloidogyne* spp.) (Tylenchida: Heteroderidae) are major pests. One of the best alternative methods for control is to use plant extracts. The aim of this study was to evaluate the effects of five different indigenous plant extracts (*Capsicum frutescens*, *Hyoscyamus niger*, *Melia azedarach*, *Xanthium strumarium* and *Achillea wilhelmsii*) on *Meloidogyne javanica* in pot trials in glasshouse conditions. Pepper cv. Charleston, commonly cultivated in Turkey, was used as the assay plant. Plants were inoculated with eggs (3000 eggs plant⁻¹, for the hatching test) or second-stage juveniles (J2) (1000 J2 plant⁻¹, for J2 mortality test) of *M. javanica*. Plant extracts (3, 6 and 12% concentrations) and nematodes were applied at the same time. Positive controls were *M. javanica* eggs or J2 only, and the negative control was water only added to the pots. All of the plant extracts showed different level of anti-nematode activity. Egg masses, plant height, plant fresh weight, plant dry weight, root fresh weight and root dry weight were evaluated and compared with controls. Results showed that, 12% concentration of *X. strumarium* and *H. niger* have marked effect on hatching (17.8 and 19.5 egg masses plant⁻¹, respectively). In the juvenile mortality studies, 12% concentrations of *M. azedarach*, *X. strumarium* and *H. niger* were found effective (21.2, 42.3 and 78.5 egg masses plant⁻¹, respectively). In the control groups, results were 191.74 egg masses plant⁻¹ for the hatching test and 247.4 egg masses plant⁻¹ for the juvenile mortality test. In both experiments, the fresh and dry weight of plant shoots and roots were not significantly different to controls.

Keywords: *Meloidogyne javanica*; pepper; plant extracts; root-knot nematode.

Sensitivity of *Meloidogyne enterolobii* and *Ditylenchus dipsaci* to Fluopyram

Sebastian Kiewnick¹, Andreas Keiser² and Alan Storelli²

¹ Agroscope, National Competence Center for Nematology, 8820 Wädenswil, Switzerland

² Bern University of Applied Sciences BFH, Centre for Food Systems, 3052 Zollikofen, Switzerland

corresponding author: sebastian.kiewnick@agroscope.admin.ch

Fluopyram, a succinate dehydrogenase inhibitor (SDHI) fungicide and recently registered as nematicide in several countries was evaluated in *in-vitro* tests for its effects on *Meloidogyne enterolobii* and *Ditylenchus dipsaci*. Assays to determine effects of Fluopyram on nematode motility and recovery were conducted in aqueous solutions with incubation times of up to 72 h. Second-stage juveniles (J2) of *M. enterolobii* were exposed to concentrations of 0, 0.01, 0.1, 0.5, 1.0 and 10 ppm active substance (a.s.), whereas mixed stages of *D. dipsaci* were exposed to 0, 1, 5, 10, 30 and 50 ppm a.s. For *M. enterolobii*, the EC50 value for immotile J2 after 24 h exposure was 0.7 ppm a.s., whereas the EC50 for J2 that did not recover after washing and additional 24 h in water was 2.71 ppm a.s.. Conversely, *D. dipsaci* was significantly less sensitive to Fluopyram. Incubation for 24 h resulted in fewer than 50% paralysed nematodes at a concentration of 50 ppm a.s. EC50 values after 48 and 72 h exposure ranged around 24 and 12 ppm, respectively. From the paralysed *D. dipsaci*, 50% did not recover at a concentration of 24.7 ppm after washing and incubation in water for an additional 24 h. These findings demonstrate that sensitivity of nematodes to Fluopyram can be very different depending on the nematode species tested. For *M. enterolobii*, calculated EC50 values do correlate with concentrations expected in soil when used under field conditions. However, EC50 values for *D. dipsaci* are 20 to about 50 times higher than the expected concentration under commercial conditions. Therefore, longer exposure at sub-lethal concentrations, or repeated applications might be needed for sufficient control of *D. dipsaci*.

Keywords: Integrated management; nematicide; root-knot nematodes; stem nematode.

Invader vs invader: Potential of *Acacia dealbata* against *Bursaphelenchus xylophilus*

João C.S. Leocádio¹, Paula C. Veríssimo^{2,3} and Cristina I.C. Galhano^{1,4}

¹ Department of Environmental Sciences, Coimbra Agriculture School, Polytechnic of Coimbra, Bencanta, 3045-601 Coimbra, Portugal

² Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

³ CNC - Center for Neuroscience and Cellular Biology, University of Coimbra, Rua Larga, 3000-504 Coimbra, Portugal

⁴ CFE - Centre for Functional Ecology, Department of Life Sciences, Calçada Martim de Freitas, University of Coimbra, 3000-456 Coimbra, Portugal

corresponding author: cicgalhano@esac.pt

Bursaphelenchus xylophilus, the pinewood nematode, native to North America, is considered one of the most dangerous pathogens for conifers and is being spread worldwide nowadays, mainly due to human activity. In Portugal it was first reported in Setúbal Peninsula, in 1999, affecting *Pinus pinaster*. Currently, all the country is considered an affected area and it was already reported as affecting another forest species, *Pinus nigra*. *Acacia dealbata*, the silver wattle, native to Australia, is present in Europe since the nineteenth century, being considered an invasive species in several countries including Portugal. Nevertheless, plants have worked as natural pesticides since ancient times. Thus, searching for sustainable alternatives to control biological invaders and simultaneously reducing the negative impact of using synthesised chemical compounds are important targets. *Acacia dealbata* does not seem to be affected by natural enemies in Portugal and other species of the genus were already reported as nematocides. The present study aims to investigate the nematocidal effect of *A. dealbata* leaves and seed extracts, obtained by maceration and infusion on *B. xylophilus*. To accomplish this goal, *in vitro* assays were conducted. Seed extracts showed mortality rates of upper 90%, unlike other extracts which showed little or no effect on *B. xylophilus*. To better understand the effects, protein profile and enzymatic activity of the extracts were determined. Extracts showed differences in protein profile as well as in enzymatic activity. Seed extract showed greater protein diversity and even enzymatic activity of proteases, chitinases and phosphatases. Thus, this preliminary study indicates that *A. dealbata* species could be explored to develop a new biological nematocide.

Keywords: Bionematicide; enzymatic activity; pinewood nematode; plant extracts; silver wattle.

Naphthoquinones from walnut husk residues – nematicidal activities against *Meloidogyne hispanica*

Carla Maleita¹, Ivânia Esteves², Rita Chim¹, Luís Fonseca², Mara Braga¹, Isabel Abrantes² and Hermínio C. de Sousa¹

¹ CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

² CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: carlammaleita@hotmail.com

Walnut, *Juglans* spp., farming practices and processing activities lead to the generation of large amounts of walnut husk residues that contain natural products that can be easily valorised by their recovery and use at distinct value-added applications. Among these natural products, several naphthoquinones having a wide range of biological activities, including nematicidal, are known to be present in significant amounts. However, their potential uses as viable alternatives to synthetic nematicides have not been fully explored so far. In addition, the scarce availability of efficient commercial nematicides to control important plant-parasitic nematodes, such as *Meloidogyne* spp., together with other environmental and health-related issues, has recently encouraged research towards the development of novel, safer and effective natural-origin nematicides. This study aimed to assess the effects of three pure naphthoquinones usually present in walnut husks (juglone, 1,4-naphthoquinone and plumbagin) on the mortality of *M. hispanica* second-stage juveniles (J2), and to evaluate the effect of extracts from in natura and dried *J. nigra* walnut husks on the *M. hispanica* life cycle. Among the tested pure compounds, 1,4-naphthoquinone was the most active substance, causing 42% J2 mortality (at 50 ppm). The extract from in natura walnut husks had similar effects on *M. hispanica* mortality to those observed for pure 1,4-naphthoquinone. The extract from dried walnut husks was classified as repellent, as it reduced the nematode penetration (by approximately 50%) but did not show effects in reproduction. The results revealed that walnut husk residues can be valorized as a renewable source of naphthoquinone-based natural products or extracts that can be potentially employed as bionematicides against the root-knot nematode *M. hispanica*.

Keywords: 1,4-naphthoquinone; extracts; *Juglans nigra*; juglone; plumbagin.

Trying to contribute to the circular economy: what is the nematicide potential of agroforestry waste on pinewood nematode?

M. Virginia Ozcariz¹, André R. Pereira², João C. S. Leocadio², Luena N. da Silva² and Cristina I.C. Galhano^{2,3}

¹ Cátedra de Micología, Escuela Técnica Superior de Ingenierías Agrarias, Campus Palencia, Universidad de Valladolid, Avenida de Madrid 57, 34004 Palencia, Spain

² Department of Environmental Sciences, Coimbra Agriculture School, Polytechnic of Coimbra, Bencanta, 3045-601 Coimbra, Portugal

³ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: cicgalhano@esac.pt

Pinewood nematode (PWN), *Bursaphelenchus xylophilus*, remains one of the most important enemies of the maritime pine, *Pinus pinaster*, causing severe losses in all the economic fields related to this forest species. In Europe, since its detection in Portugal in 1999, despite all the efforts of the scientific community, there are no effective PWN control methods. Therefore, this work aims to find an environmentally sustainable nematode control solution but, simultaneously, aims to foster the circular economy, a challenge established in December 2015 by the European Union. The adopted Circular Economy Package intends to stimulate Europe towards measures covering the whole product lifecycles, from production and consumption to waste management, promoting recycling and re-use, which will lead to a sustainable economic growth, originating new jobs. Thus, this work will evaluate the nematicidal potential of agroforestry waste, specifically those obtained from pecan nut (*Carya illinoensis*) farms, on *Bursaphelenchus xylophilus*. The tested waste were pecan nut shell, pecan nut pericarp, and wood chips of pruning branches of pecan nut. These waste were chosen based on the significant quantity of their world annual production, and also based on their already reported properties: polyphenols and tannins richness, herbicides, antibacterial and antioxidant, among others. Preliminary *in vitro* tests will be performed testing firstly the nematicidal/nematostatic effects of the above mentioned waste aqueous extracts. Briefly, this research aims to find a new bionematicide promoting the circular economy by using the pecan residues as raw material.

Keywords: Bionematicide; *Bursaphelenchus xylophilus*; *Carya illinoensis*; pecan waste.

Evaluation of the root powder of *Nepeta meyeri* against root-knot nematodes in tomato grown under natural conditions

Hayriye Didem Sağlam¹, İlker Kepenekci² and Atilla Öcal³

¹ Department of Plant Protection, Faculty of Agriculture, Ahi Evran University, Kirsehir, Turkey

² Department of Plant Protection, Faculty of Agriculture, Gaziosmanpaşa University, Tokat, Turkey

³ Bati Akdeniz Agricultural Research Institute, Antalya, Turkey

corresponding author: saglamhds@gmail.com

Vegetables are important sources of many nutrients for human. Many biotic and abiotic factors have a negative impact on yield in vegetables. Root-knot nematodes (*Meloidogyne* spp.) have become a major pest of vegetables. Management of root-knot nematodes have become difficult. Chemical controls have been effective against nematodes, but the chemicals have adverse effects on human health, the environment and non-target organisms due to high toxic residues. For this reason, one of the best alternative methods to control plant-parasitic nematodes is to use plant extracts as biopesticides. The aim of this study was to evaluate nematicidal activities of root powder of *Nepeta meyeri* against mixed populations of *M. incognita* and *M. javanica* under natural conditions between July 2014 and January 2015 in Fethiye (Muğla, Turkey). Ilgin F1-Tomatoes (*Solanum lycopersicum*) variety was used for the experiment. Plant extracts were applied at early planting, with planting, 15th day after planting, and 30th day after planting. Ql Agri 35 SL (BASF) and Vydate L (DUPONT) nematicide were used as a positive control and the negative control was water only, applied by drip irrigation. Three concentrations (0.05, 0.1 and 0.2 g plant⁻¹) of the essential oil, and chloroform, acetone and methanol extracts isolated from the root parts of *N. meyeri* were tested. The experiment was conducted four replicates for each treatment and arranged in a randomised block design. At the end of the growing season the efficiency of the applications were evaluated according to the root gall index based on 0-10 Zeck scale and yield of tomatoes were recorded. The results showed that the different extracts isolates and all doses of extracts did not show a significant effect against *M. incognita* and *M. javanica*. Positive controls suppressed the nematode populations during the experiment.

Keywords: *Meloidogyne incognita*; *Meloidogyne javanica*; *Nepeta meyeri*; plant extracts; root parts.

Screening of nematicidal activity compounds from the seeds of *Camellia oleifera* on *Meloidogyne* and *Heterodera*

Yuhong Sun¹, Liangxiong Xu², Deliang Peng³ and Yanhua Wen¹

¹College of Agriculture, South China Agricultural University (SCAU), Guangzhou City, 510642, Guangdong Province, China

²South China Botanical Garden, Chinese Academy of Sciences, Guangzhou City, 510650, Guangdong Province, China

³State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.

corresponding author: yhw@scau.edu.cn

Camellia oleifera plants has a long history of cultivation in China, the seeds are mainly used for the production of edible oil and tea saponin. In this experiment, 40 g crude extracts from the seeds of *C. oleifera* was subjected to column adsorption of AB-8 macroporous resin and eluted with aqueous EtOH(100:0,70:30,30:70,0:100). The 70% ethanol-eluted fraction was evaluated as strong nematicidal activity fractions by bioassay. From part of 70% ethanol-eluted fraction (8 g), four fractions, TS-3-1, TS-3-2, TS-3-3 and TS-3-4, were obtained by column chromatography over silica gel and eluted with ethylacetate: methanol: n-butanol: water (2: 1: 4: 7). From 3.21 g TS-3-1, six fractions, TS-31-1, TS-31-2, TS-31-3, TS-31-4, TS-31-5 and TS-31-6, were obtained by column chromatography over ODS reverse phase silica gel. From the strong nematicidal activity of fraction TS-31-2, four flavonoid glycosides were purified by HPLC method, and were identified as theaflavanoside IV, theaflavanoside III, theaflavanoside II and naringin by spectroscopic analysis including ¹H-NMR, ¹³C-NMR, ESI-MS, and the data compared with the literature. Bioassay of the nematicidal activities of the four flavonoid glycosides showed that the theaflavanoside IV, theaflavanoside III, theaflavanoside II and naringin have strong nematicidal activities against hatching of *Meloidogyne incognita* with 1 mg ml⁻¹ concentration and 9 day treatment, resulting in the inhibitory rate reaching 94.19, 90.46, 95.44 and 73.86%, respectively. The LC₅₀ of theaflavanoside III, on *Heterodera avenae*, *H. glycines* and *M. incognita*, was 4.326 g ml⁻¹, 90.275 g ml⁻¹ and 105.38 g ml⁻¹, respectively. The LC₅₀ of theaflavanoside IV, against *H. avenae*, *H. glycines* and *M. incognita*, was 9.06 g ml⁻¹, 213.92 g ml⁻¹ and 81.21 g ml⁻¹, respectively. This is the first report nematicidal activity of flavonoid compounds from *C. oleifera* seeds. This Research was supported by Chinese Special R & D Fund for Public Benefit Agriculture (201503114).

Keywords: *Camellia oleifera*; flavonoid glycosides; nematicidal activity compounds.

Induction of natural plant defences to manage root-knot nematodes: optimising application methods and concentrations of plant defence activators

Maria Clara Vieira dos Santos¹, Isabel Abrantes¹ and Rosane H. C. Curtis²

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² Bionemax UK Ltd, Rothamsted Centre for Research and Enterprise, Harpenden, AL5 2JQ, Hertfordshire, UK

corresponding author: mcvs@sapo.pt

The exploitation of natural plant defence mechanisms could lead to sustainable environmentally friendly management strategies to reduce root-knot nematode (RKN) population densities. Natural plant defence mechanisms can be triggered by the application of plant defence activators. However, their efficacy could be dependent on the amount of compound absorbed by the plants. To assess the effect of plant defence activators on the reproduction of the RKN, *Meloidogyne incognita*, tomato plants were treated with benzothiadiazole (BTH) and cis-jasmone, representatives of the salicylic acid (SA) and jasmonic acid (JA) biochemical defence pathways, respectively. In a pot trial, under controlled conditions, the defence activators were applied as foliar sprays, soil drench or seed treatment to tomato plants or seeds, cv Tiny Tim, which were then inoculated with 300 RKN eggs. Three concentrations of each plant defence activator/application method were evaluated (0.16, 0.31 and 0.63 μM for BTH and 2.5, 5 and 10 μM for cis-jasmone). The numbers of galls and egg masses per g root and the nematode reproduction factor were assessed 60 days post-inoculation. None of the plant defence activators, irrespectively of the mode of application or concentration, reduced nematode penetration. Nematode reproduction was effectively reduced by foliar applications of BTH (>20%) or cis-jasmone at 5 and 10 μM (>50%) and seed treatment with cis-jasmone (>40%). When applied as foliar sprays, both BTH and cis-jasmone reduced the number of egg masses per g root (>35%). Only foliar sprays induced an increase in shoot length and root weight. The results suggest that foliar sprays that activate the JA pathway of systemic acquired resistance induce an effect on nematode reproduction in tomato, and should be considered in the design of integrated pest management programmes.

Keywords: Benzothiadiazole; cis-jasmone; foliar sprays; *Meloidogyne incognita*.

Isolation of new entomopathogenic nematodes (EPN) from different Governorates and their production and survival parameters in Egypt

Atef S. Abdel-Razek¹, Mohamed A. Foda², Mona A. Hussein¹ and Ibrahim E. Shehata¹

¹ National Research Centre, Department of Plant Protection, Cairo, Egypt

² Al-Azhar University, Faculty of Science, Department of Zoology, Cairo, Egypt.

corresponding author: abdelrazek820@yahoo.com

Field surveys were carried out at Giza, Behera, Alexandria, Sohag, Qulupeia, El-Sadat city, Sharquia, Bani-swif, North Sinai (El- Aresh city) and South Sinai (Ras-Seder city), Tanta, Fayoum and Suez. Five isolates were isolated from 593 samples. Based on morphometric characters, three strains were found to belong to Heterorhabditidae. These isolates were from Belbies city (Sharquia) and identified as *Heterorhabditis indica* (code IB), from Kefor El-Nile (El-Fayoum) and identified as *H. bacteriophora* (4) and the third strain was from Ras-Seder city (South Sinai) and identified as *H. brevicadus* (1); this latter species was considered as the first record in Egypt. Two Steinernematid strains coded by (5) and (Sg) were isolated from Ras-Seder city (South Sinai) and identified as *Steinernema carpocapsae* and *S. glaserii*. All isolates were multiplied by *in vivo* method using *Galleria mellonella* and the reproductive rate of *H. bacteriophora* (4) was the highest $60.4 \times 10^3 \pm 668$ infective juveniles larva⁻¹ followed by *S. glaserii* (Sg) , *S. carpocapsae* (5), *H. brevicadus* (1) and *H. indica* (IB). Effects of the temperature and the storage periods on survival of the isolated strains showed that survival of Steinernematidae isolates (5) and (Sg) was higher than Heterorhabditae isolates (4), (1) and (IB) after storage at 15°C. The tested isolates were ordered according to storage at 15°C as: 5 > Sg > 4 > 1 > IB. According to tolerance of room temperature after 6 weeks storage the tested isolates were ordered as: 4 > IB > 1 > Sg > 5.

Keywords: *Heterorhabditis*; nematodes; production; Steinernematids; survival.

Cadavers as a resource: competition between entomopathogenic nematodes and free-living nematodes in the genus *Oscheius* acting as scavengers

Rubén Blanco-Pérez^{1,2}, Francisco Bueno-Pallero^{1,2}, Luis Neto² and Raquel Campos-Herrera¹

¹MeditBio, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

²Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

corresponding author: rbperez@ualg.pt

Entomopathogenic nematodes (EPN) thrive in soil from natural and agricultural areas. They are well-studied insect pathogens and their persistence is one of the key parameters for successful use as biological control agents in agroecosystems. The soil is a complex, species-rich environment and, thus, a better understanding of EPN soil food web dynamics, particularly antagonistic interactions, is critical to achieving long-term EPN persistence in crops. Previous studies suggested that free-living nematodes (FLN) in the genus *Oscheius* act as scavengers. These nematodes, also often found in soils, can interfere with EPN reproduction when exposed to live insect larvae. However, whether the EPN reproduction is successful in mixed populations if they have to reproduce in cadavers is unknown. We speculate that being EPN facultative scavengers, they will reproduce in cadavers even when competing with FLN, in a species-specific and density-dependent manner, although production will decrease if compared with the natural niche (live larva). We explored the outcome of EPN-*Oscheius* competition as scavengers by using *Galleria mellonella* larvae cadavers that were frozen and compared with interactions with live ones. The differential reproduction ability of two EPN species (*Steinernema kraussei* and *Heterorhabditis megidis*) alone and combined with two FLN (*Oscheius onirici* and *O. tipulae*) was assessed. Overall, EPN progeny was significantly higher in live larvae than in cadavers, and *H. megidis* produced more than double the number of infective juveniles (IJ) than *S. kraussei*. Similarly, in mixed treatments, the proportion of EPN progeny was significantly lower in cadavers and when *S. kraussei* was employed. The initial EPN inoculum was not critical in defining the final EPN output. Our results showed that even if their final progeny production was strongly limited as well as their ability to overcome competitions with other scavengers, cadavers can contribute to long-term EPN persistence.

Keywords: *Heterorhabditis megidis*; multitrophic interactions; *Oscheius onirici*; *Oscheius tipulae*; *Steinernema kraussei*.

The effects of endophytic bacteria on entomopathogenic nematode virulence and infectivity

Mary Jo Hurley¹, Dina Brazil¹ and Thomae Kakouli-Duarte^{1,2}

¹ Institute of Technology Carlow, Kilkenny Road, R93V960 Carlow, Ireland

² Molecular Ecology and Nematode Research Group (MENRG), enviroCORE, Department of Science and Health, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

corresponding author: maryjo.hurley@itcarlow.ie

Entomopathogenic nematode predictability and efficiency in biological control can be improved by observing the effect of soil microorganisms on nematode behaviour. This project explores the compatibility between an Irish strain of the nematode *Steinernema feltiae* (12[1]), three commercial nematode strains of *S. feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* (e-nema), and a number of endophytic bacterial isolates. Aspects of their interaction under investigation include nematode and bacterial survival, bacterial colonisation, nematode biology and behaviour, and synergism in biocontrol and in plant growth promotion. With the overall study aims being to improve nematode predictability and to assess the potential for a novel combined biocontrol and plant growth promotion agent. Results presented here are from experiments designed to (a) investigate the effects of endophytic bacteria on nematode virulence and (b) to examine the effect of endophytic bacterial exposure on *Galleria mellonella* and *Otiorhynchus sulcatus* susceptibility to nematodes. The effect of endophytes on nematode virulence varied between nematode and bacterial isolates. No individual isolate significantly and consistently affected all nematode species. Moreover, no nematode species was affected across all doses examined. Four separate experiments were carried out to investigate the effect of endophytic bacteria on nematode infectivity. For each insect, a bioassay and a plant based experiment was carried out. Bioassays indicate that there were no significant differences in insect mortality, following exposure to endophytic bacteria. In plant based experiments, oilseed rape and strawberry plants were the experimental arenas for *G. mellonella* and *O. sulcatus* respectively. *G. mellonella* mortality did not differ significantly from the control. Similarly, the presence of endophytic bacteria did not significantly affect the susceptibility of *O. sulcatus* to *S. feltiae* and *H. bacteriophora*.

Keywords: Biocontrol; *Galleria mellonella*; *Otiorhynchus sulcatus*; sustainability; synergism.

Attraction behaviours of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) to synthetic volatiles emitted by insect damaged *Brassica nigra* roots

Žiga Laznik and Stanislav Trdan

University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

corresponding author: ziga.laznik@bf.uni-lj.si

We tested the chemotactic response of infective juveniles (IJ) of the entomopathogenic nematodes (EPN) *Steinernema feltiae*, *S. carpocapsae*, *S. kraussei* and *Heterorhabditis bacteriophora* to the synthetic volatiles (dimethyl sulfide [DMS], dimethyl disulfide [DMDS], dimethyl trisulfide [DMTS], allyl isothiocyanate [AITC], phenylethyl isothiocyanate [PITC], and benzonitrile [BN]) emitted by insect-damaged *Brassica nigra* roots. We hypothesised that attraction behaviour exhibited by the EPN toward the tested volatile compounds (VOC) could be related to the species and would vary with foraging strategy, VOC, time of exposure and temperature. *Steinernema kraussei* was the most mobile species in our assay, and at 20°C, more than 35% of *S. kraussei* IJ moved to the outer circles of the Petri dishes, while among the other tested EPN species, the greatest movement was observed in *H. bacteriophora* (10%). The data showed that chemosensation is more a species-specific characteristic than a host searching strategy. The movement of different EPN species towards VOC was influenced by temperature; at the higher temperature (25°C), *S. feltiae* was the most mobile species in our investigation because 42% of IJ moved to the outer circles. All of the compounds tested in our assay repelled *S. kraussei* at both experimental temperatures, suggesting that sulphur compounds and glucosinolate breakdown products could play an important role in EPN navigation.

Keywords: *Brassica nigra*; chemosensation; *Heterorhabditis*; *Steinernema*; VOC.

Thermal plasticity of symbiont and its host: Model of growth rate for *Xenorhabdus bovienii* isolated from different latitudes

Jasmina Mackiewicz and Daniel P. Kübler

Institute of Environmental Science, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland

corresponding author: jasmina.mackiewicz@uj.edu.pl

The main aim of this study was to test whether temperature has an influence on the association between entomopathogenic nematode and its symbiotic bacteria. Symbiotic bacteria provide essential nutrients for their hosts and allow them to colonise new niches. In general, symbiotic bacteria are characterised by lower thermal plasticity than their hosts, which has been observed for example in corals and aphids. At higher temperatures symbiotic bacteria cannot survive, which in turn negatively affects host fitness. Symbionts can also decrease host's reproduction rate and longevity through competing for the same resources or through increased metabolic activity. In this study we estimated the growth curve of symbiotic bacteria *Xenorhabdus bovienii* and compared it with thermal reaction norm for its host entomopathogenic nematode *Steinernema feltiae*. We tested eight strains of *X. bovienii* isolated from different latitudes across the Europe. Firstly, we measured growth rate in exponential phase of *X. bovienii* at temperatures ranging between 2°C and 35°C. To estimate bacterial growth as a function of temperature we used the square-root model proposed by Ratkowsky (1983). Maximal, minimal and optimal temperature for bacteria's growth was estimated. Then we measured number of progeny for nematode *S. feltiae* at temperatures ranging between 16°C and 30°C to assess maximal, minimal and optimal temperature for reproduction. Finally we compared data for nematodes and symbiotic bacteria. Our results show that symbiotic bacteria *X. bovienii* has wider thermal range than its host nematode *S. feltiae*. Our results also suggest that *S. feltiae* prefers lower growth rate of its symbiont for successful reproduction.

Keywords: Entomopathogenic nematodes; growth curve; mutualism; Ratkowsky model.

Discovery of a new jumping behaviour for host-finding of an entomophilic nematode *Caenorhabditis japonica*

Etsuko Okumura, Yuko Takeuchi-Kaneko and Takuma Sugi

Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake, Sakyo, Kyoto 606-8502, Japan

corresponding author: etsuko8okumura@gmail.com

Jumping is known as one of the host-finding behaviours and a unique strategy in entomopathogenic species, especially in the genus *Steinernema*, such as *S. carpocapsae*. A bacteriovore nematode, *Caenorhabditis japonica*, forms a phoretic and necromenic association with the subsocial burrower bug, *Parastrachia japonensis* in a species-specific manner. Dauer larvae (DL) of *C. japonica* nictate and attach to nymphs of *P. japonensis* around the nest under leaf litter. Nictating behaviour is often observed in laboratory regardless of whether the host bug exists. During observation under microscopy, it sometimes happened that nictating DL disappear from sight and then we realised that *C. japonica* DL would jump by themselves. In order to capture the moment of jumping behaviour, we firstly inoculated DL in water suspension on small particles of dog-food made by crushing in a blender, which were spread on agar plate / NGM, and served for recording with a digital / a high-speed camera. As a result, it was revealed that DL of *C. japonica* jump in various styles, not only in a similar style to *Steinernema* but also twisting, and no motion without bending their body from head to tail. Moreover, *C. japonica* DL showed an amazing collective jumping behaviour, which looked like a cooperative phenomenon for survival. Secondly, we tested whether *C. japonica* is able to jump on a filter paper in a plastic plate without nutrition. As a result, *C. japonica* DL were able to jump on a filter paper, and individuals that had jumped were observed soon after inoculation. However, *C. japonica* DL had quite active movement, so that it is better to prepare a smaller filter paper than plastic plate, and not to let them climb on the wall directly and reach the lid of the plate. Furthermore, some DL showed a longer jumping distance than we expected; the longest distance was at least more than 15 times its body length of *C. japonica* DL.

Keywords: Collective/cooperative behaviour; leaping; multiple jumping patterns; new jumping behaviour.

Phenotyping for oxidative stress-tolerance as tool to screen for extended longevity in *Heterorhabditis bacteriophora* infective-juvenile populations

Nanette H Nellas Sumaya, Verena Dörfler, Mike Barg, Olaf Strauch, Bart Vandenbossche, Carlos Molina and Ralf-Udo Ehlers

e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany

corresponding author: N.Hope@e-nema.de

The nematode-bacterium association between *Heterorhabditis bacteriophora* and *Photorhabdus luminescens* is a safe control agent against insect pests. *H. bacteriophora* infective dauer juveniles (DJ) survive on the soil after application and seek for insect hosts. However, limited shelf life and restricted stability under environmental stresses are major obstacles to a widespread use of entomopathogenic nematodes in large-scale agricultural systems. In the present work, the influence of oxidative stress-tolerance as genetic selection parameter for prolonged DJ-longevity has been investigated. A core collection of natural isolates of different origin was characterised for their DJ mid-survival-time under oxidative stress at room temperature and cold storage conditions. Differences of 19 and 36 days in mean survival time were detected among the isolates stored under oxidative and control conditions, respectively. A strong correlation was determined between the DJ-longevity under oxidative-stress and the DJ-survival in populations stored without stress induction. Subsequently, a population composed of 40 different isolates (mix) was subjected to oxidative selection pressure. An increase in DJ-longevity was determined after selection. The mix pool had an extended mid-survival-time of 9 days after two selection cycles. Additionally, the selected pool was more resistant to heat and desiccation stress when compared with a wild type population. Candidate genes for longevity and oxidative stress regulation in nematodes are currently tested for their impact on phenotypic effects via RNAi and are used to genotype natural populations and genetic crosses. Our results are of relevant importance for the understanding of environmental factors regulating DJ-longevity and stress responses in entomopathogenic nematodes as well as for the generation of breeding strategies to develop biocontrol products with prolonged shelf life and soil persistence.

Keywords: Extended life span; nematode shelf life; reactive oxygen species.

Attraction of an entomopathogenic nematode to a plant-parasitic fungusSheng-Yen Wu¹, Fahiem E. El-Borai^{1,2} and Larry Wayne Duncan¹¹ University of Florida, Citrus Research and Education Center (CREC), 700 Experiment Station Rd, Lake Alfred, FL 33850, USA² Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig, Egyptcorresponding author: Fahiem@ufl.edu

Given the ubiquity of entomopathogenic nematodes (EPN) in the rhizosphere, other soil inhabitants have likely evolved methods to exploit their services and the resources in insects killed by these nematodes. For example, some free-living, bacterivorous nematode species are reported to compete effectively with entomopathogenic nematodes and some plant roots respond to herbivory by producing volatile cues that attract EPNs. In two surveys to characterise the EPN community in a citrus orchard, 42% and 14% of sentinel *Galleria mellonella* larval cadavers were infected by *Fusarium solani* without evidence of EPN reproduction, whereas EPN emerged from 28% and 44% of the cadavers, respectively. We are testing the hypotheses that *F. solani* might 1) attract EPN to insects and 2) thereafter compete with EPN in the insect cadaver. In two-choice olfactometer assays, PVC t-tubes were filled with either autoclaved or raw, sandy soil (8% moisture). Agar plugs with or without *F. solani* mycelia and conidia were added to sand in caps at opposite sides of the tubes. Larvae of *G. mellonella* were restrained with screens in both sides of tubes in some experiments but were absent in others. Three hundred infective juvenile (IJ) *Steinernema diaprepesi* were added to the centre arm of the t-tube and after 24 h, nematodes were recovered from the soil in caps on each side of the tube. When insects were present they were dissected and numbers of recovered IJ were added to that from the soil. In the presence or absence of insects, significantly more IJ were recovered from the tube side with the fungus than from that without. The habitat complexity affected the rate of attraction. The presence of the insect reduced the rate of attraction to *F. solani* by more than half and attraction in autoclaved soil was more than twice that in raw soil. Ongoing experiments are testing whether *F. solani* affects the insecticidal efficacy and/or reproductive success of EPN.

Keywords: *Fusarium solani*; habitat complexity; herbivory; *Steinernema diaprepesi*; volatile cues.

Evaluating nematode and yield data - do we need additional information?

Suria Bekker¹, Mieke Daneel², Hendrika Fourie¹ and Andre Nel³

¹ Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

² Agricultural Research Council – Institute for Tropical and Subtropical Crops (ARC – ITSC), Private Bag X11208, Nelspruit, 1200, South Africa

³ Agricultural Research Council - Grain Crops Institute (ARC – GCI), Private Bag X1251, Potchefstroom, 2520, South Africa.

corresponding author: mieke@arc.agric.za

When conducting nematode trials, nematode and yield data are collected and after statistical analysis significant differences of nematodes and yield between the different treatments are determined. However, other factors can play a significant role in yield and are often not taken into account. A 4-year trial was conducted to determine the effect of conservation agriculture (CA) on yield of maize as the main crop in the North West Province of South Africa. The trial included four replicates of 12 treatments, consisting of monoculture maize produced under conventional practices (CT) compared with monoculture maize and maize rotated with cowpea/sunflower only or cowpea/sunflower and pearl millet sequence under CA practices. Together with yield, nematode assemblages and soil chemistry were determined annually to investigate the effect of CA on the different parameters. When yield was plotted against the XY coordinates, it was obvious that the first replicate was significantly different from the other replicates since yield was much lower in these plots. When investigating soil chemistry and nematode populations, this discrepancy seemed to be linked with higher Aluminium (Al) and Potassium (K) concentrations in this replicate. Multi-table analysis was performed to determine the effect of plant-parasitic nematodes (PPN), soil chemistry and treatments on yield over the 4-year period, allowing four years to be compared in one graph known as a compromise. Data was analysed using 3 and 4 replicates to determine the impact of the first replicate on yield. Fortunately, since all treatments were represented in the 1st replicate, it did not seem to have a significant effect on the results obtained. However, when factors like excessive Al and K levels are unevenly distributed in a field, this might have a significant effect on results and inferences made, confirming the importance to collect additional soil data when conducting nematode trials.

Keywords: Conservation agriculture; maize; plant-parasitic nematodes; soil chemistry.

Root-knot nematodes parasitising African nightshades and African spinach in Kenya

Oliver Chitambo¹, Solveig Haukeland², Komi Fiaboe², Sunday Ekesi², George Kariuki³ and Florian Grudler¹

¹ INRES, Institute of Crop Science and Resource Conservation, Molecular Phytomedicine, Rheinische Friedrich-Wilhelms-Universität Bonn, D-53115 Bonn, Germany

² ICIPE - International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya

³ KU - Kenyatta University, Department of Agricultural Sciences and Technology, P.O. Box 43844-00100 Nairobi, Kenya

corresponding author: chitambooliver@gmail.com

African nightshades (*Solanum* spp.) and African spinach (*Amaranthus* spp.) are important leafy vegetables in many parts of Africa. Sustainable production of African nightshades and African spinach faces a twin challenge from both above- and below-ground pests. Root-knot nematodes (*Meloidogyne* spp.) are below-ground pests capable of parasitising many plant hosts including African nightshades and African spinach leading to severe yield loss. The epidemiology of root-knot disease on other crops is well studied, but its importance on different species of African nightshades and African spinach remains largely unknown. This study aimed at uncovering the hidden diversity of *Meloidogyne* spp. on African nightshades and African spinach. Sampling included fields located in three regions of Kenya: Eastern, Western and Central province. Standard extraction techniques were used to extract nematodes from 62 soil/root samples. Subsequently, nematodes were identified at genus level and population densities determined. Morphological characters were used to place *Meloidogyne* spp. into distinct groups. In order to confirm the species identity, mitochondrial gene sequencing was used targeting the following gene fragments: COII and 16S rRNA, COI and NAD5. *Meloidogyne* spp. were detected in all sampled regions in 95% soil and 65% of root samples. *M. javanica* was the most abundant species. African nightshades were found to be good host of *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. hapla*, but non-host to *M. incognita*, whereas African spinach were parasitised by *M. incognita* only. This is the first report of *M. enterolobii* parasitising African nightshades in Kenya. The information acquired calls for precise monitoring of *Meloidogyne* spp. on agricultural land. The identified non-host genotypes of African nightshades and African spinach may offer an unexploited opportunity for the management of *Meloidogyne* spp.

Keywords: Detection; *Meloidogyne enterolobii*; mitochondrial markers; non-host.

Field evaluation of soil nematode communities under organic and conventional farming systems in Chuka, Tharaka Nithi County, Kenya

Janet Gesare¹, Solveig Haukeland², George Kariuki¹, Edward Karanja², Martha Musyoka² Komi Fiaboe² and Noah Adamtey³

¹ Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya

² ICIPE - International Centre for Insect Physiology and Ecology, Plant Health Division, P.O. Box 30772-00100 Nairobi, Kenya

³ Department of International Cooperation, FiBL, P.O. Box 113 Ackerstrasse, Switzerland

corresponding author: shaukeland@icipe.org

A study was conducted to evaluate the effects of organic and conventional farming systems on the abundance and diversity of soil nematodes in Kenya. An on-farm experiment (site 1) was set up in April 2015 and March 2016 with four farmers (each having four 5 × 5m plots). A parallel experiment was established as a demonstration trial (site 2) consisting of four plots of the same size and replicated four times. Maize-bean intercrop was planted in the long rainy season and beans monocrop in the short rainy season. The organic plots received neem cake + compost + Tithonia + Ash; the conventional plots Marshall + Calcium Ammonium Nitrate + Diammonium phosphate, and farmers' practice Ash + Manure. Sampling was done during planting, flowering and harvesting where at least 500 g soil and 50 g roots were collected from each plot. A total of 31 genera belonging to bacterial feeders (BF), fungal feeders (FF), omnivores (OM), predators (PR) and plant-parasitic (PPN) nematodes were recovered from the samples/plots. Total abundance of nematodes in soil and roots was significantly different between the treatments where organic plots recorded the highest (2039) followed by conventional (1711), control (1594) and farmers' practice (1126). Composition of trophic groups was significantly different among the treatments. Organic had the highest BF (826) and least PPN (398.5); conversely the non-amended control had highest PPN (639.5) and low BF (284). Ecological indices including maturity index (MI), channel index (CI) and enrichment index (EI) were significantly different between the treatments and seasons but not for sites. Diversity parameters such as genera richness (d), Shannon index (H') and Renyi profile were highly significant between the treatments. In this study organic amendments appear directly or indirectly to suppress populations of PPN. Such farming systems are therefore recommended for small holder farmers and extension officers to use for awareness creation.

Keywords: Kenya; nematode diversity; nematode management; tropical farming systems.

Plant-parasitic nematodes in commercial pineapple fields in Kenya and the effect of biological control agents on *Meloidogyne* speciesAgnes W. Kiriga¹, [Solveig Haukeland](#)², George Kariuki¹ and Nikolai Van Beek³¹ Department of Agricultural Science and Technology, Kenyatta University P.O. Box 43844-00100 Nairobi, Kenya² ICIPE, Plant Health Division, P.O. Box 30772-00100 Nairobi, Kenya³ Kenya Biologics Ltd, P.O. Box 4560-01002 Thika, Kenyacorresponding author: shaukeland@icipe.org

Plant-parasitic nematodes in particular *Meloidogyne* spp. cause significant yield reduction in commercial pineapple worldwide. In Kenya few studies have been conducted on nematodes in pineapple, although the main commercial producer in Kenya has sole dispensation to use Telone II (1,3-Dichloropropene) indicating the seriousness of the problem. This study was conducted to provide an update on the occurrence of plant-parasitic nematodes in commercial pineapple and to evaluate the effect of selected bio-control agents on *Meloidogyne* species. Surveys were conducted in the plantations of Del Monte and Kakuzi (Thika and Murang'a respectively). Soil and root samples were collected from different ages of the crop. After extraction, nematodes were counted and identified to genus level using morphological features including molecular techniques for the *Meloidogyne* species. Glasshouse experiments on rooted pineapple crowns were conducted to evaluate the efficacy of three isolates of *Trichoderma* spp. two isolates of *Purpureocillium lilacinum* and a commercial *Paecilomyces lilacinus* against *Meloidogyne* spp. The survey results indicated a widespread distribution of plant-parasitic nematodes with the dominating spp. being *Meloidogyne* spp. (*M. javanica*), *Helicotylenchus* spp. and *Tylenchus* spp. both in soil and root samples. More nematodes were recovered from older fields (plant crop and ratoon crop) than in younger fields (pre-plant and new planting). Results from the glasshouse experiments showed that M2RT4 (icipe *Trichoderma* isolate), MK4 (Kenya Biologics (KBL) *Trichoderma* spp.) and KLF2 (KBL *Purpureocillium* spp.) reduced gall formation on pineapple roots as well as number of eggs compared to the untreated control. The results are promising showing possible alternative means of managing nematodes in pineapple.

Keywords: Bio-control agents; *Paecilomyces*; pineapple; *Purpureocillium*; *Trichoderma*.

Nematodes on yam in Nigeria: Situation on the ground

Yao Adjiguita Kolombia¹, Lava P. Kumar², Nicole Viaene³, Wim Bert¹ and Daniel L. Coyne⁴

¹ Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent Belgium

² IITA, International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria

³ ILVO, Institute for Agricultural and Fisheries Research, 9820 Merelbeke, Belgium

⁴ IITA, IITA-Kenya, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya

corresponding author: kolombia3@hotmail.com

Yam (*Dioscorea* spp.) is an important food crop in West Africa. Among the various pests and diseases that affect yam, nematodes are a major production constraint, which also seriously contribute to the tuber deterioration during storage. The present study was conducted in order to determine the current situation regarding nematode problems in the major yam growing of areas in Nigeria. Nematode symptoms (cracks, dry rot and galling) were visually assessed from yam tubers from heaps of yam tubers (n=1147) with vendors (n=181) in yam markets (23) in three agroecological zones. Where possible, two farmers within the vicinity of each market were visited (26 farmers) and 100 tubers scored individually for the presence of nematodes symptoms. Questionnaires were administered to determine vendor and farmer perceptions of nematodes and estimate losses due to nematodes. Symptomatic tubers were collected for nematode density estimation and identification. Percentage of galling tubers at market ranged from 52% (Derived Savannah) to 59% (Humid Forest). Dry rot incidence ranged from 17% (Southern Guinea Savannah) to 44% (Humid Forest). Incidence of cracks was greatest in the Derived Savannah (7%) and lowest in the Southern Guinea Savannah (4%). From farmers' stores, percentage galled tubers ranged from 22% (Derived Savannah) to 27% (Southern Guinea Savannah), and dry rot from 8% (Southern Guinea Savannah) to 9% (Derived Savanna). Incidence of cracks was low and varied from 1% to 3%. The symptoms of both nematodes on the same yam tubers were observed in both markets and farmers' stores. *Meloidogyne arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica* were found associated with galling on tubers whilst *Scutellonema bradys* was found associated with the dry rot. *Pratylenchus* spp. were found on both dry rot and galling tubers. Losses due to nematodes were estimated to be high and the awareness of farmers and vendors to nematodes and their damage to be low, based on the responses to the questionnaire.

Keywords: Dry rot; gall; *Meloidogyne*; *Pratylenchus*; *Scutellonema*.

Survival of *Meloidogyne javanica* during hot seasons under semiarid conditionsYuji Oka and Nadia Tkach

Gilat Research Center, M.P. Negev 8528000, Israel

corresponding author: okayuji@volcani.agri.gov.il

Root-knot nematodes (*Meloidogyne* spp.) are known to survive in soil for several months during hot seasons, also in semiarid areas. High temperatures and low water content of the soil can be the main factors for their survival. Anhydrobiosis of juveniles has been reported as a survival mechanism, and downward migration of the juveniles in the soil is generally 'believed'. If such migration exists after crop harvest, nematode control strategies should be reconsidered; for example, nematicide application immediately after the harvest. This study was performed to elucidate the survival mechanisms of *M. javanica* during hot and dry seasons under field conditions. Tomato plants were grown during May to July in nematode-infected field plots. After the plants were removed, the irrigation of a half of the plots was stopped. Soil samples were taken from five (0-10, 10-20, 20-30 30-40 and 40-50 cm) depths with intervals of a month (Aug-Oct), and processed for nematode extraction by Baermann funnels, and population estimation by a bioassay. The maximum soil temperatures during the sampling period at 10 cm depth were 43.1 and 40.4°C in non-irrigated and irrigate plots while those at 50 cm depth were 33.7 and 33.8°C. Number of extractable juveniles from all the soil depths by the funnels decreased sharply one month after removing the plants, whereas the bioassay showed gradual decreases of the nematode populations with time except for the first 10 cm layer where the nematode populations decreased drastically in the first month in both irrigated and non-irrigated plots. The most significant factor for the nematode survival was time, whereas soil depth (10-50 cm) and irrigation were weak factors. The results indicate that *M. javanica* juveniles do not migrate vertically several decimeters to avoid the harsh soil conditions. Anhydrobiosis and quiescence are probably the main survival mechanism of *M. javanica* juveniles.

Keywords: Dry soil; migration; moisture; quiescence; soil temperature.

Genome-wide association study in wheat identifies resistance to the cereal cyst nematode *Heterodera filipjevi*

Shree Pariyar¹, Abdelfattah A. Dababat², Wiebke Sannemann^{3,5}, Gul Erginbas-Orakci², Abdelnaser Elashry^{1,4}, Shahid Siddique¹, Alexei Morgounov², Jens Leon³ and Florian M. W. Grundler¹

¹ Institute of Crop Science and Resource Conservation (INRES), Molecular Phytomedicine, Karlrobert- Kreiten Strasse 13, D-53115 Bonn, Germany

² International Maize and Wheat Improvement Centre (CIMMYT), P.K. 39 06511, Emek, Ankara, Turkey

³ Institute of Crop Science and Resource Conservation (INRES), Plant Breeding, Katzenburgweg, Bonn, Germany

⁴ Agricultural Research Center (ARC), Agricultural Genetic Engineering Research Institute (AGERI), 9 Gama Street, Giza 12619, Egypt

⁵ University Halle-Wittenberg, Plant Breeding, Betty-Heimann-Strasse 3, 06120 Halle (Saale) 5, D-53115 Bonn, Germany

corresponding author: pariyar@uni-bonn.de

The cyst nematode *Heterodera filipjevi* is a plant parasite causing substantial yield loss in wheat. Resistant cultivars are the preferred method of controlling cyst nematodes. Association mapping is a powerful approach to detect associations between phenotypic variation and genetic polymorphisms; in this way favourable traits such as resistance to pathogens can be located. Therefore, a genome wide association study of 161 winter wheat accessions was performed with a 90K iSelect SNP chip. Population structure analysis grouped into two major sub-groups and first principle component accounted 6.16% for phenotypic diversity. The genome wide linkage disequilibrium across wheat was 3cM. Eleven quantitative trait loci (QTL) on chromosome 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL, and 7BL were identified using a mixed linear model false discovery rate of $P < 0.01$ that explained 43% of total genetic variation. This is the first report of QTL conferring resistance to *H. filipjevi* in wheat. Eight QTL on chromosome 1AL, 2AS, 2BL, 3AL, 4AL and 5BL were linked to putative genes known to be involved in plant pathogen interaction. Two other QTL on 3BL and one QTL on 7BL linked to putative genes known to be involved in abiotic stress.

Keywords: Association mapping; quantitative trait loci; SNP markers.

Morphological and molecular characterisation of *Xiphinema* species in Galicia (Spain)

Adela Abelleira, Olga Aguin, Andrea Abelleira-Sanmartin, Yoel Castro, Alicia Prado, Pilar Piñón and Pedro Mansilla

Estación Fitopatológica Areeiro, Deputación Pontevedra, Subida a la Robleda s/n, 36153 Pontevedra, Spain

corresponding author: adela.abelleira@depo.es

Species of genus *Xiphinema* are among the most important plant-parasitic nematodes, not only for the direct damage they caused to plants, but also for the role of some species as vectors of nepoviruses. These nematodes are divided in two groups: the *Xiphinema americanum* group (55 spp.) and the *Xiphinema non-americanum* group (200 spp.). Identification of *Xiphinema* species has been traditional based on the recognition of morphological characteristics, a sometimes difficult task that requires experienced personnel. An accurate diagnostics is especially needed when dealing with quarantine species and with those vector species of nepoviruses causing damage to economically important crops such as grapevines. Molecular methods developed in recent years have enabled and facilitated the identification of species from both *Xiphinema* groups. In this work *Xiphinema* species present in soil samples from different crops, mainly grapevine, taken in Galicia (NW Spain) during 2013-2016, are reported. The species identification was carried out by morphometric and molecular techniques. For molecular analyses, nematode DNA was extracted and D2-D3 expansion segments of 28S rDNA were amplified and sequenced. The sequences obtained were used for phylogenetic analyses. Five species of the *Xiphinema americanum* –group, namely *X. brevisicum*, *X. madeirense*, *X. pachtaicum*, *X. rivesi* and *X. santos*, and four of the *Xiphinema non-americanum*-group, *X. turdetanensis* and three populations of *Xiphinema* sp. were found.

Keywords: Americanum group; dagger nematode; grapevine; non-americanum group; phylogeny.

ITS sequences reveal genetic variation within populations of *Pratylenchus quasitereoides* and between *Pratylenchus* species in Australia

Farhana Begum¹, John Fosu-Nyarko¹, Shashi Sharma², Bill Macleod² and Michael Jones¹

¹ Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, Perth, WA 6150, Australia

² Department of Agriculture and Food Western Australia, South Perth, WA 6151, Australia

corresponding author: farhanakbd@gmail.com

Pratylenchus quasitereoides is a newly described root-lesion nematode species in Western Australia. It is widespread with high population densities in wheatbelt regions and presents a growing challenge to grain production reducing wheat and barley yields by up to 24%. The damage caused by root-lesion nematode is species-specific, hence, it is essential to identify the species of infested fields as a pre-requisite to developing appropriate control strategies. There is very little morphological variation in root-lesion nematodes making it difficult to distinguish species using this feature alone. The aim of this project was therefore to identify *P. quasitereoides* from among nematode populations from four wheat fields in Western Australia using sequences of the partial 18S-ITS1–5.8S-ITS2-partial 28S regions of genomic DNA as a first step to quantifying the extent of infestation. Using morphometric parameters and DNA sequencing, *P. quasitereoides* was distinguished from 30 *Pratylenchus* species; its sequences indicated it was more closely related to *P. bolivianus* (85% identical). *P. quasitereoides* from two locations formed a sub-group in a phylogenetic analysis with 8% variation within nematodes of the other locations. It was also clearly distinguished from *P. thornei* present in Western Australia with as much as 37% variation in the ribosomal sequences compared to *P. neglectus* and *P. penetrans*. Interestingly, morphometrically identified Australian populations of *P. neglectus* and *P. penetrans* were respectively only 57% and 56% identical to sequences of similar species elsewhere, whereas the sequences of *P. thornei* from Western Australia was 99.6% identical to those identified from Italy, Spain and USA. This knowledge of genetic variation of root-lesion nematode populations in Western Australia wheatbelt is important: it will help in addressing yield losses associated with particular species of root-lesion nematodes and in developing specific methods for their control.

Keywords: Genetic variation; ITS; nematode population; *Pratylenchus* spp.

New microsatellite loci and population genetics analysis of *Heterodera schachtii* (Nematoda: Heteroderidae) in South KoreaJiyeon Kim¹, Taeho Kim², Haena Kwak¹, Soyeong Jin¹ and Joong-Ki Park¹¹ Division of EcoScience, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea² Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, 145 Anamro, Seongbuk-gu, Seoul 02841, Republic of Koreacorresponding author: godspeedtaeho@gmail.com

The sugar beet cyst nematode, *Heterodera schachtii* (Schmidt, 1871), is a major agricultural pest that is difficult to track and even more challenging to eradicate. Genetic markers can be used to infer population dynamics, track invasive species, and prevent ecological damage and economic loss. In 2011, *H. schachtii* infestations were discovered in South Korea for the first time. We developed fifteen new microsatellite markers for this species and used them to track this invasion in its early stage. We examined the genetic diversity and structure of three populations and confirmed the most likely point of entry. Populations had low heterozygosity levels, which may be due to population structure, history, and inbreeding. Our analysis suggested that the nematode may have entered South Korea from a single source population of mixed ancestry, or from multiple sources. The microsatellite loci we developed could be useful for population genetics studies and phytoparasitic nematode monitoring in various areas of pest management, and contribute to the study of the invasion biology of nematodes.

Keywords: Genetic diversity; *Heterodera schachtii*; microsatellite markers; simple sequence repeat; South Korea.

The BIOCOTES project: Overview of the research focused on the application of entomopathogenic nematodes in Europe

Itamar Glazer¹, Dana Ment¹, Duarte Toubarro², Nelson Simoes², Bart Vandebossche³, Carlos Molina³, Ralf-Udo Ehlers³, Olaf Strauch³, Christine Griffin⁴, Apostolos Kapranas⁴, Julien Chuche⁴, Iwona Skrzecz⁵ and Pdraig O'Tuama⁶

¹ ARO, Volcani Centre, HaMaccabim Road 68 , P.O.B 15159 Rishon LeZion 7528809, Israel

² Fundação Gaspar Frutuoso, University of Azores Rua Mãe de Deus n° 13, 9500-321 Ponta Delgada. S. Miguel – Açores, Portugal

³ e-nema GmbH, Klausdorfer Str. 28 -36, 24223 Schwentinental, Germany

⁴ NUIM, Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland

⁵ IBL, Forest Research Institute, Sekocin Stary, Braci Lesnej 3, 05-090 Raszyn, Poland

⁶ Coillte, Dublin Road, Newtownmountkennedy, Co. Wicklow, Ireland

corresponding author: c.molina@e-nema.de

Within the EU-BIOCOTES project, twenty-seven international partners aim to develop eleven new biological control agents in Europe. Three work packages have as objective to improve the use of entomopathogenic nematodes (EPN) in agriculture and forestry. Regarding genetics for trait improvement, three teams are involved characterising longevity and virulence related genes in *Heterorhabditis bacteriophora*. Predictive dauer juvenile (DJ) longevity bioassays were developed and natural isolates have been characterised for longevity and virulence (E-nema, Germany). Research on desiccation-tolerance related genes has been carried out extensively. Candidate markers with differential expression after desiccation were determined (Volcani Centre, Israel). Furthermore, differentially-expressed virulence genes were identified via RNA-seq (University of Azores, Portugal). In the area of biocontrol of forest pests, the efficacy of EPN against the large pine weevil (*Hylobius abietis*) in clear-felled coniferous forests has been assessed. *Steinernema* and *Heterorhabditis* EPN were compared for their effect on pine weevils in roots and stumps. In addition, the effect of soil type and application method was analysed. Field trials were carried out by the Maynooth University and Coillte in Ireland, and by the Forest Research Institute (Poland). Both EPN species were effective in deep peat and in mineral soils in field tests and resulted in considerable mortality of *Hylobius* larvae in treated stumps. In industrial production, the improvement of the downstream processing of *H. bacteriophora* is a main objective (E-nema, Germany). Two principles for separation of DJ from the culture broth were compared and a small-scale system was developed to monitor the shelf-life and virulence of stored EPNs after production. The support by the EC to the BIOCOTES project will mark a milestone in the further establishment of EPN as alternative control measure to chemical pesticides in Europe.

Keywords: Biocontrol; *Heterorhabditis*; pest management; *Steinernema*.

Effect of entomopathogenic nematodes on three soft scale insect species (Hemiptera: Coccidae)

Nikola S. Grujic¹, Branimir M. Nježić², Marina M. Dervišević¹ and Draga Đ. Graora¹

¹ University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade, Serbia

² Faculty of Agriculture, University of Banja Luka, Bulevar vojvode Petra Bojovića 1A, Banja Luka, Bosnia and Herzegovina

corresponding author: grujic@agrif.bg.ac.rs

Virulence and pathogenicity of commercial strains of entomopathogenic nematode species *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* were tested on young females of *Pulvinaria hidrangeae*, *Partenolecanium corni* and *Coccus pseudomagnoliarum*. All three scale insect species are polyphagous, with variety of ornamental plants as their hosts. The latter two can be serious pests in orchards, especially in organic ones. *C. pseudomagnoliarum* is known as pest of citrus and *P. corni* of various continental fruit tree species in Serbia. The laboratory tests were carried out in plastic Petri dishes with infective juveniles applied on filter paper. Treatments with different nematode concentrations and control were replicated five times with 10 insects in each Petri dish. After 7 days all insects were dissected. Only dead insects with presence of nematodes and/or clear symptoms of infestation were considered killed by nematodes. Results showed that all scale insects are hosts for all tested nematode species, since second generation of infective juveniles was observed in each case. Highest insect mortality was observed within highest concentration (50 infective juveniles per insect) in all combinations. *Partenolecanium corni* had the greatest overall nematode presence (90-100%) with *H. bacteriophora* causing 100% mortality of young females. *Pulvinaria hidrangeae* mortality was overall 80%, while highest mortality was 92% with *S. carpocapsae*. The least effect nematodes had on *C. pseudomagnoliarum* causing overall mortality of about 70%, which is probably due to high mortality rate in first days of experiment, as in control of 32%. Our results show that entomopathogenic nematodes caused high mortality rate of examined species. Further work is needed for developing strategies for field application. Potential inclusion of entomopathogenic nematodes in strategies for scale insects control could be of great importance, especially in organic orchards and urban areas.

Keywords: Biological control; entomopathogenic nematodes (EPN).

Entomopathogenic nematodes in East Africa

Solveig Haukeland

ICIPE - International Centre for Insect Physiology and Ecology, P.O. Box 30772 00100 Nairobi, Kenya

corresponding author: shaukeland@icipe.org

One of the first entomopathogenic nematodes species (*Steinernema kariii*) to be reported from Africa was in Kenya in the early 1990s. Since then a few surveys have been conducted in the region currently showing the presence of six *Steinernema* (three new species) and three *Heterorhabditis* species in the region. Kenya currently holds over 30 isolates of EPN in culture, of which *S. kariii*, *S. yirgalemense*, *S. weiserii*, *Heterorhabditis bacteriophora*, *H. indica* and *H. taysarae* have been reported/described. The characterisation of the remaining isolates is underway. In the neighbouring countries, Tanzania has reported *S. pwaniensis* and several unidentified *Steinernema* and *Heterorhabditis*. In Ethiopia *S. ethiopiense*, *S. yirgalemense* and *H. bacteriophora* are reported. Rwanda recently reported *S. carpocapsae* and *H. bacteriophora*, as well as unidentified isolates of both genera. Finally there are indirect reports of *Heterorhabditis* sp. in Uganda. Research regarding use of EPN in biological control is scanty and there is much potential to gain more knowledge for applied use in various crops. Only in Kenya is there commercial production of EPN by 'Dudutech IPM Solutions' (the only producer in Africa) targeting commercial horticulture farming systems against a few target pests. In the increasingly intensive agriculture with irrigated crops, EPN have good potential against new target pests, although applied data are lacking. Studies coupled with bio-prospecting and screening against selected target hosts could lead to successful use of EPN in the region if the production costs and product prices allow.

Keywords: Biological control; East Africa; entomopathogenic nematodes; *Heterorhabditis*; *Steinernema*.

Entomopathogenic nematode: A novel biocontrol technology for the management of arecanut whitegrub, *Leucopholis lepidophora* (Coleoptera: Scarabaeidae)

Jagadeesh Patil, Vijayakumar Rangasamy and Abraham Verghese

ICAR-National Bureau of Agricultural Insect Resources, P. Bag No: 2491, H.A. Farm Post Bellary Road, Bengaluru - 560 024. Karnataka, India

corresponding author: patiljaggi@gmail.com

Areca nut (*Areca catechu* L.) is one of the important commercial crops grown in India and is largely cultivated in the plains and foothills of Western Ghats and North Eastern regions of India. Arecanut is prone to attack from insect pests and diseases. Whitegrub, *Leucopholis lepidophora*, is a major pest causing a yield reduction of 39.79 to 41.60%. The main aim of the study was to develop an efficient sustainable control method against this pest. The efficacy of infective juveniles (IJ) of entomopathogenic nematodes against this pest in the laboratory and arecanut field was evaluated. In laboratory assays, lethal nematode concentration for 50% grub mortality values showed that *Heterorhabditis indica* was more virulent against both first and second instars grub than *S. abbasi*. Among the EPN species tested, *H. indica* caused significantly greater mortality (30-95%) than *S. abbasi* (15-70%) against first instar grubs, whereas *H. indica* caused significantly greater mortality (20-80%) than *S. abbasi* (5-55%) against second instars grubs at concentration of 200-3200 IJ grub⁻¹, respectively. Significant differences were found in the percentage reductions of the grubs at different larval stage applications. First instar grubs were more susceptible than second instar. In field experiment, *H. indica* caused second instar grub mortality of between 63% and 81% at concentrations of 1.25 and 2.5 x 10⁹ IJ ha⁻¹, respectively. The arecanut yields from the nematode-treated plots at 2.5 x 10⁹ IJ ha⁻¹ were at least 56 and 14% higher than those from the water control and the chlorpyrifos treated plots. The cost-benefit analysis showed that *H. indica* is promising agents for *L. lepidophora* grub control in arecanut fields. It is concluded that *H. indica* has good potential for safe management of *L. lepidophora* in arecanut production.

Keywords: arecanut; *Heterorhabditis indica*; instars; *Steinernema abbasi*; whitegrub.

Distribution and diversity of entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) from the north-eastern parts of South Africa

Willem Steyn¹, Antoinette Malan², Mieke Daneel¹ and Retha Slabbert³

¹ Agricultural Research Council – Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa

² Department of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa

³ Department of Horticulture, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

corresponding author: willems@arc.agric.za

A survey was conducted during 2014/15 in the north-eastern parts of South Africa to determine the diversity and frequency of entomopathogenic nematodes (EPN) on subtropical fruit tree crops in the Mpumalanga-, Limpopo- and Kwa Zulu Natal provinces. A total of 136 soil samples were randomly collected from both cultivated and uncultivated habitats, including subtropical fruit tree crops (avocado, litchi, macadamia and guava) and natural vegetation. EPN were isolated from 13 of the samples by means of baiting with the larvae of *Tenebrio molitor* (mealworm). This represents a 9.5% recovery rate. *Heterorhabditis* was the most common genus isolated from the samples while only two *Steinernema* was isolated. The most common *Heterorhabditis* species isolated was *H. noenieputensis* which were isolated from four different soil samples. Other species identified were two unknown *Heterorhabditis* species, *H. zealandica* and two unknown *Steinernema* species. The isolation of native isolates and species from the north-eastern parts of South Africa not only provides a new alternative for consideration in terms of biological control and integrated pest management programmes, but it also contributes to the existing knowledge regarding the distribution and diversity of EPN in South Africa. Various studies are currently underway to evaluate such local isolates against the false codling moth on subtropical fruit tree crops.

Keywords: Entomopathogenic nematodes; *Heterorhabditis*; South Africa; *Steinernema*; survey.

Molecular identification of stem and bulb nematode (*Ditylenchus dipsaci*) on garlic in Turkey

Zeki Mutlu Akar¹, Ozlem Sonmezoglu² and Elif Yavuzaslanoglu³

¹ Karamanoglu Mehmetbey University, Institute of Science, Karaman, Turkey

² Karamanoglu Mehmetbey University, Engineering Faculty, Bioengineering Department, Karaman, Turkey

³ Karamanoglu Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey

corresponding author: zekimutluakar@gmail.com

Garlic is one of the oldest vegetables used in cooking and medicine. Major production areas in Turkey are Kastamonu, Kahramanmaras, Balıkesir, Bursa, Gaziantep and Karaman provinces. There are many factors that affect the production of garlic. We examined stem and bulb nematode *Ditylenchus dipsaci*. Stem and bulb nematode causes rot and bruising on garlic plants. Yield and market value reduce. In this study, plant and soil samples from wide garlic production areas in Turkey, Hatay, Balıkesir, Aksaray, Karaman and Kastamonu, were collected in April and May 2016, one sample from every 1000 ha. Species identification from collected samples is going to be carried out using species-specific molecular markers which can be found on ITS-rDNA region in the nematode genome. The first large-scale distribution of the nematode on garlic in Turkey will be determined by the study.

Keywords: *Ditylenchus dipsaci*; garlic; molecular identification; stem and bulb nematode; Turkey.

Population dynamics of *Paratylenchus* spp. and *Pratylenchus penetrans* in glasshouse lettuce

Jolien Claerbout¹, Anneleen Volckaert², An Decombel³, Peter Bleyaert³, Sofie Venneman⁴, Isabel Vandeveldel⁴, Monica Höfte⁵ and Nicole Viaene¹

¹ ILVO (Institute for Agricultural and Fisheries Research), Burgemeester Van Gansberghelaan 96, 9000 Merelbeke, Belgium

² PCG (Provinciaal Proefcentrum voor de Groenteteelt Oost-Vlaanderen vzw), Karreweg 6, 9770 Kruishoutem, Belgium

³ Inagro vzw, Ieperseweg 87, 8800 Rumbeke-Beitem, Belgium

⁴ PSKW (Proefstation voor de groenteteelt), Duffelsesteenweg 101, 2860 Sint-Katelijne-Waver, Belgium

⁵ University Ghent, Coupure Links 653, geb. A, 9000 Ghent, Belgium

corresponding author: jolien.claerbout@ilvo.vlaanderen.be

A recent survey of 38 glasshouses where lettuce is produced as monoculture showed that pin nematodes, *Paratylenchus* spp., and the root-lesion nematode, *Pratylenchus penetrans*, are present in 47% and 18% of the samples, respectively. In some cases these nematodes seemed to be the only cause of reduced growth, resulting in small, unmarketable, lettuce heads. We found in an earlier study that high densities (> 2000 (100 ml soil)⁻¹) of pin nematodes are needed to reduce lettuce weight and that the build-up of such population densities probably takes months. As very little information is available on the influence of chemical soil disinfestation, temperature, soil type and cropping system on the population dynamics of these two nematodes, we are monitoring nematode densities in six glasshouses (five with *Paratylenchus* spp. and one with *Pratylenchus penetrans*). Our first results indicate that following chemical soil disinfestation (using 1,3-dichloropropene, metam-potassium), a considerable number of nematodes still survive in the deeper soil layer (30-60 cm), from where they can migrate to newly planted lettuce roots. We also observed that black fallow for several months did not reduce the number of vermiform stages of *P. penetrans* extracted from soil. This is possibly due to survival in organic debris and hatching. We plan to determine the damage threshold for these nematodes in lettuce under glass. The knowledge gained on nematodes will be integrated with results from our investigations on fungal diseases. Together these data will provide growers with the necessary information to reduce the use of chemicals in glasshouse lettuce.

Keywords: Flanders; glasshouse; leafy vegetable.

Development of molecular marker for the *Mi-1* gene using KASP genotypingZübeyir Devran

Department of Plant Protection, Faculty of Agriculture, University of Akdeniz, Kampüs-Konyaaltı, 07058 Antalya, Turkey

corresponding author: zdevran@akdeniz.edu.tr

Tomato is one of the important vegetables grown in the world. Root-knot nematodes cause serious problems in tomato growing areas. Improvement of tomato varieties resistant to root-knot nematode provides an environmentally sound crop management strategy. In tomato, resistance to root-knot nematodes is controlled by a single dominant *Mi-1* gene that was introgressed into cultivated tomato from *Solanum peruvianum*. The tomato *Mi-1* gene confers resistance against *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. The *Mi-1* gene is currently the only source of root-knot nematode resistance in cultivated tomatoes. Molecular markers within or tightly linked to the *Mi-1* gene have provided rapid and accurate screening of resistance without requiring nematode inoculation. In the present study, user-friendly marker was described for the *Mi-1* gene using Kompetitive Allele-Specific PCR (KASP) genotyping. Sequences of SNP markers related to *Mi-1* gene locus located on chromosome 6 were converted to KASP and tested in different tomato varieties. Our results showed that the KASP genotyping is processed rapidly and easily scored for high-throughput screening in tomato breeding.

Keywords: Genotyping; resistance gene; root-knot nematodes; SNP.

**Root-lesion nematodes, *Pratylenchus neglectus*, *P. penetrans* and *P. thornei*:
reproduction on *Solanum sisymbriifolium* cultivars**

Ivânia Esteves¹, Maria José Cunha², Isabel Abrantes¹ and Luci Conceição¹

¹ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

² CERNAS - Department of Agronomic Sciences, High School of Agriculture, Bencanta, 3045-601 Coimbra, Portugal

corresponding author: iesteves@uc.pt

Root-lesion nematodes (RLN), *Pratylenchus* spp., are important root parasites of several economically important crops. On potato, RLN have been found coexisting with potato cyst nematodes (PCN), *Globodera* spp., and therefore, effective nematode management strategies that comprise the control of these nematodes in potato fields are desired. *Solanum sisymbriifolium* is a trap crop that can successfully reduce the PCN densities in soil. However, the effects on RLN are not well known. The objective of the work was to assess the reproduction of three RLN species (*P. neglectus*, *P. penetrans* and *P. thornei*) on two *S. sisymbriifolium* cultivars (Melody and Sis 6001). Plants were grown in pots (500 cm³) containing autoclaved soil and were inoculated with 2000 nematodes of each species. Potato plants (*S. tuberosum*, cv. Desireée) were also inoculated with 2000 nematodes/plant and used as control. Five replicates/cultivar/nematode species were performed. After 60 days, nematodes were recovered from infected plant roots, using the Baermann funnel method, and from soil, using an adaptation of the Tray method. Nematode population densities in roots and soil were determined and the reproduction factor calculated. The results-revealed that RLN were able to penetrate both *S. sisymbriifolium* cultivars. However, the reproduction factors were <1 and much lower than the control, indicating that the two *S. sisymbriifolium* cultivars are poor hosts for these RLN species. Therefore, they can be used as trap crops for the management of RLN and PCN.

Keywords: Biological control; potato crop; reproduction factor; suppressive crops; trap crop.

Molecular identification of stem and bulb nematode (*Ditylenchus dipsaci*) on onion in Turkey

Nimet Genc¹, Ozlem Sonmezoglu² and Elif Yavuzaslanoglu³

¹Karamanoglu Mehmetbey University, Institute of Science, Karaman, Turkey

²Karamanoglu Mehmetbey University, Engineering Faculty, Bioengineering Department, Karaman, Turkey

³Karamanoglu Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey

corresponding author: nimet_genc91@outlook.com

Onion plant, which is important for both human nutrition and economy, grows in temperate zones in the World and in wide areas in Turkey. The stem and bulb nematode, *Ditylenchus dipsaci*, is one of the important biotic stresses that restrict onion growing. Stem and bulb nematode is a quarantine nematode and to know the distribution on the most important host plant, onion, is important. In this study, it is aimed to identify *D. dipsaci* on the fields where market-oriented onions grow in Turkey. Plant and soil samples were collected once for every 1000 ha from Adana, Aksaray, Amasya, Ankara, Eskisehir, Karaman, Konya and Tokat provinces, in 2016 April and May. Species identification is going to be carried out with the species specific molecular markers, which can be found on ITS-rDNA areas in the nematodes collected from the plant and soil samples. The study will help to identify stem and bulb nematode in the biggest onion growing fields in the country and facilitate control measures more efficiently.

Keywords: *Ditylenchus dipsaci*; molecular identification; onion; stem and bulb nematode; Turkey.

Tripartite interaction among *Pratylenchus* sp., *Bradyrhizobium japonicum* and soybean

Ahmed Elhady Goma¹, Johannes Hallmann² and Holger Heuer¹

¹Julius Keuhn-Institute, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12 , 38104 Braunschweig, Germany

²Julius Keuhn-Institute, Institute for Epidemiology and Pathogen Diagnostics Toppheideweg 88, 48161 Münster, Germany

corresponding author: ahmed.elhady@fagr.bu.edu.eg

Soybean is economically the most important legume in the world. One of the most yield limiting factors are plant-parasitic nematodes, especially in narrow rotations. Recent findings report that *Pratylenchus* spp. are wide spread in soybean fields in Germany and multiply well in the roots of this crop. However, it is not well studied which species of *Pratylenchus* are mainly affecting soybean fields, and what are their population dynamics and damage threshold levels. Considering the temperate climate conditions, only very early ripening soybean varieties can be grown in Germany. The susceptibility of these soybean varieties to the abundantly occurring *Pratylenchus* nematodes needs to be investigated, and whether *Pratylenchus* affects the nitrogen (N) fixation by the symbiotic bacterium *Bradyrhizobium japonicum*. Our results suggest that the symbiotic association between *B. japonicum* and soybean in fixing N is significantly affected by *P. penetrans*. Interestingly, introduction of *P. penetrans* before *B. japonicum* resulted in formation of deformed and non-functional nodules leading to reduced N fixation. By contrast, roots colonised with *B. japonicum* significantly decreased penetration of *P. penetrans*. This suggested that *B. japonicum* plays an important role in the triggering of soybean defence against *P. penetrans*. A better understanding of interactions between *P. penetrans* and *B. japonicum* in soybean plants may unravel new strategies for improvement of N fixation under pathogen pressure and nematode management.

Keywords: *Bradyrhizobium japonicum*; interaction; *Pratylenchus* spp.; soybean.

Molecular identification of root-lesion nematodes (*Pratylenchus* spp.) on cereals in Konya and Karaman Provinces in Turkey

Mehmet Said Karaca¹, Elif Yavuzaslanoglu², Ozlem Sonmezoglu³

¹ Karamanoglu Mehmetbey University, Institute of Science, Karaman, Turkey

² Karamanoglu Mehmetbey University, Technical Sciences Vocational School, Plant and Animal Production Department, Karaman, Turkey

³ Karamanoglu Mehmetbey University, Engineering Faculty, Bioengineering Department, Karaman, Turkey

corresponding author: eyavuzaslanoglu@kmu.edu.tr

Cereals are important commodity in Turkey for human consumption and also for animal nutrition. The need for increased production is an issue to consider. Plant diseases and pests are one of the factors affecting production increase. Cereals are grown in wide areas in Konya and Karaman provinces. Soil borne diseases and nematodes affect production rate depending on the climate and agronomic practices year to year. Plant and soil samples were collected in April and May 2016 from cereal growing areas in Konya and Karaman provinces. Root-lesion nematodes are going to be identified using species-specific primers on the ITS-rRNA regions of nematodes collected from samples. The current situation of root-lesion nematode damage in Konya and Karaman provinces will be updated with this study.

Keywords: cereal; molecular identification; *Pratylenchus* spp.; root-lesion nematode; Turkey.

Investigation of multiple resistance on some wheat genotypes against root-lesion nematodes, *Pratylenchus thornei* and *P. neglectus*

Ece B. Kasapoğlu¹, Mustafa İmren² and Ibrahim Halil Elekcioğlu¹

¹ Cukurova University, Faculty of Agriculture, Department of Plant Protection, 01330-Sarıcam, Adana, Turkey

² Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Gölköy Kampüsü, 14280 Bolu, Turkey

corresponding author: ecekasapoglu@gmail.com

Root-lesion nematodes (*Pratylenchus* spp.) are known as the most important plant-parasitic nematodes attacking mainly cereals such as wheat, barley and oats. Both root-lesion nematode species, *P. thornei* and *P. neglectus* were detected mostly in mixed population densities in wheat cropping areas in Turkey. In this study, 20 wheat genotypes were screened against *P. thornei* and *P. neglectus* to determine multiple resistance response under climatic controlled conditions in Turkey. Additionally, these genotypes were tested with root-lesion nematodes individually. Reproduction rate of *P. thornei* and *P. neglectus* were significantly different when wheat genotypes were infected with both species, a phenomena thought to be due to inter-specific competition. Reproduction rate was negatively correlated in mixed infection. Varieties, Fuatbey and Yelken, were found to be resistant against both species and could be nominated as multiple resistant lines.

Keywords: Multiple resistance; root-lesion nematodes; wheat.

Efficacy of nematicidal compounds on hatching and mortality of *Heterodera schachtii* infective juveniles

Jeongeun Kim¹, Abraham O. Mwamula¹, Faisal Kabir¹, Young Hwa Choi², Jae-Kook Lee³ and DongWoon Lee¹

¹ Department of Ecological Science, Kyungpook National University, Sangju, Gyeongsangbukdo 37224, Republic of Korea

² School of Ecology and Environmental System, Kyungpook National University, Sangju, Gyeongsangbukdo 37224, Republic of Korea

³ Crop Protection Division, National Academy of Agricultural Science, RDA, Republic of Korea

corresponding author: kjeqaz6715@naver.com

The study investigated the efficacy of selected nematicidal compounds on hatching and mortality of *Heterodera schachtii* infective juveniles isolated from Chinese cabbage field of Korea. Acute toxicity of the nematicidal compounds on individual infective juveniles was also tested by subjecting them to serial dilutions of the respective chemical compounds to find the LC50 (Lethal dose causing 50% mortality). Treatment of healthy *H. schachtii* cysts with the different nematicidal compounds (Fluopyram 40% SC, Imicyafos 30% SC, Fosthiazate 30% SC, Abamectine 1.8% EC, Terthiophene and Eclipta prostrata extract) caused varying levels in hatching and mortality rates of cysts and infective juveniles, respectively. Fluopyram and Imicyafos resulted in the lowest number of juveniles hatching, and total hatched juvenile numbers were significantly different between the different nematicidal compounds. A positive correlation in percentage reduction of hatching was observed in Fluopyram. Furthermore, the mortality test conducted also emphasised highest mortality in treatments with Fluopyram and Imicyafos (LC50 of 0.00119 and 0.00095, respectively). The study therefore demonstrated available alternative nematicidal compounds that could be used in control of *H. schachtii* and highlighted the need for further field tests.

Keywords: Acute toxicity; Chinese cabbage; cyst; lethal dose; serial dilution.

Distribution and control of sugar beet cyst nematode (*Heterodera schachtii*) in highland Chinese cabbage fields of Korea

Soon-Bae Kwon¹, Yun-Gi Moon¹, Nam-Gil Lee¹, Dong-Woon Lee² and Jae-Kook Lee³

¹Gangwon Provincial Agricultural Research & Extension Service, 83 Chungyeolro, Chuncheon, 200-150, Korea

²Department of Ecological Science, Kyungpook National University, Korea

³Crop Protection Division, National Academy of Agricultural Science, RDA, Korea

corresponding author: snbkwon@korea.kr

Sugar beet cyst nematode (SBCN), *Heterodera schachtii*, is a well-known parasite of Cruciferae family. It causes serious stand and yield reductions wherever the plants are grown. Since the first report in 2011, it was only detected in the highland vegetable cultivation areas of Gangwon province. Since then, several field surveys have been carried out in highland Chinese cabbage fields of South Korea. Chinese cabbage is the main produce of highland area as it has a multipurpose use in Korea especially for the production of Kimchi. In 2013 the total production of Chinese cabbage was 205,390 tons from 5,603 ha. However, due to the attack of SBCN, the production rate has been decreasing in recent years. In the surveyed area, symptoms of SBCN showed poor stands and growth at localised areas of infestations, leaves of severely affected plants become yellowish and wilting. Excess of fine lateral roots are also observed. In this study, distribution of SCBN was surveyed according to annual scheme of 2011, 2013, 2014 and 2015 and the SCBN was recorded from the areas of 11.6, 51.2, 65.4 and 85.1 ha, respectively. However, the distribution of SCBN is limited to the surrounding area of the first occurrence. The surveys aimed at finding a control measure for SBCN. The control methods include a sanitation, such as the prevention of contaminated soil, rotation with non-host crops such as corn, soybean, potato and red pepper etc., cultivation of 'trap crops' such as oil radish (cv. Adios etc.) or white mustard (cv. Braco) and nematicides (fumigant and non-fumigant) treatment. The effective chemicals (Dazomet, Metham sodium, Imicyafos) treatment could reduce the density of nematodes by 90%. In addition, the cultivation of trap crops could reduce the density of SCBN eggs by 69-97%.

Keywords: Nematicides; non-host crops; rotation; soil sanitation; trap crops.

NSL-1 is a susceptibility factor for cyst nematode infection in *Arabidopsis* roots

Sina-Valerie Mahlitz, Zoran S. Radakovic, Muhammad Shahzad Anjam, Shahid Siddique and Florian M. W. Grundler

¹ Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

corresponding author: s7simahl@uni-bonn.de

The NESTOR project's main objective is identifying and characterising host genes that play a role in establishing nematode-induced feeding sites. To begin, we selected 100 genes that are highly up-regulated in syncytium induced by the beet cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. Using a filtering pipeline, we discarded genes that have no homologues in sugar beets or those that show a strong developmental phenotype. Consequently, we identified three genes that impact nematode infestation and development in the roots. These genes were renamed as Nematode Susceptible Locus 1-3 (*NSL-1-3*). The focus will be only on NSL-1, a member of the highly conserved spermatophyte cell wall DUF642 protein family. This protein has been shown to display differential expression patterns upon infection by different pathogens and is located in the cell wall. NSL-1 contains a carbohydrate binding domain, and previous analysis showed that it interacts with cell wall polysaccharides particularly cellulose. The underlying mechanism behind the role of NSL-1 as a nematode susceptibility factor will be discussed.

Keywords: DUF642 protein family; nematode susceptible gene.

Cereal cyst nematodes in Algeria: main species, biology and effect on yields

Aissa Mokabli¹, Nadia Tirchi¹, Kada Righi², Fatiha Labdelli³, Fatima Haddadi⁴ and Djamel Smaha⁵

¹ University Djilali Bounaama of Khemis Miliana, Ain Defla, Algeria

² University of Mascara, Algeria

³ University of Tiaret, Algeria

⁴ University of Sciences and Technology Houari Boumed Youiène, Algeria

⁵ National Institute of Plant Protection, El Harrach, Algeria

corresponding author: mokaissa@yahoo.fr

In Algeria, cereals (wheat, barley and triticale) occupy 3,5 millions ha practised by 600 000 producers, using more than 35% of the arable land. The main factors limiting the production are bound to the technical itinerary and the irregularity of rains. Among the devastating problems, the extensively distributed cereal cyst nematodes (CCN) generate considerable losses in cereal regions. The main identified species are *Heterodera avenae*, *H. latipons*, *H. hordecalis* and *H. filipjevi*. They can occur in mixed populations in some fields of cereals. The phosphoglucose isomerase differentiates the species of *Heterodera* easily. *H. avenae* is the most dominant in Algeria. It characterised by three cycles of hatching at the rate of one cycle per year. The juvenile activity is very important in winter period with low temperatures, essentially in the months of January and February. A perfect synchronisation exists between the biologic cycle of the nematode and that of the cereal. For example, in the region of Tiaret with cereal cultivation, *H. avenae* generates yield losses on hard wheat, tender wheat and barley that vary according to the densities of nematodes between regions and localities. The extensive practice of monoculture constitutes the main reason for these losses.

Keywords: Algeria; biology; cereal cyst nematodes (CCN); identification.

Current knowledge of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) on wheat in China

Deliang Peng¹, Ricardo Holgado², Jingwu Zheng³, Shulong Chen⁴, Jinling Liao⁵ and Hongmei Li⁶

¹ State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

² Norwegian Institute of Bioeconomy Research, Pb 115, NO-1431 Ås, Norway

³ Institute of Biotechnology, College of Agriculture and Biotechnology Zhejiang University, Zijingang Campus, Hangzhou 310058, China

⁴ Institute of Plant Protection, Hebei Academy of Agriculture and Forestry Sciences, Baoding 071000, China

⁵ College of Agriculture, South China Agricultural University, Guangzhou 510642, China

⁶ Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China

corresponding author: dlpeng@ippcaas.cn

Cereal cyst nematode *Heterodera avenae* was first reported in China in 1989; now its distribution and occurrence has been confirmed in 16 provinces of China. *H. filipjevi* was first reported in Henan Province, China in 2010 and was recently discovered on wheat in Anhui, Qinghai and the Ningxia Hui Autonomus region. The yield losses caused by cereal cyst nematodes (*H. avenae* and *H. filipjevi*) were estimated to be up to 18-35% in Henan and 15-20% in Hebei. Both species completed only one generation during the whole growing season of wheat. Diagnostic methods based on LAMP were developed for detecting infested seedling of *H. avenae* and *H. filipjevi*. Two new pathotypes of *H. avenae* were defined as Ha43 and Ha91. Integrated management strategies were developed for controlling cereal cyst nematodes including rotation, planting tolerant and resistance varieties, seed coatings and biocontrol. A cDNA library of *H. avenae* (second- and fourth-stage juveniles) were constructed for exploring parasitism genes. Several genes including (*Ha-eng-7a*) were cloned and their functions were demonstrated by RNAi. *H. avenae* is the dominant species on wheat in China, which is confirmed with identification of large populations using morphological, biological and molecular characterisation. Although the ITS sequences of *H. avenae* from China is heterogeneous and has some differences from that of European and Indian populations, we think those molecular variations are intra-species variations of *H. avenae*. The morphological characters strongly supported the species status as *H. avenae*. Identification of the newly nominated *H. sturhani* depended purely on molecular data of ITS rRNA and *cox1*, because of the lack of morphological, biological and ecological differences from *H. avenae*; there is insufficient evidence that the molecular differences justify species level distinction, so we think that *H. sturhani* is an invalid species and should be considered as a junior synonym of *H. avenae*.

Keywords: Diagnosis; *Heterodera avenae*; *Heterodera filipjevi*; management; synonymisation.

Assessing the resistance of bean cultivars (*Phaseolus* spp.) to *Meloidogyne javanica*

David Pires¹, Sofia R. Costa^{1,2}, Isabel Mourão³ and Maria Teresa Almeida¹

¹CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

²Mountain Research Center (CIMO), Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

³Mountain Research Centre (CIMO)/ Instituto Politécnico de Viana do Castelo, Escola Superior Agrária, Refóios, 4990-706 Ponte de Lima, Portugal

corresponding author: david.pires89@gmail.com

Among the numerous pests and diseases that affect common bean (*Phaseolus* spp.), root-knot nematodes, *Meloidogyne* spp., stand out for their devastating effects on horticultural crops. The main control strategy consists on the application of chemical nematicides, which are being progressively restricted. Alternative nematode management methods have been considered, such as vegetable grafting using resistant cultivars. The present study aimed: 1) to assess the reaction of nine bean cultivars (*P. vulgaris* and *P. coccineus*) to *M. javanica*, considering their degree of susceptibility and resistance; 2) to determine the severity of damage to the roots; and 3) to infer on the potential use of these cultivars as commercial bean rootstocks. A pot experiment was carried out under controlled conditions in a culture room, and each treatment consisted of five replicates. Plants were inoculated with 5000 eggs and second-stage juveniles of *M. javanica*, with non-inoculated plants serving as negative control and susceptible tomato plants cv. Tiny Tim being used as positive control. Sixty days after inoculation, roots were observed to determine the number of galls and egg masses. None of the tested cultivars was completely resistant to the nematode. However, a potential for resistance was detected in Bencanta and Oriente cultivars, with levels of nematode-induced galls and egg masses comparable to those of cultivars classified as resistant in the previous studies. The Bencanta and Oriente cultivars showed promising results regarding their use as resistant rootstocks to *M. javanica*, constituting an alternative control strategy and justifying further research to test and assess the feasibility of their use in bean grafting, under controlled conditions and in the field, and in the presence of other species of root-knot nematodes.

Keywords: Host status; resistant cultivars; root-knot nematode; rootstock; sustainable control.

Nematode Susceptibility Targets for a durable Resistance - current achievements of the NESTOR project

Zoran S. Radakovic, Muhammad Shahzad Anjam, Shahid Siddique and Florian M. W. Grundler

Rheinische Friedrich-Wilhelms-University of Bonn, INRES – Molecular Phytomedicine, Karlrobert-Kreiten-Straße 13, D-53115 Bonn, Germany

corresponding author: s7zorada@uni-bonn.de

Root-knot and cyst-forming nematodes are economically important pathogens. They establish interactions with their hosts inducing specific organ-like hyperplastic, hypertrophic and hypermetabolic long-term feeding structures in the roots. Natural plant resistance is an available and safe option for nematode control, but is strongly limited by the number of available genotypes and the occurrence of resistance breaking nematode populations. Therefore, novel breeding strategies must be developed. The NESTOR project combines the efforts of five public research labs and three private companies to (i) discover and characterise *Arabidopsis* genes that are essential for susceptibility towards the root-knot nematode (RKN), *Meloidogyne incognita*, and the beet cyst nematode (BCN), *Heterodera schachtii*, and (ii) to discover target sequences in crops based on a small set of genes for which an important role in feeding cell development has been shown in this and earlier studies. The final objective is to generate crop plants with reduced activity of the selected genes. Our contribution to the NESTOR project is the identification and characterisation of essential nematode susceptibility (NS) genes in *Arabidopsis* towards BCN. So far, we have identified three genes *NSL-1* (Nematode Susceptibility Locus-1), *NSL-2* and *NLS-3*, which act as susceptibility factors for nematode infection in *Arabidopsis*. Loss-of-function mutation in a single NS-gene leads to a strong decrease in susceptibility to nematodes without performing an altered growth phenotype. The mechanistic details underlying the reduced susceptibility in these mutants will be discussed.

Keywords: *Arabidopsis*; *Heterodera schachtii*; plant parasite; sugar beet; susceptibility genes.

Induced resistance of chemical elicitors to *Heterodera glycines* in soybean

Hongli Shi, Zhongling Tian, Hongxue Zhu and Jingwu Zheng

Institute of Biotechnology, C601, Agri-Sciences Complex, Zhejiang University, Zijingang Campus, Hangzhou 310058, China

corresponding author: jwzheng@zju.edu.cn

Soybean cyst nematode, *Heterodera glycines*, is one of the most economically destructive pests of soybean. Safe and effective measures are urgently needed to control the nematode. Chemical elicitors, riboflavin, salicylic acid (SA), jasmonic acid (JA), DL- β -aminobutyric acid (BABA) and 2, 6-dichloronicotinic acid (INA), were applied to soybean as soil-drench one day before inoculation with *H. glycines*. Nematode reproduction was evaluated by counting white female in treated and untreated soybean roots 30 days post inoculation (dpi). The results showed that riboflavin, SA, JA, BABA and INA reduced reproduction of *H. glycines* by 25, 29, 38, 51 and 53%, respectively. BABA and INA were more effective, while INA most significantly inhibited the growth of soybean through reducing fresh shoot and root weight and dwarfing the shoot. Different application methods of BABA on nematode control showed that seedling root-dip, foliar spray and soil-drench reduced nematode reproduction by 12, 19 and 45%, respectively. Apparently, root-dip caused plant dwarf, soil-drench reduced the fresh root weight, and foliar spray deceased fresh shoot weight. We suggest that BABA applied as soil drench at the rate of 20 mM is a potential measure for control of soybean cyst nematode.

Keywords: BABA; chemical elicitors; *Heterodera glycines*; induced resistance.

Tuber yield, infestation and quality assessment of potato genotypes, partially resistant to *Meloidogyne chitwoodi*

Misghina Goitom Teklu^{1,2}, Corrie. H. Schomaker¹ and Thomas H. Been¹

¹ Wageningen University and Research Centre, Agro-Systems Research, 6700 AA, Wageningen, The Netherlands

² Laboratory of Nematology, Wageningen University, 6708 PD Wageningen, The Netherlands

corresponding author: misghina.goitomteklu@wur.nl

As part of developing a partial resistance test for potato genotypes resistant to *Meloidogyne chitwoodi*, growth, fresh tuber weight, level of tuber infestation and quality loss were studied under glasshouse conditions. Results from four successive experiments were combined. The study involved seven genotypes with resistance to *M. chitwoodi* and one with resistance to *Globodera pallida*. Cultivar Désirée was used as a control. Plants were inoculated with second-stage juveniles (J2) at densities ranging from 0.0625 to 256 J2 (g dry soil)⁻¹ in a 2 log series. Height as growth indicator was measured every week, while fresh tuber weight, tuber infestation and quality were measured at harvest. Also, pots with different volume 10, 5, 3 and 2 kg were used to explore the idea of testing both resistance and yield plus quality loss in smaller and cheaper pots without affecting the nematode-plant system. All data were treated equally in a meta-analysis and relevant models were used to describe the pattern of the data. Plant height showed normal logistic growth in all experiments. Plant height was positively affected by increasing Pi and negatively by pot size. In general growth was delayed. Tuber yield was not affected in four out of eight genotypes. The four other genotypes and cv. Désirée showed a decrease of fresh tuber weight with minimum yields ranging from 0.40 to 0.84 and 0.75, respectively. In two tested starch potato genotypes an additional decrease of starch content was measured. Tuber infestation levels were reduced and quality was improved compared to the susceptible control and MDG2, with more than 90% of the pots containing clean tubers, with the exception of genotype 2011M1. Quality, expressed as Tuber-Knot Index, was well below < 10, indicating that all genotypes were acceptable for industrial processing.

Keywords: Clean tubers; minimum yield; modelling; tolerance limit; Tuber-Knot Index.

Performances of some East Anatolian wheat landraces and wild relatives against root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*)

Halil Toktay¹, Atilla Öcal², Mustafa İmren³, Refik Bozbuga⁴, Abdelfattah A. Dababat⁵ and Halil Elekcioglu⁶

¹ Nigde University, Ayhan Sahenk Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, Central Campus, 51240, Nigde, Turkey

² Bati Akdeniz Agricultural Research Institute, Plant Protection Laboratory, Antalya, Turkey

³ Abant İzzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, İzzet Baysal Campus, 14280, Gölköy Bolu, Turkey

⁴ Biological Control Research Institute, 01321, Adana, Turkey

⁵ International Maize and Wheat Improvement Center, P.K. 39 Emek, 06511, Ankara, Turkey

⁶ Cukurova University, Faculty of Agriculture, Department of Plant Protection, 01330, Sarıcam, Adana, Turkey

corresponding author: mustafaimren@ibu.edu.tr

Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) cause significant yield losses in wheat fields all over the world as well as in Turkey. Unfortunately, there is no commercial cultivar that is resistant to this noxious pest. The wild relatives of wheat have evolved in infested soil for millennia and may have superior levels of resistance that can be transferred to commercial wheat. *Triticum dicoccoides*, occurs naturally across the East Anatolia and is a rich genetic resource to improve resistance to diseases and pest for breeding programmes. To evaluate this hypothesis, a collection from Tubitak project 112O565 accessions were tested in controlled room conditions. Turkish wild Emmers (*T. dicoccoides*) and national spring wheat varieties for resistance to both *P. thornei* and *P. neglectus*. In separate experiments, 32 wild Emmers and 42 wheat cultivars with seven replicates were inoculated with individuals of *P. thornei* or *P. neglectus*. At the end of experiment result showed that 25 and 35 wheat varieties were moderately resistant to *P. thornei* and *P. neglectus*, respectively. In total, 17 lines exhibiting multiple resistance reactions to both nematodes were in the same group with the control lines.

Keywords: *Pratylenchus* spp.; resistance; root-lesion nematodes; wheat.

Fumigation of garlic seed: a tool for eradication of *Ditylenchus dipsaci*

Miloslav Zouhar¹, Ondřej Douda², Milan Dlouhý³, Jana Lišková³, Marie Maňasová¹ and Václav Stejskal²

¹ Czech University of Life Sciences Prague (Faculty of Agrobiolgy, Food and Natural Resources, Department of Plant Protection), Kamýcká 129, Prague 6, 16521, Czech Republic

² Crop Research Institute Prague (Division of Crop Protection and Plant Health), Drnovská 507/73, 161 00 Prague, Czech Republic

³ Lučební závody Draslovka a.s. Kolin, Havlíčkova 605, Kolín IV, 280 99, Czech Republic

corresponding author: zouharmiloslav@seznam.cz

The stem and bulb nematode (*Ditylenchus dipsaci*) is a very important quarantine organism spreading worldwide via infested seeds and planting materials. Plant protection against this serious pest is rather strange and complicated because of wide host range of this nematode species and its ability to enter a state of anhydrobiosis. Generally, if the nematode occurs in the soil, it is possible to eradicate it only by application of toxic nematicides. However, the number of usable nematicides is reduced. Consequently, there is only one way to protect cultivated plants against to *D. dipsaci* - seed and seedling treatment. Currently, it is possible to use hot water technique as the seed pre-treatment, which is very difficult to implement in practice and the risk of seed damage is rather high. The objectives of this study were to evaluate 1) penetration of gaseous hydrogen cyanide (HCN) into garlic tissues, 2) HCN phytotoxicity, and 3) nematicide potential of HCN against *D. dipsaci*. HCN penetration into the core of the garlic clove was approximately 30% of the concentration inside the fumigation chamber after 30 h of exposure. HCN phytotoxicity was observed only during exposure treatment lasting 16 h and more. The garlic cloves naturally infested by *D. dipsaci* were treated with HCN at a concentration of 20 g m³ for 12, 18 and 24 h in a fumigation chamber, and 99% mortality of nematodes was achieved in all three exposure times.

Keywords: *Ditylenchus dipsaci*; garlic; HCN; plant protection.

Metagenomics analysis applied to *Anisakids* populations reveals an extraordinary symbiotic microbial diversity

Susana C. Arcos¹, Felipe Lira², Lee Robertson¹, Rosa González¹, Yolanda Jiménez-Ruiz¹, José Luis Martínez² and Alfonso Navas¹

¹ Museo Nacional de Ciencias Naturales, CSIC, calle José Gutiérrez Abascal 2, Madrid 28006, Spain

² Departamento Biotecnología Microbiana. Centro Nacional de Biotecnología, CSIC, calle Darwin 3, Campus de Cantoblanco, Madrid 28049, Spain

corresponding author: anavas@mncn.csic.es

The V4 variable region of the microbial 16s RNA present in 113 anisakids population was amplified using the standard Illumina primers. The resultant libraries were sequenced at the facilities from the Fundación Parque Científico de Madrid (FPCM, Spain) using the Illumina platform in a pair-end reads with 280 cycles. Approximately 25 Mb were sequenced from 113 microbiota with an average of ~250.000 reads for each sampled microbiota (151.171 bp to 412.396 bp) and were analysed using MOTHUR v.1.35.1 software following the standard procedure (SOP) for Illumina MiSeq sequences. The paired-end reads were assembled and sequences were removed if they did not have a length ranging between 280 and 320 bp. After assembling, all sequences were trimmed to start and finish at the same coordinates. Unique sequences from all samples were merged and aligned to the SILVA bacteria reference database. The resulting sequences that were not classified at least at the kingdom level or identified as Archaea, Eukaryota, chloroplast or mitochondria were removed from the analysis. After, the sequences were classified into groups corresponding to their taxonomy at different levels (kingdom, order, family and genus) and then assigned to operational taxonomic units (OTU) with a similarity level $\geq 97\%$. After OTU assignation, the data were corrected to fix the misallocated taxa adjusting it to the right level using a custom Perl script. All taxa were counted in respect to their presence in each sample and the taxa with fewer than 20 sequences counted in all 114 samples: *Anisakis simplex* ss (100), *A. pegreffii* (7), hybrid (1) and *Pseudoterranova decipiens* (6) were considered less representative and then were culled to avoid misunderstood analysis. Taxa with ≥ 20 sequences were maintained for further analysis. 18 microbiota Phyla are present in the studied populations distributed according to 58 orders, 126 families and 240 genera, which are structured in four different groups.

Keywords: *Anisakis*; microbial 16s RNA; *Pseudoterranova*.

Nematodes as bioindicators of soil health in some of South Africa's major irrigation schemes

Gerhard du Preez¹, Hendrika Fourie¹, Mieke Daneel² and Victor Wepener¹

¹ Unit for Environmental Sciences and Management, Potchefstroom, North West University, 2520 South Africa

² Agricultural Research Council - Institute for Tropical and Subtropical Crops (ARC-ITSC), Potchefstroom Campus, North-West University, 1200 South Africa

corresponding author: gc.dpreez@gmail.com

Ensuring food security is becoming increasingly difficult as Earth's freshwater resources are deteriorating at an alarming rate. Anthropogenic activities associated with agriculture, mining, and industry pose a severe threat to the quality of water utilised by some of South Africa's major irrigation schemes. This includes the Hartbeespoort Dam, Crocodile West and Marico-Bosveld schemes, which in total accounts for more than 33 000 ha of irrigated land and forms part of the greater Crocodile (West) Marico water management area. Although studies have reported on the presence of metals, nutrients, pesticides and faecal coliform, especially in the Hartbeespoort Dam and Crocodile West Schemes, the effect of water quality on agroecosystem (soil) health remains unknown. Subsequently, a probabilistic based risk assessment will be undertaken to predict the threats posed to soil health in Soybean lands associated with the Crocodile (West) Mario water management area. We will report on the identified pollutants that pose a threat to soil health, as well as irrigated areas of concern (potential pollution hotspots). Also, predicted results will be evaluated and verified using a TRIAD assessment that includes a physico-chemical, ecotoxicological and ecological line of evidence. While physico-chemical analyses of collected water, sediment and soil samples represent the 1st line of evidence, single-species toxicity test of environmental samples using *Caenorhabditis elegans* encompasses the ecotoxicological component. The ecological line of evidence will be evaluated using community and nematode specific indices. Furthermore, the occurrence, abundance, and distribution of nematodes (with focus on plant-parasitic nematodes) in the respective irrigation schemes will also be considered.

Keywords: Crocodile (West) Marico water management area; irrigation schemes; soil health; TRIAD assessment.

Evaluation of the bioremediating ability of bacterial endophytes using nematodes as bioindicators in a bioenergy crop

Aoife Egan and Thomae Kakouli-Duarte

Molecular Ecology and Nematode Research Group, enviroCORE, Dargan Centre, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

corresponding author: aoife.egan@itcarlow.ie

Nematodes are representative of their habitat, respond quickly to disturbance in the soil composition and are therefore well suited as bio-indicators for environmental biomonitoring. Bioenergy crops, hosting certain pollutant degrading bacterial endophytes, can be both utilised for bioenergy production and in bioremediation efforts, if they are grown on contaminated land. The work presented here utilises nematodes as indicators of soil health to evaluate the effectiveness of the bioremediation process of the bioenergy crop oilseed rape. This work is currently ongoing. The plants were grown in nickel contaminated soil in the presence of endophytic *Pseudomonas* isolates, from the enviroCORE stock collection. The evaluation of nematodes as indicators of bioremediation efficiency was conducted by: 1) morphological characterisation of nematode assemblages in the rhizosphere of oilseed rape crop. Trophic grouping, coloniser-persister values, ecological indices and diversity indices were assigned to the data; and 2) molecular analysis of the nematode assemblages using polymerase chain reaction of the 18S rDNA gene, followed by denaturing gradient gel electrophoresis. The morphological results for the test samples (containing nickel and treated with bacterial endophytes) were indicative of a recovering nematode assemblage, free from stresses. The most commonly occurring coloniser-persister value was 5 (control value was 4), with a maturity index of 3.8- 4.8 (control value was 4). The Shannon wiener index was 0.8-1.37 and the Simpson index was 0.39-0.63 (compared to 1.73-2.54 and 0.8-0.99 in the control, respectively). Both approaches aim to indicate the level of soil health and evaluate the phytoremediating capacity of oilseed rape in the presence of endophytic bacteria. The ability of nematodes as indicators of soil health in this system will be discussed.

Keywords: Environmental biomonitoring; molecular analysis; morphological characterisation; nickel contamination; oilseed rape.

A comparative approach using ecotoxicological methods from single-species bioassays to model ecosystems

Arne Haegerbaeumer¹, Sebastian Höss², Peter Heininger³ and Walter Traunspurger¹

¹ University Bielefeld, Morgenbreede 45, 33615 Bielefeld, Germany

² ECOSSA, Giselastr. 6, 82319 Starnberg, Germany

³ German Federal Institute of Hydrology, Am Mainzer Tor 1, 56002 Koblenz, Germany

corresponding author: a.haegerbaeumer@uni-bielefeld.de

The pollution of sediments with chemical contaminants is a major environmental concern and considerably contributes to the chemical and ecological status of aquatic ecosystems. Therefore, a thorough ecotoxicological assessment in sediments can help to identify causes of stress in benthic habitats and to improve ecosystem health. Effects on natural nematode assemblages to chemical stress were assessed in this study in complex sediment microcosms and in acute community toxicity tests. Responses of the various freshwater nematode species in both approaches were compared to effects on the well-established model organism *Caenorhabditis elegans* in standardised toxicity tests (DIN ISO 10872). The findings of the acute tests only partly reflected classifications of nematode species according to the NemaSPEAR[%]-index but underlined the function of *C. elegans* representing the sensitivity of freshwater nematodes. Over the course of the microcosm study, strong dose-dependent effects on nematode communities, as well as on the NemaSPEAR[%]-index, could be noticed. Additionally, standardised *C. elegans*-toxicity tests with whole sediment samples were conducted to estimate bioavailability and direct toxicity, indicating that concentrations causing sublethal effects in *C. elegans* have already severe effects on natural nematode communities. This study aimed for a better interpretation of toxicity bioassays in terms of sediment quality assessment. Combining sophisticated experimental tools with field observations allows for more accurate decision making in environmental risk assessment. Data on chemical concentrations, single-species and community toxicity, and *in-situ* assemblages can be integrated as single lines of evidence in a weight-of-evidence approach. As suitable ecotoxicological tools and ecological indices (NemaSPEAR[%]-index) for nematodes are already available, this organismal group should be used more often in sediment quality assessments.

Keywords: Acute tests; microcosms, nemaSPEAR[%]-index; nematodes; sediments.

The receiver operating characteristic curve application in environmental nematology

Thomae Kakouli-Duarte and Eileen Kelly

Molecular Ecology and Nematode Research Group, enviroCORE, Dargan Centre, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

corresponding author: Thomae.Kakouli@itcarlow.ie

Various ecological studies using predictive modelling have found bias in systems when assessing the accuracy performance of a test, where there are only two classifiers, false positives and false negatives. Receiver operating characteristic curves do meet the criteria of an unbiased system, as they function with four classifiers and their combinations. Predictive accuracy is of great importance when evaluating and comparing laboratory tests, and receiver operating characteristic curves are a valuable practical tool for this purpose. This system was applied to data generated from toxicity bioassays using the nematode *Steinernema feltiae*. This study analysed data from bioassays testing heavy metal effects on the nematode, and included several nematode behavioural parameters, such as nematode infectivity, pathogenicity, fecundity, attraction to host and mobility. Results show that the areas under the curve for all bioassays ranged from 0.78 to 1.00. The false alarm rate was set at 9%, or lower, for a good balance between sensitivity and specificity. Factors, such as sample size, can affect test performance. This work concluded that receiver operating characteristic curve analysis is a useful technique that can be utilised toward improving the understanding of the validity of toxicity bioassays and in comparing their accuracies. The curve analysis also indicated that the bioassays used to observe (1) nematode movement towards the host, (2) their ability to seek and invade their hosts, and (3) nematode fecundity, in the presence of heavy metals, were suitable for predicting heavy metal contamination effects in soil.

Keywords: Ecotoxicology; heavy metals; predictive modelling.

Assessment of soil nematode communities in northern meadows and agroecosystems under different land use intensity

Elizaveta M. Matveeva, Anna A. Sushchuk and Daryia S. Kalinkina

Institute of Biology of Karelian Research Centre, Russian Academy of Sciences, Pushkinskaya St. 11, Petrozavodsk, 185910, Republic of Karelia, Russia

corresponding author: elizaveta.matveeva@gmail.com

Soil nematode communities were studied in ecosystems varying in intensity of human intervention: natural meadows (n=56), hay meadows (n=17) with slight human impacts and agroecosystems with monocultures (n=31) heavily impacted by crop management. It was shown that nematode fauna in the natural and hay meadows were similar and more diverse than in the agroecosystems (30–31 genera against 24). Nematode abundance had the lowest values in the agroecosystems, and gradually rose with decreasing land use intensity. Trophic structure of soil nematode communities in all biotopes was dominated by bacterial feeders, with the highest percent of the group (61%) in agroecosystems. The relative abundance of predators and nematode associated with plants was significantly lower in agroecosystems as compared with natural and hay meadows. Plant parasites showed a tendency to increase populations and, consequently, a higher share in the nematode community structure in agroecosystems as compared with meadows (16% vs 11–14%). Nematode communities under heavy human impact were characterized by high EI and low CI values in compared with natural and hay meadows (EI: 57 vs 40–48; CI: 18 vs 25–28). However, using discriminant analysis it was found that only SI index enables clear differentiation between nematode communities of agroecosystems and meadows (52,9% of cases were correctly classified). Agroecosystems had low SI values indicating soil food web simplification or degradation. Based on the taxonomic richness, community structure and ecological indices, hay meadows, formed by perennial grasses, were similar to the natural meadows. Thus, hayfields with low level of disturbance may be suitable (more than meadows that have never been cultivated) for use as reference sites in monitoring the ecological condition of soils under annual crops. Study was carried out under state order (№ 0221-2014-0004, 0221-2015-0006) and partially supported by the Russian Foundation for Basic Research (№ 15-04-07675).

Keywords: Agroecosystems; ecological indices; soil nematodes; taxonomic diversity; trophic structure.

Animal trampling effects on soil free-living nematodes

Stanislav Pen-Mouratov^{1,2}, Roi Meller¹, Rentao Liu^{1,3} and Yosef Steinberger¹

¹ The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel

² The Steinhardt Museum of Natural History and Israel National Center for Biodiversity Studies, Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, 6997801, Israel

³ Key Laboratory for Restoration and Reconstruction of Degraded Ecosystem in Northwestern China of Ministry of Education, Ningxia University, Yinchuan 750021, China

corresponding author: stanislavpm@post.tau.ac.il

The main goal of the present study was to evaluate the effect of ungulate trampling intensity on the abundance and diversity of a soil free-living nematode community under different soil conditions in a man-made safari-zoo area (the Ramat Gan Safari Park, Israel). Our results revealed the impact of trampling on both soil properties and soil nematode communities. Nematode density was found to be lowest in the trampling area during the study period. However, the negative impact of animal trampling on soil nematode communities has been attenuated due to the tree canopy protective effect and seasonal fluctuations. In contrast to the *Cupressus sempervirens* and *Eucalyptus camaldulensis*, the *Tamarix aphylla* reduced the trampling effect on soil nematode communities in a cold-wet period and *vice versa*, increased this effect in the warmer-wet period. During the hottest period of the year, with the external adverse factors of trampling domination, the ability of the trees to protect nematode communities was significantly reduced. Of all the coloniser–persister (cp) continuum of nematode functional guilds, only bacteria-feeding nematodes belonging to the cp⁻¹ guild was positively affected by trampling during the study period. The results showed that about 62% (48% negatively and 14% positively) of the observed nematode species were affected by either direct trampling effect or soil property changes. The applied ecological indices confirmed a negative impact of animal trampling on the soil biota in the study area.

Keywords: Bio-indicator; nematode; safari-zoo area; soil compaction; trampling.

Terrestrial nematodes as part of indication system for ecosystem functions/services assessment in the frame of STACCATO project

Vlada Peneva¹, Stela Lazarova¹, Milka Elshishka¹ and Tanya Bileva² and Josef Settele

¹ Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Y. Gagarin Street, 1113 Sofia, Bulgaria

² Agricultural University, Mendeleev Blvd 12, 4000 Plovdiv, Bulgaria

corresponding author: vpeneva@ecolab.bas.bg

STACCATO project (SusTaining AgriCultural ChAnge Through ecological engineering and Optimal use of natural resources) was funded by the BiodivERsA programme. One of the objectives of the project is to test and improve some indicators for ecosystem functions/services (ESF/ESS) and their values - building upon but going beyond existing indicators sets like those of the CBD (Convention on Biological Diversity) and SEBI (Streamlining European Biodiversity Indicators). Beyond the applicability of the existing ones, specific integrative indicators for each of the strands will be tested for their suitability, e.g., the diversity of indicator plants (Provisioning /Regulating Services (RS), pollinators, and natural enemies of crop pests (RS). Nematodes are major component of soil microfauna and food web. They are both taxonomically and functionally diverse and play significant ecological role as pests and in organic matter transformation. Studies on nematode communities in relation to environmental changes/disturbances showed that nematodes can be useful indicators of ESF/ESS. The contribution of nematodes to ESS is related to resource transfer through herbivore and detritivore channels, nutrient mineralisation, distribution of bacterial and fungal propagules to new resources, regulatory services through predation on opportunistic species; however, they are also involved in a number of disservices such as overgrazing, plant damaging herbivory reducing carbon fixation and availability to other organisms in the food web (. The conceptual framework includes assessment of ESF/ESS in two types of agricultural landscapes dominated by annual crops e.g., winter wheat or oilseed rape, and semi-natural grasslands in different proportions in five countries. The sampling design and protocols for ESF assessment based on terrestrial nematodes are elaborated. Acknowledgements: The authors thanks STACCATO project (<http://www.staccato-project.net/>) for supporting this presentation.

Keywords: Ecology; grasslands oilseed rape; sustainable agriculture; winter wheat.

Communities of terrestrial nematodes after different approaches to heathland restoration

Petra Radochová and Jan Frouz

Institute for Environmental Studies, Charles University in Prague, Benatska 2, 12800 Prague, Czech Republic

corresponding author: radochovap@gmail.com

Since the 20th century, the distribution of European heathlands rapidly decreased due to agricultural intensification, heavy use of artificial fertilisers or acidification. Therefore, various attempts of heathland restoration are presently under way. Analysis of nematode community composition can be one of the tools suitable for succession evaluation. In 2011, 2013 and 2014, soil samples were collected from heathland restoration experiment (launched in 2011) where different restoration methods were applied in a 3 × 3 factorial experiment; existing heathlands were also sampled to identify the target community both in dry and wet heathland. A total of 173 samples of extracted nematodes were analysed for absolute abundance, trophic groups, and genera dominance. Various indices were calculated to describe the nematode community. We were able to prove faster development of wet heathlands towards the target community. However, because of large data variability, there was no significant difference between treatments. Development of wet and dry heathlands differed also in increased proportion of omniphagous nematodes in 2013 and predators in 2014 in dry heathlands. After three years of heathland restoration, the nematode community has not yet reached parameters of the target community.

Keywords: Acidification; alkalization; plant and soil addition; treatments.

Nematode communities associated with crops in organic and conventional farms

Andreia Teixeira¹, Maria Teresa Almeida¹ and Sofia R. Costa^{1,2}

¹ Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

² Mountain Research Center (CIMO), Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

corresponding author: andrea_se_teixeira@hotmail.com

Nematodes are involved in many processes in soil and have been used as bioindicators of soil conditions. Their communities include free-living organisms mostly involved in nutrient mineralisation and also plant-parasitic nematodes that negatively affect production in agroecosystems. Our aim was to compare the structure and dynamics of nematode communities associated with organic and conventional farming and to evaluate the functional diversity of free-living nematodes and the population density of plant-parasitic nematode genera. Soil samples were collected in two farms in geographical proximity, one with organic certification and another under conventional production, along two vegetable crop cultures: pea cv. Maravilha de Kelvedon and lettuce cv. Maravilha das Quatro Estações, both improved for conventional production. Rhizosphere soil was collected at the beginning, at full development and at the end of the cultural cycle, for extraction, identification and quantification of nematodes. Crop yield was recorded, and the dry weight of produce was determined. The density of plant-parasitic nematode populations in the pea culture was significantly larger in conventional production, whereas in lettuce it was similar in both systems. Several genera of plant-parasitic nematodes were associated with both crops, the most abundant genera being *Tylenchus*, *Tylenchorhynchus* and *Pratylenchus*. The analysis of the nematode communities, through the community indices on the structure and enrichment of the soil food web revealed that the soil from both farms had small disturbance and an even activity of decomposition pathways. In the organic farm peas had smaller water content and crop yield was significantly higher. Although the water content of lettuce was also smaller in the organic farm, crop yield was higher in the conventional farm. This may be attributed to the slower growth rate of lettuce grown under the organic regime that however yielded better quality produce.

Keywords: Community indices; crop yield; functional diversity; plant-parasitic nematodes; sustainability.

DNA metabarcoding to characterise soil nematode communities

Lieven Waeyenberge, Annelies Haegeman, Nancy de Sutter, Nicole Viaene and Martine Maes

ILVO, Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

corresponding author: lieven.waeyenberge@ilvo.vlaanderen.be

Eight hundred million years of evolution has resulted in nematodes being dominant among Animalia both in terms of densities and diversity in a wide range of different habitats. Because of their presence in each trophic level associated with these habitats and their broad-range differential lifestyle (from colonist to persister, from plant-feeder to predator), nematode communities are considered as bio-indicators of ecosystem health. By extension, an awareness is increasing to regard compositions of nematode communities in arable land as an important component in the new vision of integrated pest management. The main method to characterise nematode communities is still a morphological one. Such an approach is time-consuming, labour-intensive and requires specialised personnel. DNA metabarcoding based on amplicon sequencing could replace the traditional identification method, although there are some challenges associated with it. The most important one is finding a suitable DNA marker or barcode. This barcode should be composed of variable regions, covering the required taxonomic range of nematodes, flanked by conserved regions needed for universal primer design. We have tested a 18S rRNA primer set to evaluate its usefulness in characterising and quantifying extracted soil nematode communities. An amplicon of 490 bp was created and compared with an in-house curated database based on SILVA. The primers can be accepted as nematode-specific as 80% of the sequences derived from nematodes. However, preliminary results showed a rather poor correlation between morphological identification and counts on the one hand and molecular detection and relative abundance on the other hand. This can be due to several reasons like differences in DNA extraction efficiency among nematode genera, variations in rRNA copy number, incompleteness of the DNA database, etc. We are proceeding with our research in order to address these problems and to further optimise the DNA metabarcoding approach.

Keywords: 18S rRNA; amplicon sequencing; ecological bio-indicators; molecular identification; relative abundance.

Characterisation and functional validation of key RNAi pathway genes in root-knot nematode, *Meloidogyne incognita*Prakash Banakar and Uma Rao

Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

corresponding author: prakashbanakar@gmail.com; umarao@iari.res.in

The southern root-knot nematode, *Meloidogyne incognita*, is one of the most devastating species of plant-parasitic nematodes with a host range that spans most cultivated plants. Since the first report of successful *in-vitro* RNAi in *M. incognita* in 2003, the literature is replete with the application of RNAi for functional genomics of several nematode genes. However, the transcriptional evidence for the complete gamut of genes involved in RNAi is lacking. Difficulties in the application of RNAi to some parasitic nematodes show that not all the nematodes are amenable to RNAi-based target validation. *In-silico* comparison of RNAi pathway genes in several nematode genomes showed that some of the genes encoding upstream components of long dsRNA processing were either lacking or unrecognisable. In order to establish the transcriptional evidence of RNAi pathway in *M. incognita*, we employed the primary sequence similarity-based approach to identify putative orthologs of *Caenorhabditis elegans* RNAi pathway genes in *M. incognita*. Seventy-six predicted protein sets from *C. elegans* were used to identify 28 RNAi pathway genes in *M. incognita* genome *in-silico*. We show transcriptional evidence for 28 RNAi pathway genes in *M. incognita*, including four new genes *csr-1*, *nrde-3*, *sago-1* and *ppw-1*, which were not identified in the previously published *M. incognita* draft genome. The differential gene expression in the four developmental stages revealed higher expression of these genes in the egg stage, indicating the involvement of these genes in embryonic development. Further, *in vitro* RNAi silencing of the five key pathway genes affected the RNAi processing of a target gene in *M. incognita* juveniles, confirming the presence of an active RNAi pathway in *M. incognita*. Our results indicate that in spite of the lack of all the *C. elegans* RNAi pathway gene orthologues, *M. incognita* possess the machinery required to facilitate an RNAi response.

Keywords: *Meloidogyne incognita*, RNA interference, RNAi pathway

Identification and molecular characterisation of the calreticulin gene in *Globodera pallida*

Aida Duarte and Louise-Marie Dandurand

PSES, University of Idaho, ID 83844-2339 Moscow, USA

corresponding author: aduarte@uidaho.edu

Potato cyst nematode (PCN), *Globodera pallida*, is a serious pest of potatoes world-wide and is subject to quarantine procedures wherever it occurs. PCN can be a devastating pest if not controlled. *G. pallida* was first detected in the United States in 2006 in Idaho. The impact of this nematode in potato crop producing areas reinforces the urgent need for development of new control strategies. The purpose of this study is to identify protein-coding genes in *G. pallida* that could be established as new nematode targets for the development of resistant potato. Calreticulin is a Ca^{2+} binding multifunctional protein, isolated and cloned from *G. pallida* and designated (*Gp-crt*). In order to determine whether this gene is differentially expressed during nematode development, cDNA was amplified from RNA isolated from eggs, second-stage juveniles (J2), and females. qPCR is being performed to analyse *Gp-crt-1* expression, and preliminary studies revealed that, this gene is expressed in all developmental stages tested but has higher levels of expression in J2. Biological assays in potato genotypes are being conducted to further understand the nature and function of this gene.

Keywords: cDNA; potato cyst nematode; qPCR.

Effect of different temperatures on the root-lesion nematode *Pratylenchus vulnus* by measuring the expression levels of genes involved in the parasitism

Elena Fanelli, Alberto Troccoli and Francesca De Luca

Institute for Sustainable Plant Protection, CNR, Via Amendola 122/D, 70126, Bari, Italy

corresponding author: elena.fanelli@ipsp.cnr.it

Climate changes induced by temperature variations, with increasing summer temperatures and alterations in rainfall patterns, are expected in the next decades. They will increase economic damage caused by plant-parasitic nematodes all over the world. Higher soil temperatures will affect nematodes, plant-parasitic, predatory and free-living, by increasing the rate of development of some species and consequently more damage to host crop. The aim of the current research is to investigate the relationships between temperature changes and the life cycle and reproduction of the root-lesion nematode *Pratylenchus vulnus*. An Italian population of *P. vulnus* was grown on carrot disks and exposed to different temperature conditions (23, 28 and 33°C). The reproduction at 28°C increased two fold compared to that at 23°C and dramatically decreased at 33°C. The cellular response to temperature changes in *P. vulnus* was also investigated by measuring the transcription level of heat shock protein 90 (*hsp90*) gene, which plays a key role in many cellular processes and promptly responds to temperature variations. In addition the expression levels of two effector genes, *Pv-eng-1*, and *Pv-eng-8*, involved in the early parasitism of *P. vulnus* were determined. Results obtained in this study are reported and discussed. Study partially funded by CISIA-Metagerm.

Keywords: Climate change; effector genes; *hsp90*.

Molecular characterization of a novel effector expansin-like protein from *Heterodera avenae* that induces cell death in *Nicotiana benthamiana*

Jing Liu¹, Huan Peng¹, Jiangkuan Cui¹, Wenkun Huang¹, Lingan Kong¹, Jihong Liu
Clarke², Heng Jian³, Guo Liang Wang^{1,4} and Deliang Peng¹

¹ Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

² Plant Health and Biotechnology Division Virus, Bacteria and Nematodes, Norwegian Institute of Bioeconomy Research, Høgskoleveien 7, 1430 Ås, Norway

³ Department of Plant Pathology and Key Laboratory of Plant Pathology of Ministry of Agriculture, China Agricultural University, Beijing 100193, China

⁴ Department of Plant Pathology, Ohio State University, Columbus, OH 43210, USA

corresponding author: dlpeng@ippcaas.cn

Cereal cyst nematodes are sedentary biotrophic endoparasites that maintain a complex interaction with their host plants. Nematode effector proteins are synthesised in the oesophageal glands and are secreted into plant tissues through the stylet. To understand the function of nematode effectors in parasitic plants, we cloned predicted effectors genes from *Heterodera avenae* and transiently expressed them in *Nicotiana benthamiana*. Infiltration assays showed that Ha-EXPB2, a predicted expansin-like protein, causes cell death in *N. benthamiana*. *In situ* hybridisation showed that the transcripts of Ha-EXPB2 are localised within the subventral gland cells of the pre-parasitic second-stage nematode. HaEXPB2 has the highest expression in parasitic second-stage juveniles. Subcellular localisation assays revealed that HaEXPB2 is localised in the plant cell wall after *H. avenae* infection. The cell wall localisation is mediated by its N terminal and C terminal regions. In addition, we found that Ha-EXPB2 bound to cellulose and its carbohydrate-binding domain is required for this binding. This study demonstrate that Ha-EXPB2 may play an important role in the parasitism of *H. avenae* through targeting the host cell wall.

Keywords: Cell death; effector; expansin protein.

Molecular characterisation and functional importance of cellulose-binding protein secreted by the root-knot nematode *Meloidogyne enterolobii*

Haibo Long¹, Deliang Peng², Shazi Weili¹ and Yanfang Sun¹

¹ Environmental and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences Xueyuan Road No.4, 571101, Haikou, China

² Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Yuanminyuan West Road No. 2, 100193, Beijing, China

corresponding author: hb_long@yeah.net

A cDNA encoding a secretory cellulose-binding protein was isolated from the root-knot nematode *Meloidogyne enterolobii*. The putative full-length cDNA, designated *Me-cbp1*, encoded a 208 amino acid protein containing an N-terminal secretion signal peptide and a cellulose-binding module (CBM). *In situ* hybridisation showed that the *Me-cbp1* transcripts specifically accumulated in the two subventral gland cells of the second-stage juveniles. Developmental expression confirmed that its transcript abundances were high in the motile juvenile stages and low in the sedentary stage of the nematode. Post-transcriptional gene silencing by soaking the nematodes in double-stranded RNA against the gene reduced nematode infectivity by 27%, suggesting that the cellulose-binding protein may have an important role in pathogenesis.

Keywords: Cell wall modifying; effectors; *Meloidogyne enterolobii*; RNAi.

Msp40 effector of root-knot nematode manipulates plant immunity to facilitate parasitism

Junhai Niu^{1,2}, Pei Liu¹, Qian Liu¹, Changlong Chen¹, Quanxin Guo¹ and Heng Jian¹

¹ Department of Plant Pathology, China Agricultural University, Beijing 100193, China

² Tropical Crops Genetic Resources Institute, Danzhou, Hainan 571737, China,

corresponding author: hengjian@cau.edu.cn

Root-knot nematodes (RKN) are obligate biotrophic parasites that invade plant roots to transform cells near the vascular cylinder into a permanent feeding site, and engage in prolonged and intimate relationships with their host plants. The nematode secretions with the activity of suppressing host immunity play essential roles in successful parasitism and are largely unknown. We show here that a potential RKN-specific secreted protein gene 40 (MiMsp40) cloned from *Meloidogyne incognita*, expresses exclusively in subventral esophageal gland secretory cells and is strongly upregulated during the early parasitic stages. The *Arabidopsis thaliana* overproducing MiMsp40 were more susceptible to nematode infection relative to wild type (WT, Col-0) plants, as reflected by the increased number of root galls and eggs. Conversely, host-derived RNAi of MiMsp40 suppressed nematode parasitism and/or propagation. Moreover, MiMsp40 transient expression prevented the pro-apoptotic protein Bax-triggered defence-related programmed cell death (PCD). Overexpression of MiMsp40 in plants suppressed the callose deposition and the expression of marker genes for bacterial elicitor elf18-triggered immunity. Coagroinfiltration assays indicated MiMsp40 also suppress macroscopic cell death triggered by MAPK cascades or ETI cognate elicitors R3a/Avr3a. The collective results demonstrated that MiMsp40 could be a novel *Meloidogyne*-specific secreted effector, injected into plant cell mainly during the early parasitic stages, playing a role in suppressing PTI and/or ETI signal to facilitate RKN parasitism.

Keywords: Effector; *Meloidogyne incognita*; MiMsp40; PTI.

Utility of transcriptome of *Heterodera avenae* for identification and functional validation of gene targets using RNAiPradeep Papolu, Divya Singh, Vikas Patel and Uma Rao

Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

corresponding author: umarao@iari.res.in

The cereal cyst nematode (CCN), *Heterodera avenae*, is a major biotic constraint for crop production in wheat and barley growing regions around the world. To gain insight into its repertoire of parasitism genes, we identified 40 genes from the CCN transcriptome that were cloned from cDNA of second-stage juveniles of *H. avenae*. These 40 genes were classified into four major groups viz., transcripts belonging to neuropeptides, proteases, cell wall degrading enzymes and secretory proteins. All the 40 genes were subjected to *in vitro* RNAi by soaking in double stranded RNA of the each of the gene. The gene silencing was evaluated for short and long term effects in terms of penetration and reproduction ability of *H. avenae* on wheat. The results revealed that RNAi silencing of different genes led to about 7-76% reduction in penetration and 10-80% reduction in the number of cysts over control. It is very interesting to note that a transient silencing by dsRNA soaking could still provide such a long-term effect on the nematode reproduction. However, genes belonging to cell wall degrading enzymes were more effective in reducing both the penetration and reproduction. The potential genes identified in the present study could be used to determine their role in the pathogenicity of three known pathotypes of *H. avenae*. This is a pioneer study in *H. avenae* involving such a large number of genes at a time. The study is very significant due to the synchronisation of its life cycle with the crop leading the completion of only one life cycle and the difficulty in inducing hatching under laboratory conditions due to its high sensitivity to temperature.

Keywords: Cereal cyst nematode; functional validation; *Herodera avenae*; RNAi.

Characterisation of three novel fatty acid- and retinoid-binding protein genes (*Ha-far-1*, *Ha-far-2* and *Hf-far-1*) from the cereal cyst nematodes *Heterodera avenae* and *H. filipjevi*

Fen Qiao¹, Lilian Luo^{1,2}, Huan Peng¹, Wenkun Huang¹, Lingan Kong¹, Daohong Jiang², David J. Chitwood³ and Deliang Peng¹

¹ The State Key Laboratory for Biology of Insect Pests and Plant Disease, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing 100193, China

² College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, 430070, Hubei Province, China

³ Nematology Laboratory, USDA-ARS, Building 011A, BARC-West, Beltsville, Maryland 20705, USA

corresponding author: dlpeng@ippcaas.cn

Heterodera avenae and *H. filipjevi* are major parasites of wheat, reducing production worldwide. Three novel FAR genes (*Ha-far-1*, *Ha-far-2*, *Hf-far-1*) were cloned from *H. avenae* and *H. filipjevi*. *Ha-far-1* and *Ha-far-2*, encoding proteins of 191 and 280 amino acids, respectively, and sequence identity of 28%. Ha-FAR-1 sequence is 78% similar to the Gp-FAR-1 protein from *G. pallida*, whilst Ha-FAR-2 is 30% similar to Rs-FAR-1 from *Radopholus similis*. Only one FAR protein Hf-FAR-1 was 96% sequence identity to Ha-FAR-1. The three proteins are alpha-helix-rich and contain the conserved domain of Gp-FAR-1, but Ha-FAR-2 had a remarkable peptide at the C-terminus which was random-coil-rich. Both Ha-FAR-1 and Hf-FAR-1 had casein kinase II phosphorylation sites, while Ha-FAR-2 had predicted N-glycosylation sites. Phylogenetic analysis showed that the three proteins clustered together, although Ha-FAR-1 and Hf-FAR-1 adjoined each other in a plant-parasitic nematode branch, but Ha-FAR-2 was distinct from the other proteins in the group. Fluorescence-based ligand binding analysis showed the three FAR proteins bound to a fluorescent fatty acid derivative and retinol and with dissociation constants similar to FAR from other species, though Ha-FAR-2 binding ability was weaker than that of the two others. *In situ* hybridisation detected mRNAs of *Ha-far-1* and *Ha-far-2* in the hypodermis. The qRT-PCR results showed that the *Ha-far-1* and *Ha-far-2* were expressed in all developmental stages; *Ha-far-1* expressed 100 times more than *Ha-far-2* in all stages. The highest expression level of *Ha-far-1* was observed in fourth-stage juveniles, whereas the highest expression level of *Ha-far-2* occurred in second-stage juveniles. This research work was supported by the NSFC(31571988), the 973 Program (2013CB127502) and the SFASRPI (201503114).

Keywords: Binding; FAR protein; fatty acid; *Heterodera avenae*; *Heterodera filipjevi*.

Identification of parasitism-related genes in *Pratylenchus penetrans*

Paulo Vieira^{1,2}, Thomas Maier³, Inga A. Zasada⁴, Thomas Baum³, Kathryn Kamo² and Jonathan D. Eisenback¹

¹ Virginia Tech, Department of Plant Pathology, Physiology and Weed Science, Blacksburg, Virginia, USA

² USDA ARS, Floral and Nursery Plants Research Unit, BARC, Maryland, USA

³ Department of Plant Pathology, Iowa State University, Ames, Iowa, USA

⁴ USDA ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon, USA

corresponding author: pvieira.us@gmail.com

Worldwide crop losses due to plant-parasitic nematodes have been estimated at \$118 billion annually, with *Pratylenchus* spp., commonly known as the root-lesion nematode (RLN), ranking third in terms of economic losses. Currently, the most common strategies used for RLN control are genetic resistance, nematicide application, and rotation with non-host crops. Host resistance to *Pratylenchus* spp. is very limited, as only a few loci have been linked to resistance/tolerance to some RLN species. Application of new technologies to control RLN is needed, since most of the chemicals currently used present negative effects to the environment and increase production costs. Using a comparative transcriptomic approach and a nematode secreted proteins identification pipeline, we identified numerous candidate genes, which may be important for the mediation of *P. penetrans* with its host. The candidate nematode genes identified so far represent a range of putative biological functions, such as genes encoding cell wall-degrading enzymes, proteases, putative suppression of the host defenses, and pioneer genes specific to this group of nematodes. In order to validate our *in silico* analyses, a subset of genes were studied by RT-qPCR analyses and *in situ* hybridisation. Our results revealed the identification of several genes whose expression was restricted to the oesophageal glands of *P. penetrans*, and the expression patterns of the different candidate genes confirmed their expression in planta. Currently we are performing plant-mediated gene silencing for some nematode candidate genes, in order to evaluate their importance for nematode establishment and parasitism. This analysis sheds light on putative parasitism-related genes of *P. penetrans*, and will aid in the identification of potential gene targets for selection and use to design effective control strategies against root-lesion nematodes.

Keywords: Nematode effectors; root-lesion nematode; transcriptome.

From wild to cultivated olive: how anthropogenic changes impact the diversity of plant-parasitic nematode communities?

Nadine Ali¹; Johannes Tavoillot²; Guillaume Besnard³, Bouchaib Khadari⁴, Ewa Dmowska⁵, Grazyna Winiszewska⁵, Odile Fossati², Mohamed Ater⁶, Mohamed Aït Hamza⁷, Abdelhamid El Mousadik⁷, Aïcha El Oualkadi⁸, Abdelmajid Moukli⁸, Ahmed Elbakkali⁹, Elodie Chapuis^{2,10} and Thierry Mateille¹¹

¹ Tishreen University, Faculty of Agriculture, Latakia, Syrian Arab Republic

² IRD, UMR CBGP, 34988 Montpellier-sur-Lez Cedex, France

³ CNRS, UMR EDB, Université Toulouse III Paul Sabatier, 31062 Toulouse Cedex 9, France

⁴ SUPAGRO, UMR AGAP, 34398 Montpellier Cedex 5, France

⁵ Museum and Institute of Zoology PAS, 00-679 Warsaw, Poland

⁶ Université Abdelmalek Essaadi, Faculté des Sciences et Techniques, 93030 Tétouan, Morocco

⁷ Université Ibn Zohr, Faculté des Sciences d'Agadir, 80000 Agadir, Morocco

⁸ INRA, CRRA, 40000 Marrakech, Morocco

⁹ INRA, UMR APCRPG, 50000 Meknès, Morocco

¹⁰ IRD, UMR PVBMT, 97410 Saint Pierre, La Réunion, France

¹¹ IRD- (CBGP), 911 Av Agropolis, 34394 Montpellier, France

corresponding author: Mateille@ird.fr

Plant-parasitic nematodes (PPN) are major crop pests. On olive (*Olea europaea*), they contribute significantly to economic losses in the ten-top olive producing countries in the world, especially in the Mediterranean basin. Damage increases with cropping intensification and in nurseries. The diversity of their communities respond to evolutionary, environmental and anthropogenic forces. The olive tree is a good host plant model to understand the impact of such forces on PPN diversity since it grows according to different modalities (wild, feral and cultivated). A wide survey (213 soil samples) was conducted in several olive-growing regions in Morocco. The diversity, the functional and trophic composition as well as the patterns of nematode communities were described and then compared between the different olive modalities. The results revealed a high diversity of PPN communities with the detection of 117 species. Some taxa were recorded for the first time on olive trees worldwide and new species were also identified. Anthropogenic factors (wild vs cultivated conditions) strongly impacted the PPN diversity because the species richness, the local diversity and the evenness were significantly decreased and the total abundance of nematodes significantly increased in cultivated conditions and vice-versa in wild conditions. This impact was also remarkable on functional and trophic composition of communities because cultivated olive exhibited many more obligate and coloniser PPN and less persister PPN. Community patterns were also impacted: genera such as *Xiphinema* and *Heterodera* were enhanced in wild olive, whereas others such as *Meloidogyne* and *Tylenchorhynchus* were especially enhanced in high-density orchards. The study underlined that PPN diversity is a relevant indicator to assess soil health and that PPN community structures could be taken into account in order to design control strategies based on the management of their diversity.

Keywords: Anthropisation; biodiversity; community; olive; plant-parasitic nematodes.

Diversity of meiofauna, temporal variations and vertical distribution of free-living marine nematodes from the intertidal zone of the Ría de Ares-Betanzos (Galicia, NW Iberian Peninsula)Luiz Henrique Carvalho¹ and Celia Besteiro^{2,3}¹ Instituto Federal do Maranhão (IFMA), Campus São Luis, Avda. Getúlio Vargas, 4, Monte Castelo, São Luis de Maranhão, CEP 65030-005, Brazil² Departamento de Zooloxía e Antropoloxía Física, Facultade de Veterinaria, Universidade de Santiago de Compostela, Campus Universitario, Ramón Carballo Calero, s/n, 27002 Lugo, Spain³ Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela, Rúa da Ribeira, 1 e 4, 15590 A Graña, Ferrol, A Coruña, Spaincorresponding author: celia.besteiro@usc.es

In this communication we present our information on the free-living nematode communities from the Ría de Ares-Betanzos. We have studied a total of 61.265 individuals of meiofauna, distributed in 20 major taxa, from 879 samples obtained in the intertidal zone of seven beaches of Seselle and were taken during 1993, 1994, 1995, 1998 and 2003. Nematodes were the dominant group in the meiofauna of the Ría de Ares-Betanzos; they completely dominate the samples from the beaches of Ber, Sada and San Pedro de Veigue and also dominate the remaining ones except for the following samples: W03 (winter 2003) from the beach of Seselle, SM93 (summer 1993) from the beach of A Magdalena and SM93 (summer 1993) from the beach of Gandarío. For the study of temporal variations we used the samples obtained with syringes (one sample of 100 cc and three samples of 50 cc in each locality and sample period). Nematodes from the Ría de Ares-Betanzos seem not to have any set distribution pattern. For the vertical distribution study, we used the samples obtained with a corer 30 cm high, divided into sections of 5 cm. Nematodes seem to be mainly distributed in the two upper fractions, 0-5 and 5-10 cm, in which they represent 49.62% and 36.33% of the communities respectively. Those results are different from those found in the contiguous Ría de Ferrol and in the rías of Foz and O Barqueiro, where nematodes comprise the main part of the meiofauna (89%) and were restricted to the upper horizon (0-5 cm). The majority of individuals were concentrated in the 10 upper cm, without relation to the sediment type. The most important factors driving the vertical distribution of the nematodes (and meiofauna) should be Ph and the desiccation of the sediment during the low tide. Other factors, such as the presence of seagrasses, can locally modify this distribution.

Keywords: Ares-Betanzos; Galicia; horizontal distribution; marine nematodes; vertical zonation.

Free-living nematodes communities at the Ría de Ferrol (Galicia, NW Península Ibérica): temporal variations, vertical distribution and diversity of meiofauna

Wilma dos Santos Eugênio¹ and Celia Besteiro^{2,3}

¹ Universidade Federal de Maranhão (UFMA), Avda. dos Portugueses, 1966, Bacanga, São Luis de Maranhão, CEP 65030-805, Brazil

² Departamento de Zooloxía e Antropoloxía Física, Facultade de Veterinaria, Universidade de Santiago de Compostela, Campus Universitario, Ramón Carballo Calero, s/n, 27002 Lugo, Spain

³ Estación de Bioloxía Mariña da Graña, Universidade de Santiago de Compostela, Rúa da Ribeira, 1 e 4, 15590 A Graña, Ferrol, A Coruña, Spain

corresponding author: celia.besteiro@usc.es

For a long time we have studied the meiofauna of the Ría de Ferrol. In this communication we present our information on the free-living nematode communities from those localities. We have studied a total of 126,142 individuals of meiofauna, distributed in 26 major taxa, from 1.044 samples obtained in the intertidal zone of seven localities, four sandy beaches and three muddy inlets and were taken in 1990, 1991, 1993, 1994, 1995, 1998 and 2003. Nematodes were the dominant group in the meiofauna of the Ría de Ferrol; they completely dominate the samples from the inlets of A Cabana, Maniños and Mugaridos and also dominate many of the samples from the beaches of Cariño and San Felipe, but not for the samples from the sandy beaches of Batel and Chanteiro. For the study of temporal variations we used the samples obtained with syringes (one sample of 100 cc and three samples of 50 cc in each locality and sample period). Nematodes from the Ría de Ferrol seem not to have any set distribution pattern. For the vertical distribution study, we used the samples obtained with a corer 30 cm high, divided into sections of 5 cm. Nematodes seem to be mainly distributed in the upper fractions, 0-5 cm, where the nematodes comprise 87.73% of the communities. Those results are different from those found by Carvalho (2008) in the Ría de Ares-Betanzos, where he founded the main part of the nematodes distributed between the two upper horizons (0-5 and 5-10 cm). The majority of individuals were concentrated in the 5 upper cm, without relation to the sediment type. The most important factors driving the vertical distribution of the nematodes (and meiofauna) are probably Ph and the desiccation of the sediment during the low tide. Other factors, as the presence of seagrasses, can locally modify this distribution.

Keywords: Ferrol; Galicia; horizontal distribution; marine nematodes; vertical zonation.

Identification of nematodes associated with *Rhynchophorus ferrugineus* in South Italy

Elena Fanelli¹, Monica Oreste², Alberto Troccoli¹, Gianluca Scarcia^{1,2}, Alessio Vovlas⁴, Nicola Trisciuzzi³, Eustachio Tarasco^{1,2} and Francesca De Luca¹

¹ Institute for Sustainable Plant Protection, CNR, Via G. Amendola 122/D, 70126, Bari, Italy

² DiSSPA, University of Bari "Aldo Moro", Via G. Amendola 125/A, 70126 Bari, Italy

³ Centro Ricerca e Sperimentazione in Agricoltura (CRSA) "Basile Caramia", Via Cisternino, 281 Locorotondo (BA), Italy

⁴ A.P.S. Polyxena, Via Donizetti 12, 70014, Conversano, Bari, Italy

corresponding author: francesca.deluca@ipsp.cnr.it

The nematode fauna associated with the red palm weevil *Rhynchophorus ferrugineus*, is important as indicator of biodiversity, potential genetic resources and potential biological agents. A close association of the diplogastrid *Mononchoides macrospiculum* and the rhabditid *Teratorhabditis synpapillata* with *R. ferrugineus* has been reported previously on palm trees from Apulian region (southern Italy). During a palm survey for the palm weevil *R. ferrugineus*, carried out in 2015 in South Italy, the associated nematode fauna was extracted and characterised at the molecular level. The ITS containing region, the 18 S rRNA gene and the D2-D3 expansion segments of the 28S rRNA gene were amplified and sequenced. The association of *M. macrospiculum* and *T. synpapillata* was confirmed in the new survey; however, five additional nematode species including rhabditids, diplogastrids, aphelenchoidids and few plant-parasitic species were also found. The authors report the biodiversity of the nematodes associated with *R. ferrugineus* from field surveys and the current knowledge on the 'specific habitat' of specialised and divergent entomophilic nematodes.

Keywords: DNA amplification; nematode fauna; red palm weevil; sequencing.

Interesting findings of soil-inhabiting, including plant parasites, nematodes in the botanical gardens of the North-West Russia

Daryia S. Kalinkina, Anna A. Sushchuk and Victoria V. Lavrova

Institute of Biology of Karelian Research Centre Russian Academy of Sciences, 185910
Petrozavodsk, Russia

corresponding author: danial_22@mail.ru

Soil nematodes are closely related with plants. Introduction of new plant species in biocenoses can lead to appearance of unusual nematode species, including dangerous parasites that had not been revealed before. In order to find new species in North-West of Russia soil nematode fauna in the rhizosphere of introduced trees were investigated in botanical gardens of Republic of Karelia (61° N), Arkhangelsk (65° N) and Murmansk regions (67° N). As a result rare and unusual species were found. Nematodes of the genera *Nagelus*, *Cephalenchus*, *Rotylenchus*, *Paratrichodorus* and *Longidorus* were found in the rhizosphere of introduced trees in the investigated botanical gardens. These genera are unusual for natural biocenoses and previously only found on urban areas. The last two genera are specific pathogens, ectoparasitic pests of plant roots and vectors of plant viruses. In addition, entomopathogenic nematode species *Steinernema affine* was detected for the first time in Russia, in botanical garden of Big Solovetsky island. Another interesting phenomenon, endotokia matricida, was noted for *Rhabditis* from Polar-Alpine Botanical Garden, located above the Arctic Circle (67 N). Matricidal hatching can be the result of unfavourable environmental conditions, for example, insufficient supply of food resources. Thus, these interesting findings of nematodes can be evidence of human activities related to the accidental introduction and spread of dangerous parasites. The study was supported by the RFBR (№ 15-04-07675; № 16-34-00650).

Keywords: Endotokia matricida; human activity; *Steinernema affine*; unusual species of nematodes.

Plant-parasitic nematode communities associated to wild and cultivated olives (*Olea europaea*) in South Italy

Alberto Troccoli¹, Elena Fanelli¹, Nicola Vovlas¹, Thierry Mateille², Vincenzo Radicci¹
and Francesca De Luca¹

¹ Institute for Sustainable Plant Protection, CNR, Via G. Amendola 122/D, 70126, Bari, Italy

² IRD, UMR CBGP (INRA/IRD/CIRAD/Montpellier SupAgro), 755 Avenue du Campus Agropolis, CS30016, 34988 Montferrier-sur-Lez Cedex, France

corresponding author: alberto.troccoli@ipspp.cnr.it

Olive (*Olea europaea* L.) is one of the most ancient and economically important crop of Mediterranean area. Olive trees serve as hosts to a large number of plant nematodes, the most important and widely distributed of which are root-knot (*Meloidogyne* spp.), root-lesion (*Pratylenchus* spp.), needle (*Xiphinema* spp.) and spiral nematodes (*Helicotylenchus* spp.). A study was conducted in the springs of 2013-2015 in South Italy, which is the largest olive growing area within the country, to analyse the diversity of plant-parasitic nematodes associated to cultivated olive in comparison to wild olive in Italy, aiming to understand the contribution of olive domestication and human impact in plant-parasitic community and thus in their pathogenicity. Plant-parasitic nematode species inhabiting the rhizosphere of wild and cultivated olives of southern Italy were surveyed and characterised at morphological and molecular level. More than 100 soil samples collected from the rhizosphere of olive feeder roots in rainfed middle density and irrigated middle density olive orchards, as well as in spotted wild olive areas, resulted in the detection and identification of 25 species belonging to 12 genera, most of which were already known, with the exception of a needle and a spiral nematode, possibly representing new species. The most frequently occurring nematode genera were *Helicotylenchus*, both in cultivated and wild olives. Ectoparasites such as *Xiphinema* and *Tylenchidae* in general and, to a lesser extent, *Pratylenchus*, were also frequently encountered. Conversely, *Meloidogyne* species were only rarely found. *Rotylenchulus macrodoratus* and *Heterodera mediterranea* seems to be restricted to Apulia region only.

Keywords: Mediterranean area; morphological and molecular analysis; olive; plant-parasitic nematodes.

NEMARACE – genomic sequencing and polymorphism analysis of the different races of *Meloidogyne incognita*

Erika V. S. Albuquerque¹, Regina M.G.D. Carneiro¹, Eder Marques¹, Maria C.M. Silva¹, Andressa Machado³, Maria F. Grossi-de-Sá¹, Pierre Abad², Philippe Castagnone-Sereno² and Etienne Danchin²

¹ Embrapa Recursos Genéticos e Biotecnologia, PqEB Av W5 Norte, 70770-917 Brasília, Brazil

² INRA, UMR 1355, 400 route des Chappes, 06903, Sophia Antipolis, França

³ IAPAR, Rodovia Celso Garcia Cid, km 375 86047-902 - Londrina, Brazil

corresponding author: erika.freire@sophia.inra.fr

The concept of host-race for several species of root-knot nematodes (RKN) is based on the variability of parasitic success among populations subjected to a differential host test. No correlation between phylogeny and host-races has been reported to date. However, detailed studies based on the genome-wide analysis of single nucleotide polymorphisms (SNP) from different races of RKN were never performed. Knowledge of the genetic variability between populations of different races may indicate whether there is an association of this host classification with the corresponding nematode genome sequences. This collaborative work will consist in the large-scale genomic sequencing and comparative analysis of several populations of each of the four *Meloidogyne incognita* races. The populations will be investigated at the intraspecific polymorphism level which may be related to the host range phenotypes of *M. incognita*. In the first stage of the research, we identified the race status of 18 *M. incognita* populations from different cultures, using the North Carolina differential host test. In the second stage, an adapted DNA extraction protocol yielded large quantities of high quality samples. In the third stage, we used a set of PCR/SCAR markers to verify the specific purity of the populations. In the fourth stage, samples from 12 populations are being sequenced to be analysed by bioinformatics PoolSeq approach. The data generated should contribute to the development of potential marker sequences for *M. incognita* molecular pathotyping and durable control strategies

Keywords: Genomics; molecular marker; PoolSeq; root-knot nematode.

Identification and characterisation of candidate effector genes in the transcriptome of the root-knot nematode *Meloidogyne enterolobii*

Sebastian Kiewnick¹, Jürg Frey², Christian Ahrens², Andrea Braun-Kiewnick¹ and Abdelnaser Elashry³

¹ Agroscope, National Competence Center for Nematology, Schloss 1, 8820 Waedenswil, Switzerland

² Agroscope, Institute for Plant Production Sciences, IPS, Schloss 1, 8820 Waedenswil, Switzerland

³ University of Bonn, Molecular Phytomedicine, Karlrobert-Kreiten-Strasse 13, 53115 Bonn, Germany

corresponding author: sebastian.kiewnick@agroscope.admin.ch

The root-knot nematode *Meloidogyne enterolobii* is a polyphagous species, attacking an extremely wide range of host plants including ornamentals and important agricultural crops. It is considered one of the most pathogenic and virulent root-knot nematode species, as it is able to develop and reproduce on host plants carrying resistance to the major tropical root-knot nematode species. The objective of this project was to conduct *de novo* sequencing of the transcriptome of the Swiss *M. enterolobii* population. Sequencing was done on an Illumina MiSeq platform using the 2 × 300 bp paired end reads kit. RNA was obtained from two life stages, eggs and second-stage juveniles, of a well-characterised *M. enterolobii* reference population present in Switzerland. Our assembly resulted in the identification of homologues of known effectors from other root-knot and cyst-nematodes. We were able to identify approximately 200 *M. enterolobii* putative secretory proteins. *De novo* transcriptome sequencing will provide significant new information on candidate effector genes to better understand the interaction of this nematode with host plants and to develop durable control strategies based on plant resistance.

Keywords: Control; effectors; resistance.

***De novo* analysis of the transcriptomes of *Heterodera avenae* at five developmental stages**

Huan Peng, Jiangkuan Cui, WenKun Huang Ling'an Kong and Deliang Peng

State Key Laboratory for Biology of Insect Pests and Plant Disease, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing 100193, China

corresponding author: hpeng@ippcaas.cn

The cereal cyst nematode *Heterodera avenae* is one of the important pests of cereal. Little is known about the molecular mechanism of *H. avenae* parasitism. In this study, we investigated the transcriptomes of *H. avenae* at different developmental stages including preparasitic second-stage juveniles, parasitic second-stage juveniles, third- and fourth-stage juvenile, adult females and eggs, resulting in 82549 transcripts with an average transcript length of 1 546 bp with Trinity assemblers. Of these, 54.37%, 45.54% and 48.23% of transcripts were successfully annotated in Nr, Swiss-Prot and Go databases, respectively. In addition, 1642 putative effectors that were similar to known phytonematode effectors and 117 putative novel effectors that had no match sequence to known phytonematode effectors were identified in *H. avenae* transcriptomes. Further, 216 transcripts without orthologous sequence to Embryophyta, Craniata and Insecta were found to have putative lethal RNAi phenotypes. These data lay the foundation for future study of *H. avenae*-cereal interactions and *H. avenae* parasite control.

Keywords: Cereal cyst nematode; differentially expressed genes; effectors; lethal RNAi phenotypes.

Expression of *Meloidogyne incognita* genes in the interaction with *Rhizophagus intraradices* and water stress on tomato

Isabella Pentimone¹, Laura C. Rosso¹, Mariantonietta Colagiero¹, Pasqua Veronica¹, Maria Teresa Melillo¹, Francesca De Luca¹, Elena Fanelli¹, Raffaella Balestrini² and Aurelio Ciancio¹

¹ Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Unit of Bari, 70126, Italy

² Istituto per la Protezione Sostenibile delle Piante (IPSP), CNR, Unit of Turin, 10125, Italy

corresponding author: aurelio.ciancio@ipsp.cnr.it

A transcriptomic study was carried out on the interaction of *Meloidogyne incognita* with the arbuscular mycorrhizal fungus *Rhizophagus intraradices* and water stress, on *Solanum lycopersicum* cv. San Marzano nano. RNA was extracted from 6-week-old roots (with/without mycorrhizal inoculum or mild water stress), 1 and 2 weeks after inoculation with juveniles. The RNAs were sequenced with Illumina Next Generation Sequencing, and CLC™ analysis was applied to the reads obtained. After filtering reads mapping on the tomato genome (SL2.40.26), the *M. incognita* ASM18041v1 genome was used to identify the nematode expressed contigs. The tomato unmapped reads ranged from 7.35×10^5 (galls with *M. incognita*, *R. intraradices* and water stress) to 4.13×10^6 (nematodes only). The reads mapping on ASM18041v1 ranged from 43.8 % (*M. incognita* plus *R. intraradices*) to 71.9% (nematode only), and 41.6% were annotated. Seven days after inoculation all treatments shared 4964 contigs (76.6% of overall unique elements) out of 6474, with 185 transcripts (2.8%) uniquely expressed in roots with nematodes, 300 (4.6%) in the nematode and mycorrhiza association and 326 (5%) found only in roots with *M. incognita*, mycorrhiza and water stress. Two weeks after inoculation, 5216 contigs (84.3%) were commonly expressed, with 191 (3.1%) uniquely expressed in roots with nematodes, 138 (2.2%) only in roots with nematode and mycorrhiza, and 144 (2.3%) only in roots with *M. incognita*, *R. intraradices* and water stress. Gene expression was compared at the two times in more stringent conditions (RPKM>100). In roots with nematodes only, 99 transcripts (69.8%) were always expressed, with 20 and 48 uniquely expressed at 1 and 2 weeks, respectively. Addition of *R. intraradices* reduced commonly expressed genes (89), and increased those unique at 1 and 2 weeks (48 and 43, respectively), in a situation similar to presence of water stress. Study partially funded by CNR, Progetto Premiale Aqua.

Keywords: Arbuscular mycorrhiza; endosymbiont; root-knot nematode; transcriptome; water stress.

New or old problem? The occurrence of new virulent populations of potato cyst nematodes

Loes J.M.F. den Nijs

NPPO, Geertjesweg 15, 6706 EA Wageningen, The Netherlands

corresponding author: l.j.m.f.dennijs@nvwa.nl

Potato cyst nematodes (PCN) are a known problem in The Netherlands. Farmers have learned to contain and manage these nematodes by using a combination of control measures. Management practices all rely heavily on the use of resistant potato varieties. In the starch potato growing area a 1: 2 cropping system is possible using these varieties. For *Globodera rostochiensis* the resistance is based on the *H1* gene, which gives an almost absolute resistance. For *G. pallida* the resistance is based on at least two major genes and some minor genes. They all play a role in the process resulting in varying degrees of resistance, expressed as relative susceptibility in comparison to a fully susceptible variety. Growing the correct resistant variety for the population of PCN nematodes in a specific field can reduce the population density below the EU described detection level. However, repeated cropping of the same resistant variety puts selection pressure on the nematode population causing a shift in the virulence within the population. History has shown that these processes have occurred in the past (shifts from pathotype Ro1 to Ro2, Pa2, Pa3) and by using the same approach these processes are still ongoing. Recent information received from various sources within The Netherlands gives evidence that new virulent populations have evolved recently. This process will go on due to the frequent growing of resistant potato varieties. The approach to follow these developments in the field and the first results will be presented. The possible management options to address this new but old problem will be discussed.

Keywords: *Globodera pallida*; *Globodera rostochiensis*; pathotypes; resistance.

Overview of plant-parasitic nematode interceptions in UK trade 2014-2016

Rebecca Lawson and Thomas Prior

Fera, National Agri-Innovation Campus, Sand Hutton, York, YO41 1LZ York, UK

corresponding author: bex.lawson@fera.co.uk

Annually, Fera processes over 40,000 statutory samples for plant pests and diseases, around 2000 of which are for plant-parasitic nematodes. Data and trends on nematode interceptions for trade routes including large and small ornamentals, potato residues, aquatics and other growing media over the last two years will be presented.

Keywords: Ornamentals; quarantine; soil.

Summary of recent outbreaks of *Meloidogyne fallax* in the UK

Thomas Prior, Rebecca Lawson and Jennifer Hodgetts

Fera, National Agri-Innovation Campus, Sand Hutton, York, YO41 1LZ York, UK

corresponding author: thomas.prior@fera.co.uk

Meloidogyne fallax is a I/A2 EU annex listed species, with an EPPO A2 designation. In 2013, a UK outbreak of this pest species was reported from an organic crop of leeks (*Allium ampeloprasum*) in one field of 12.5 ha in Staffordshire, with the total area affected approximately 1 ha. The leek plants were supplied by a UK propagator in March 2013 and had been grown from seed in peat blocks. One possibility is that the pest could have been introduced into the infested field with plant waste and soil resulting from the on-site processing of leeks produced in other EU member states. Following additional UK outbreaks on amenity turf, Defra carried out specific surveys, trace back and trace forward activities, which will be presented.

Keywords: Quarantine; risk; root-knot nematode.

Durability of resistance in tomato cultivars and rootstocks bearing the *Mi* gene to populations of *Meloidogyne arenaria*, *M. incognita* and *M. javanica*

Gökhan Aydınli¹ and Sevilhan Mennan²

¹ Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

² Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

corresponding author: gokhanay@omu.edu.tr

Using resistance tomato cultivars and rootstocks have been increasing in glasshouses in the Middle Black Sea Region of Turkey. Resistance in these tomatoes is based on the presence of the *Mi* gene that confers resistance to the three major root-knot nematode species *Meloidogyne arenaria*, *M. incognita* and *M. javanica*. The objectives of this study were to screen the *Mi* gene expressed as the homozygous or heterozygous forms in four tomato cultivars and four rootstocks by PCR-based co-dominant SCAR marker Mi23, and to determine the reaction of these tomato plants to eight different local populations including *M. arenaria* (four populations), *M. incognita* (two populations) and *M. javanica* (two populations). Ten plants from each tomato cultivar and rootstock were inoculated with 3000 eggs per pot. Tomato cv. Barbaros was used as a susceptible control. Five plants were harvested after 8 weeks for short period and the remaining five plants after 16 weeks for long period. According to the Mi23 marker, all tomato cultivars and a rootstock displayed two bands of 380 bp and 430 bp in size indicating that the *Mi* locus was in heterozygous form. Among all cultivars and rootstocks, three rootstocks were homozygous as only a single band of 380 bp was amplified. All tomato cultivars and rootstocks tested for both short and long period were showed low root gall indexing and supported low reproduction to all *Meloidogyne* populations except one *M. incognita*. This population of *M. incognita* was detected as partially virulence in our previous study. Rootstocks were resistant to all the populations, whereas cultivars had reduced resistance to partially virulent population. Acknowledgement: This study was supported by TÜBİTAK-TOVAG (1110793).

Keywords: *Mi* gene; resistance; rootstocks; tomato cultivars.

Plant-parasitic nematodes associated with grafted bean

Sofia R. Costa^{1,2}, João Capitão³, António Ferreira³, Francisco Vaz³, J. Raul Rodrigues⁴, Luísa Moura⁴, L. Miguel Brito⁴ and Isabel Mourão⁴

¹ Mountain Research Center (CIMO), Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

² CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

³ Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Refóios, 4990-706 Ponte de Lima, Portugal

⁴ Mountain Research Centre (CIMO)/Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Refóios, 4990-706 Ponte de Lima, Portugal

corresponding author: sofia.costa@bio.uminho.pt

In Portugal, green beans are predominantly produced from glasshouse-grown common beans (*Phaseolus vulgaris*). Intensive farming has increased the incidence of diseases, including plant-parasitic nematodes (PPN), whereas fertiliser and pesticide applications are increasing soil salinity. Vegetable grafting is an emerging technique to alleviate soil-borne diseases and abiotic stress. Although PPN resistant rootstocks have been developed for some crops, they are not available for common bean. With this aim, we tested three *P. coccineus* cultivars as rootstocks for two widely used common bean cultivars (commercial hybrid 'Oriente' and standard Portuguese traditional 'Vagem Rajada'): the commercially-available 'Aintree' and 'White Emergo' (TozerSeeds) and the Portuguese landrace 'Feijão de 7 anos'. Plants grafted onto each rootstock, and non-grafted or self grafted plants (controls) were grown in two separate glasshouse trials: site PV under conventional commercial management and site PL under low-input management. Plants were grown for a full cropping cycle, in completely randomised plots, replicated three times in PL and four in PV. Soil samples were collected to extract, identify and quantify PPN genera. In site PV, populations of the most abundant PPN, *Pratylenchus* and *Heterodera*, were significantly smaller in grafted than in control plants, with minimal numbers in the rhizosphere of plants grafted onto 'Feijão de 7 anos' ($P < 0.05$). In site PL, the most abundant PPN genera were *Tylenchorhynchus*, *Pratylenchus* and *Xiphinema*. Population densities of these nematodes were not always significantly smaller in grafted plants. The most successful rootstock differed depending on the grafted cultivar and PPN genus considered. Yield could not be correlated with nematode density in either site. Grafting is a promising technique for sustainably granting PPN resistance to crops, but the biotic and abiotic factors that modulate its success in field conditions needs further research.

Keywords: *Phaseolus coccineus*; *Phaseolus vulgaris*; *Pratylenchus* sp.; protected culture; rootstock.

Survey of potato cyst nematode populations in Great Britain for sustainable crop management

Katarzyna J. Dybal-Lima¹, Ivan G Grove¹, Simon G Edwards¹, Vivian C Blok² and Matthew A. Back¹

¹ Crop and Environment Sciences Department, Harper Adams University, Newport, Shropshire, TF10 8NB, UK

² The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

corresponding author: kdybal-lima@harper-adams.ac.uk

The potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are destructive pests of potato crops. Estimates show that potato cyst nematodes cause annual crop losses of approximately £25.9 million. Development of potato genotypes with the *H1* resistance gene have proven to be highly effective in controlling *G. rostochiensis* but at the same time increase the risk of selection of *G. pallida*, which is more challenging to manage. Most recent survey identified *G. pallida* as being the dominant species and indicated that the species distribution of potato cyst nematodes has been changing. The objective of this research was to provide an insight of the current potato cyst nematodes occurrence and distribution. A stratified survey was conducted in England and Wales based on total potato growing area and 423 soil samples from ware potato fields were collected. Deoxyribonucleic acid was extracted from samples containing nematode cysts and an independent qPCR-based assay was performed to confirm species presence. Additionally data from a national annual survey for 68 fields was provided by Animal and Plant Health Agency and included in the study. The survey showed that potato cyst nematodes were present in 48% of sites sampled and of the populations found, 89% were *G. pallida*, 5% were *G. rostochiensis* and 6% contained both species. The results showed a decrease in the incidence of potato cyst nematodes compared with the previous survey and confirm the continuous species shift towards *G. pallida* as the predominant species. Understanding the species composition and factors affecting species variability is highly beneficial for the development and preservation of resistant varieties. Furthermore, the survey samples may generate other pertinent information about the virulence and viability. Survey data for Scotland provided by Science and Advice for Scottish Agriculture will be included and an up-to-date potato cyst nematodes distribution map for Great Britain will be produced.

Keywords: Distribution map; *Globodera*; identification; management.

Novel method for phenotyping yam for nematode resistance

Yao Adjiguita Kolombia^{1,4}, Abiodun O. Claudius-Cole², Nicole Viaene^{3,4}, Lava P. Kumar¹, Wim Bert⁴ and Daniel L. Coyne⁵

¹ IITA, International Institute of Tropical Agriculture, PMB 5320, Oyo Road, Ibadan, Nigeria

² Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria

³ ILVO, Institute for Agricultural and Fisheries Research, 9820 Merelbeke, Belgium

⁴ Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent Belgium

⁵ IITA, IITA-Kenya, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya

corresponding author: kolombia3@hotmail.com

Yam (*Dioscorea* spp.) is the second most important tuber crop globally and a major staple food in West Africa, providing a valuable source of carbohydrates for over 60 million people from an estimated annual production of 44 million MT. Two nematode genera, *Scutellonema* and *Meloidogyne* are the main nematode pests of yam and are responsible for dry rot disease and galling and/or crazy root syndrome, respectively, on tubers. However, breeding for resistance against these nematodes has been a slow process. One of the major constraints is the lack of a rapid and accurate yam phenotyping procedure for resistance to nematodes. To alleviate this shortcoming, a screen house experiment was conducted to (1) develop a rapid and efficient assessment method using vine cuttings, and (2) characterise the response of “popular landraces” and “promising breeding lines” to yam nematodes. Nematode-free plant material from 26 yam cultivars (7 landraces, 18 improved cultivars and 1 released cultivar), initiated from vine cuttings were established in 1 l nursery bags. At 4 weeks after planting, plants were inoculated with *M. arenaria*, *M. enterolobii*, *M. incognita*, *M. javanica* and *S. bradys* and evaluated for nematode damage and reproduction at 8 weeks after inoculation. For *M. arenaria*, *M. javanica*, *M. incognita* and *M. enterolobii* 88, 58, 50 and 46% of accessions were resistant, respectively, and 46% of accessions were resistant to *S. bradys*. The study determined that vine cuttings can serve as a rapid, flexible screening method which can be used for assessing nematode resistance on yam. Further validation using conventional planting material needs to be carried out to corroborate the findings of the present experiment.

Keywords: Damage index; *Meloidogyne*; reproduction factor; *Scutellonema*; vines.

The effects of root-knot nematodes (*Meloidogyne incognita*, *M. arenaria* and *M. javanica*) initial populations density on plant growth and damage on cucumberSemiha Şeker¹, Sevilhan Mennan¹ and Gökhan Aydınlı²¹ Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey² Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

corresponding author: smennan@omu.edu.tr

Damage level of root-knot nematodes depend on various factors including initial population level of nematode. In this study it was aimed to research the effects of initial population density of three different root-knot nematode species (*Meloidogyne javanica*, *M. incognita*, *M. arenaria*) on plant growth of both susceptible (cv. Mir) and tolerant (cv. Gordion) cucumber plants that are commonly grown in Samsun province, applying 0, 20, 40, 80 and 240 eggs (100 cc soil)⁻¹. Two sets of experiment were performed in the controlled glasshouses; in the first set, plant were grown for 5 weeks and in the second set for 12 weeks after artificial inoculations. Plant growth parameters and yields were determined both sets. In tolerant cucumber variety, the values relating to Reproduction criteria (Ro) of root-knot nematodes in the first set, were the lowest for *M. incognita* at 240 eggs (100 cc soil)⁻¹ inoculum level, whereas they were the highest for *M. javanica* and *M. arenaria*. It was found that the reduction of plant growth started at 20 eggs (100 cc soil)⁻¹ inoculum level for *M. arenaria* in the short term and 40 eggs (100 cc soil)⁻¹ for *M. javanica* at the long term. In summary, *M. arenaria* and *M. javanica* were more harmful than *M. incognita* for all sets, and plant development decreased with increasing inoculums levels of all three species.

Keywords: Cucumber; population density; root-knot nematode.

Host suitability of some weeds to *Meloidogyne arenaria* and *M. ethiopica*

Şeyma Toksöz¹, Sevilhan Mennan¹, Emine Kaya Altop¹, Hüsrev Mennan¹ and Gökhan Aydın²

¹ Ondokuz Mayıs University, Agricultural Faculty, Plant Protection Department, 55139 Samsun, Turkey

² Ondokuz Mayıs University, Vocational High School of Bafra, 55040 Samsun, Turkey

corresponding author: smennan@omu.edu.tr

Many weed plants have been known as a host for different root-knot nematode species. These plants provide the means of survival for nematode populations, which are sources of infection of lots of cultivated plants. *Meloidogyne ethiopica* was recently found in Turkey, whereas *M. arenaria* is well established. However, there is insufficient data about weed hosts for these species. Host suitability of common weed species in Turkey (*Cyperus esculentus*, *C. difformis*, *Taraxacum officinale*, *Xanthium strumarium*, *Euphorbia helioscopia*, *Matricaria chamomilla*, *Conyza caedensis*, *Plantago lanceolate*, *Lolium perenne*, *Mercurialis annua*, *Vicia ludoviciana*) was evaluated for *M. ethiopica* and *M. arenaria* in two glasshouse trials with four replications. Tomato (cv. Falcon) was used as a susceptible host for comparison. Plants were inoculated with 1000 eggs of *M. arenaria* or *M. ethiopica*, and were left to grow for 8 week. The experiment was terminated, and roots of each plant species were evaluated using 0-10 gall index. Eggs were extracted by shaking the roots in 1% NaOCl solution and counted. Egg production and galling differed significantly among weed species. Weed species were classed as poor to good host for the two root-knot nematode species after the experiment. Among weed species, *Cyperus esculentus* was a poor host for both *M. arenaria* and *M. ethiopica*. This is the first research on *M. ethiopica* and some weed species in Turkey.

Keywords: Host; *Meloidogyne arenaria*; *Meloidogyne ethiopica*; weed.

Glance at Dunes of Corrubedo Natural Park, Galicia (NW Iberian Peninsula): free-living marine nematodes (Nematoda) from Ladeira beach

Mario Ayora^{1,2}, Maria Teresa Losada^{1,2}, Diego Carreira^{1,2} and Célia Besteiro^{1,2}

¹ Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Santiago de Compostela, Campus of Lugo, Avda. Ramón Carballo Calero, s/n 27002, Lugo, Spain

² Marine Biology Station of A Graña, University of Santiago de Compostela, Rúa da Ribeira, 1 and 4, 15590 A Graña (Ferrol), Spain

corresponding author: mario.ayora@usc.es

Dunes of Corrubedo Natural Park is situated at the very end of Barbanza Peninsula, Galicia (NW Iberian Peninsula). It is a natural protected area of 1000 ha, in which includes some sand beaches, freshwater and seawater lagoons and marshlands, with a high variety of fauna and flora. Samples were taken in Ladeira beach. It is a fine sand beach of 1000 m length open to the Atlantic Ocean. To extract the marine meiofauna, the sample was washed on a small 63 μm sieve, first with MgCl_2 and then with filtered freshwater. The free-living nematodes were picked up from the rest of the meiofauna using soft forceps. Then, we prepared the slides using glycerol and paraffin and mounted the specimens on it. Once mounted, we scanned the slides using the microscope objectives from 3.2 \times to 40 \times or 100 \times to determine the genus. We found a total of 304 specimens, which belong to 6 different orders, 11 different families and 30 different genera. Some of them were new citations for the checklist of free-living marine nematodes. In conclusion, with the results obtained in this study, the checklist of free-living marine nematodes of Galicia increases by 3 new subfamilies and 13 new genus. Therefore, the actual checklist is composed of 50 genera and 21 species.

Keywords: Corrubedo; free living marine nematodes; Galicia; Nematoda; taxonomy.

Checklist of the free-living marine nematodes of the Iberian Peninsula (N.E. Atlantic)

Celia Besteiro^{1,2}, Mario Ayora^{1,2}, Diego Carreira^{1,2} and Maria Teresa Losada^{1,2}

¹ Department of Zoology and Physical Anthropology, Faculty of Veterinary, University of Santiago de Compostela, Campus of Lugo, Avda. Ramón Carballo Calero, s/n 27002, Lugo, Spain

² Marine Biology Station of A Graña, University of Santiago de Compostela, Rúa da Ribeira, 1 and 4, 15590 A Graña (Ferrol), Spain

corresponding author: celia.besteiro@usc.es

Faunal data related to a specific zoological group are usually scattered in studies of various kinds, such as faunal publications, ecological works, results of oceanographic expeditions, doctoral theses and even grey literature, which hinders the accurate knowledge of the fauna in a particular geographical area. It is also essential to note that in old work either obsolete generic and specific names are used or new species of sometimes questionable validity are described. Therefore, the existence of updated inventories contributes to a better understanding of the species and facilitates the carrying out of zoogeographical and ecological studies. For the preparation of this Checklist we have reviewed all published papers in which species of free-living marine nematodes located on the coasts of the Iberian Peninsula are mentioned. All references to date provide a total of 237 species for the Checklist of Free-Living Marine Nematodes of the Iberian Peninsula. The signposts on the coast of the Iberian Peninsula of each species are collected and ordered chronologically. As for the literature, we have reviewed all work containing citations of free-living Iberian marine nematodes, and also those whose titles suggest that they contain those citations, although it may not be so. Also, the preparation of the present Checklist allowed us to clarify some errors that have been dragging on for some time and make a few clarifications, all based on a review of the original papers.

Keywords: Inventory; Iberian Peninsula; marine nematodes.

Molecular and phylogenetic analysis of *Acrobeles singulus* (Nematoda: Cephalobidae) based on D2-D3 segment of 28S rDNA

Nafise Divsalar¹, Ebrahim Shokoohi² and Hendrika Fourie²

¹ Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

² Unit for Environmental Sciences and Management, Potchefstroom, North West University, South Africa

corresponding author: Ebrahim.Shokoohi@nwu.ac.za

Acrobeles singulus was collected in a forest in northern part of Iran (Nowshahr, Mazandaran province). The species was recognised according to morphological and morphometric characters. For detailed study, the D2D3 segment of 28S rDNA was amplified using specific primers and sequenced. Then, phylogenetic relationships with other closed taxa was studied. The Nblast result showed that studied population resembles American population *A. singulus* (DQ145622) with 98% identity. Phylogenetic analysis using Neighbor Joining methods placed the Iranian population together with the same species (DQ145622) in a group with 99 bootstrap values. The result indicates close relationship of *A. singulus* and *A. complexus*. In addition, genetic distance using Maximum Composite Likelihood showed that Iranian isolate of *A. singulus* has the lowest genetic distance with Bulgarian (0.011; DQ145622) and the highest genetic distance with Spanish isolate (0.152; HM055392).

Keywords: 28S rDNA; *Acrobeles*; nematode; phylogeny.

New data of *Enchodelus signyensis* (Nematoda: Nordiida) from two Antarctic islands

Milka T. Elshishka, Stela S. Lazarova, Georgi A. Radoslavov, Petar I. Hristov and Vlada K. Peneva

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Y. Gagarin Street, 1113 Sofia, Bulgaria

corresponding author: melshishka@gmail.com

Enchodelus signyensis was described by Loof in 1975 when studying Spaull's collections from various islands of Maritime Antarctic and was named after its type locality Signy Island. It is endemic for the Maritime Antarctic and is the only nordiid species present in Antarctica. The species is widespread and have been recovered from different microhabitats – bare soil, algae, moss and grass communities. Here, we present morphological (including SEM), morphometric, postembryonic and sequence data (18S rDNA and the D2-D3 expansion fragment of 28S rDNA) of this species from Livingston and King George Islands along with comments on its taxonomy and distribution. A number of morphological characters, such as the presence of moderately developed cuticularised pieces around oral aperture, peculiarities of pharynx expansion, size and position of posterior pair of pharyngeal nuclei, less complex uterus, and position of posterior ventromedian supplement, showed that this species differs substantially from the other members of the genus *Enchodelus*. Similarly, our molecular analyses supported this finding. In the 28S rDNA-based phylogenetic tree the *Enchodelus* sequences available in the GenBank formed two distinct clusters with *E. signyensis* being a part of a well supported group with two *Pungentus* spp., therefore we hypothesise that the species could be a member of a different genus and its taxonomic position should be reconsidered. Acknowledgements: The present study was supported by the project No 64/27.04.2016, Program for support of young scientists, Bulgarian Academy of Sciences.

Keywords: 18S rDNA; D2-D3 28S rDNA; morphology; SEM; taxonomy.

***Bursaphelenchus luxuriosae*: from Japan to Portugal?**

Maria L. Inácio¹, Filomena Nóbrega¹, Manuel Mota^{2,3} and Paulo Vieira²

¹ INIAV, Instituto de Investigação Agrária e Veterinária. Quinta do Marquês, 2780-159 Oeiras, Portugal

² Lab. Nematologia/ ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora, Portugal

³ Departamento de Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal

corresponding author: lurdes.inacio@iniav.pt

Bursaphelenchus luxuriosae was recently identified in maritime pine trees (*Pinus pinaster*) in Gois, central Portugal. This species belongs to the xylophilus-group and has been identified and reported in Japan alone associated with the cerambycid beetle, *Acalolepta luxuriosa* emerging from *Aralia elata* (Araliaceae). The occurrence of *B. luxuriosae* in Portugal might be seen as unexpected, since members of *Acalolepta* are distributed in the Indomalaya ecozone (Southeast Asia and in Australia), and are found mainly associated with broad-leaved forests. It is difficult to ascertain whether *B. luxuriosae* was introduced or already occurred as a native species in Portugal. This finding could also be an indicator for the introduction of its insect vector, as recently a beetle of this group as been detected in Germany as well. Following the several thousands of pinewood samples processed since the detection of the PWN in Portugal, this constitutes the first report and occurrence of this species in Portugal, and in Europe, including its natural association with a conifer tree. This finding demonstrates the wide dissemination of *Bursaphelenchus* species, and their high plasticity, enabling establishment in different geographic areas and colonisation of different types of host trees. Up to date, *B. luxuriosae* was never related to the induction of pine wilt. However, its status as an introduced or native species to Portugal and Europe is still unknown. Efforts should be stepped up to determine the insect vector for *B. luxuriosae* in Portugal, maintaining close surveillance for the presence of this nematode in samples from the national survey programme.

Keywords: *Acalolepta*; forest nematode; maritime pine; xylophilus-group.

Multi-gene phylogenetics in combination with morphology allow species delineation and DNA barcode based diagnostics within a cryptic *Pratylenchus* species complex

Toon Janssen¹, Gerit Karszen^{1,2}, Valeria Orlando¹ and Wim Bert¹

¹ Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

² National Plant Protection Organization, Wageningen Nematode Collection, P.O. Box 9102, 6700 HC Wageningen, The Netherlands

corresponding author: toon.janssen@ugent.be

Root-lesion nematodes of the genus *Pratylenchus* are an important pest parasitising a wide range of plants including several economically important crops. Currently, the genus comprises more than 70 morphologically defined species. However, the taxonomy of the genus is impeded by the lack of diagnostic characters, morphological interspecific plasticity, and incomplete taxonomic descriptions. In order to complement inconvenient morphological diagnostics with barcoding-based diagnostics a link between morphology and molecules has to be established. We reconstructed a multi-gene phylogeny of clade IV root-lesion nematodes using nuclear ribosomal and mitochondrial gene sequences. A combination of this phylogenetic framework with molecular species delineation, morphometrics, ecological information and sequences from type location material allowed us to clarify long-standing debates about the taxonomic status of *P. penetrans*, *P. fallax* and *P. convallariae*. In these species and several other related *Pratylenchus* species a substantial amount of cryptic biodiversity was recovered, confirming that morphology based identification is cumbersome in this taxonomically confusing genus.

Keywords: Cryptic speciation; phylogeny; *Pratylenchus penetrans* group; species delineation; systematics.

A preliminary survey of nematodes associated with the syconia of *Ficus sur*Natsumi Kanzaki¹ and Meike Kruger²¹ Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan² Department of Genetics, University of Pretoria, Pretoria 0002, South Africa

corresponding author: nkanzaki@affrc.go.jp

Fig (*Ficus* spp.) syconia inhabiting nematodes, which are transported by fig wasps (Agaonidae), have been an interesting system to study the evolution of pathogenicity/parasitism, morphology, and phenotypic plasticity. Thus far, four aphelenchids (*Schistonchus*, *Ficophagus*, *Martininema* and *Bursaphelenchus*), a tylenchid (*Ficotylus*), five diplogastrids (*Parasitodiplogaster*, *Teratodiplogaster*, *Acrostichus*, *Pristionchus* and *Rhabditolaimus*) and a rhabditid (*Caenorhabditis*) have been described from figs, i.e., multiple nematode colonisations must have occurred. However, because the genus *Ficus* comprises more than 700 species worldwide, the global diversity of fig-associated nematodes has not been established yet. Here, we preliminary examined the nematode species associated with *F. sur* in South Africa as the reference for a larger scale survey. Syconia of *F. sur* were collected on 14 Nov. 2015 from trees planted on the campus of the University of Pretoria and examined for their associating nematodes. The isolated nematodes were heat-killed and fixed in formalin or soaked in DESS. The formalin-fixed materials were processed into permanent slides and used for microscopic observation, while the DESS vouchers were casually examined, morphotyped under a microscope and their molecular barcoding loci (near-full-length of small subunit and D2-D3 expansion segments of ribosomal RNA gene and partial code of mitochondrial cytochrome oxidase subunit I) sequenced. Based on the morpho- and genotypes, seven nematode species were recognised, namely, *Bursaphelenchus* sp., *Ficophagus* sp., two *Acrostichus* spp., *Teratodiplogaster* sp., *Pristionchus* sp. and *Rhabditolaimus* sp. Based on morphological characters, *Teratodiplogaster* sp. is probably conspecific to *T. martini* and the others were considered as undescribed species. Proper taxonomic descriptions are necessary for those species to provide basic information for more detailed ecological and evolutionary studies in the future.

Keywords: Diversity; *Ficus*; fig wasp; parasite; phoretic associate.

Three new species *Acrobeloides* n. sp., *Acrobeloides* n. sp. 2 and *Pseudacrobeles* n. sp. from South Korea

Taeho Kim¹, Jiyeon Kim², Haena Kwak² and Joong-Ki Park²

¹ Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, 145 Anamro, Seongbuk-gu, Seoul 02841, Republic of Korea

² Division of EcoScience, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

corresponding author: godspeedtaeho@gmail.com

Acrobeloides n. sp., *Acrobeloides* n. sp. 2, and *Pseudacrobeles* n. sp. are described and illustrated from samples collected from rice, chrysanthemum and potato farmland, respectively, in Gyeongsangnam-do, Republic of Korea. *Acrobeloides* n. sp. is a typical acrobeloid with a swollen metacarpus and lateral lines that extend to the tail end region. However, this species is distinctly different from other acrobeloids species in having lateral lines that range from 1 to 5 in number, three knob-shaped low rounded labial probolae and a conical and pointed tail end with a small spike-shaped mucro. *Acrobeloides* n. sp. 2 and *Acrobeloides* n. sp. are similar to each other, but can be distinguished by total body length, proportion of oesophagus length to total body length, ratio of corpus length to stoma length and location where the lateral lines change in number. *Pseudacrobeles* n. sp. share morphologic characters typical of the genus *Pseudacrobeles* and other pseudacrobeloids, such as three lateral lines that fade out near the phasmid region. However, this new species is distinguishable from other species of the genus by various morphometrics: it has a relatively smaller body size and comparatively shorter corpus length relative to stoma length. Analysis of molecular sequence data from the D2-D3 regions of 28s ribosomal DNA, 18s ribosomal DNA, ITS2-5s-ITS1 region of ribosomal DNA and partial cox1 region of mitochondrial genome for these three new species provide additional evidence to confirm their status as new species.

Keywords: *Acrobeloides*; Cephalobidae; morphology; morphometrics; *Pseudacrobeles*.

***Meloidogyne megadora* - Biochemical, molecular and phylogenetic analyses**

Carla Maleita¹, Ana M. S. F. de Almeida², Nicola Vovlas³ and Isabel Abrantes⁴

¹ CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

² Instituto Piaget, Escola Superior de Saúde de Silves, Enxerim, 8300-025 Silves, Portugal

³ Institute for Sustainable Plant Protection, 70126 Bari, Italy

⁴ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: carlammaleita@hotmail.com

Meloidogyne megadora infects coffee trees, an economically important crop worldwide. This root-knot nematode (RKN) species is regularly confused with other species, such as *M. decalineata*. An accurate identification of *M. megadora* is essential for the development of preventive measures to avoid the dispersion of this pathogen and establishment of efficient and sustainable integrated pest management programs. Coffee plants with large root galls were found in a nursery and in coffee plantations in Democratic Republic of São Tomé and Príncipe. Single egg-mass of one population was used to establish a pure culture on bean. The purposes of this research were to characterise *M. megadora* isolate by biochemical and molecular analyses [random amplified polymorphic DNA (RAPD) and polymerase chain reaction of internal transcribed spacer region between 18S and 28S of ribosomal RNA genes (PCR ITS region)] and to evaluate the genetic diversity and relationships between *M. megadora* and the most common *Meloidogyne* spp. A characteristic non-specific esterases phenotype (Me3), clearly differentiate *M. megadora* from other species found associated with coffee. Three RAPD primers produced specific bands to *M. megadora*, *M. arenaria*, *M. incognita* and *M. javanica*. Molecular analysis of ITS region resulted in amplification product of 700 bp. Phylogenetic relationship between *M. megadora* and several *Meloidogyne* spp. sequences was analysed revealing that *M. megadora* differs from other RKN species. Based on the studies conducted, isozyme analysis remains a useful and efficient methodology for *M. megadora* identification when females are available. Further studies will be needed to convert *M. megadora* differential DNA fragment obtained by RAPD and develop a species-specific sequence-characterised amplified region-PCR assay for its diagnosis based on second-stage juveniles.

Keywords: Democratic Republic of São Tomé and Príncipe; ITS region; RAPD; root-knot nematodes.

A new species of *Laimaphelenchus* associated with *Quercus suber*

Carla Maleita¹, Sofia R. Costa^{2,3} and Isabel Abrantes³

¹ CIEPQPF - Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II, Pinhal de Marrocos, 3030-790 Coimbra, Portugal

² Mountain Research Center (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

³ CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

⁴ CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: carlammaleita@hotmail.com

The genus *Laimaphelenchus* comprises non-pathogenic species mostly associated with moss, algae and lichens on conifers and encompasses 17 valid species. The diagnosis of *Laimaphelenchus* species has been commonly based on presence or absence of vulvar flap and on shape structure of the tail tip. During a field survey of nematodes on cork oak, *Quercus suber*, in Alentejo region, Portugal, two *Laimaphelenchus* isolates were obtained from bark of declining cork oaks and established in *Botrytis cinerea* grown on Malt Extract Agar. The objective of this research was to characterise these isolates by morphological and molecular criteria. They are included in the *Laimaphelenchus* species group without vulvar flap (*L. australis*, *L. heidelbergi*, *L. pannocaudus*, *L. patulus*, *L. phloesini* and *L. pini*), but can be distinguished from those by the tail tip shape structure that have a stalk-like terminus (visible by light microscopy) and three diffuse tubercles with 4-6 finger-like protrusions (visible only by scanning electron microscopy). The mitochondrial DNA region from the cytochrome oxidase subunit I (mtCOI), the D2/D3 expansion segments of the large subunit (LSU) of ribosomal DNA and small subunit (SSU) of ribosomal RNA gene were amplified and sequenced. In phylogenetic analyses, using the D2/D3 expansion segments of LSU, sequences of the *Laimaphelenchus* isolates clustered separately from all *Laimaphelenchus* species with available sequences in Genbank. Nevertheless, they formed a 92% supported clade with *Laimaphelenchus* spp. with vulvar flap. Phylogenetic trees, from Maximum Likelihood method analysis of sequences of LSU and SSU ribosomal RNA gene, were similar, but differed when compared with mtCOI phylogenetic trees. According to the results an undescribed *Laimaphelenchus* sp. was found associated with cork oak.

Keywords: Cork oak; cytochrome oxidase subunit; large subunit of ribosomal DNA; morphology; small subunit ribosomal RNA.

Morphological and molecular characterisation of *Cryptaphelenchus* associated with *Pinus* species in Tunisia

Manel Mejri¹, Luís Fonseca², Joana M. S. Cardoso², Mohamed L. Ben Jamâa¹ and Isabel Abrantes²

¹ INRGREF - National Research Institute of Rural Engineering, Water and Forests, Carthage University, HediKarray Street, 2049 Ariana, Tunisia

² CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

corresponding author: luisbidarra@gmail.com

Pine forests, in Tunisia, correspond to 57.6% of the total forest area and are mainly composed of *Pinus halepensis* (53.7%), *P. pinea* (3.1%) and *P. pinaster* (0.8%). In order to identify the nematofauna associated with the main pine species, wood samples were taken and nematodes were extracted using the tray method. Nematodes of the genus *Cryptaphelenchus* were found associated with the three *Pinus* species. A *Cryptaphelenchus* isolate obtained from *P. pinaster* was established on *Botrytis cinerea* cultures and characterised morphologically, morphometrically and molecularly by cloning and sequencing of the large subunit (LSU) of the rDNA. The morphological and morphometrical diagnostic characters confirm its identity as *Cryptaphelenchus*: Body length short ($\leq 500 \mu\text{m}$), C-shaped not slender ($\alpha \pm 20$); lips rounded forming a cap, slightly offset; stylet delicate ($\leq 10 \mu\text{m}$) with small rounded basal knobs. Females with vulva posterior ($V \geq 75\%$) without vulvar flap; tail short and conical. Males with spicules with prominent and narrow rostrum; bursa absent and tail gradually attenuated to point. The phylogenetic analysis, from multiple sequence alignment between LSU sequences of *Bursaphelenchus*, *Ektaphelenchus*, *Devibursaphelenchus* and other *Cryptaphelenchus* species, with available sequences in GenBank, revealed that the Tunisian isolate clusters together with a Japanese, an Australian and an Iranian *Cryptaphelenchus* sp. isolates, forming a separate phylogenetic group from the other analysed genera. Further morphological (scanning electron microscopy) and molecular studies will be performed to identify the species.

Keywords: Large subunit of ribosomal DNA; morphology; phylogenetic analysis; pine trees.

A tentative new member of seed-gall nematode, *Anguina* sp. (Tylenchidae: Anguinidae) from northern Iran

Mahyar Mobaseri, Majid Pedram and Ebrahim Pourjam

Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

corresponding author: mj_pedram@yahoo.com

Anguina sp., a tentative new species of the genus, causing seed gall formation in foxtail weed plants (*Alopecurus myosuroides*), growing in a garden in Mazandaran province, northern Iran is studied with its morphological and molecular characters. Juveniles of the species were recovered during preliminary analyses of a soil sample. Females, males, eggs and juveniles were recovered from the purple galls during the subsequent studies. The present Iranian population of *Anguina* is characterised by its 1516-2564 μm long obese females irregularly curved after fixation, and having finely striated cuticle, 7-9 μm long stylet, muscular median bulb located at proximal third of the pharynx, prodelphic reproductive system, with the ovary reaching base of pharynx and reflexed once or twice, 72-164 μm body width at vulva, the latter at 85.1-91.2%, 51-77 μm long post vulval uterine sac and 60-80 μm long conical tail. Males of this population are characterised with their slender body and 25-30 μm long tylenchoid spicules. From 13 known species of the genus, nine of them are sequenced for their of internal transcribed spacer region of ribosomal RNA gene, and comprising of the aforementioned sequence of the present Iranian population, revealed it fits to none of them. Morphological comparisons with the rest four species, *Anguina agropyronifloris*, *A. amsinckiae*, *A. tumefaciens* and *A. paludicola* are discussed. The phylogenetic analyses showed this population falling into a clade including *A. agrostis*, *A. funesta*, *A. graminis*, *A. phalaridis* and some unidentified species, with 0.97 Bayesian posterior probability.

Keywords: *Alopecurus myosuroides*; Bayesian; taxonomy.

Morphological and molecular characterisation of two new species of *Bursaphelenchus* intercepted at Ningbo Port, ChinaMunawar Maria¹, Yiwu Fang¹, Xuan Wang¹, Jianfeng Gu² and Hongmei Li¹¹ Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China² Technical Centre, Ningbo Entry-Exit Inspection and Quarantine Bureau, 9 Mayuan Road, Ningbo 315012, Zhejiang, China

corresponding author: lihm@njau.edu.cn

Bursaphelenchus species are mycophagous/plant-parasitic or both, either associated or presumed to be associated with insect vectors and have been considered as a potential threat to cultivated plants, particularly conifers. The economic importance of quarantine species *B. xylophilus* and *B. cocophilus* attracted global attention and more descriptions of new species have been published in recent years, which enriched the taxonomic status of the genus *Bursaphelenchus*. Although molecular characterisation provides quick identification, the species assemblage in the "groups" based on morphological diagnostic characters support the appropriate identification. As *Bursaphelenchus* species can be introduced to exotic places through imported wood materials, the special international regulations have been implemented to restrain its distribution worldwide. During quarantine inspections at Ningbo Port, China, two new species, *B. parantoniae* and *B. saudi*, were intercepted from the wood packaging material imported from Belgium and Saudi Arabia, respectively. The phylogenetic analyses were carried out through 18S, ITS and D2-D2 28S rDNA gene sequences. *B. parantoniae* n. sp. belongs to the *hylobianum*-group and phylogenetically clusters with *B. antoniae*, *B. chengi*, *B. hylobianum* and *B. niphades*. It is characterized by having 2 lateral lines, 7 caudal papillae, 15-17 µm long spicule with flattened cucullus, small vulva flap and female tail conical tail with a hyaline region and terminus bluntly rounded. *B. saudi* n. sp. belongs to the *africanus*-group and is closed to *B. burgermeisteri*, *B. obeche* and *B. paraburgeri*. It is characterised by having 4 lateral lines, 7 caudal papillae, spicule transversely striated with 21-26 µm long, thick vulva flap and female tail hook shaped with rounded tip. Our results suggested that the concomitant use of morphological and molecular characterisation provides better insights in taxonomic and phylogenetic relationships among species of *Bursaphelenchus*.

Keywords: phylogeny; quarantine; taxonomy.

New record of *Trichodorus variabilis* (Triplonchida: Trichodoridae) from Iran and its phylogenetic study

Majid Pedram, Ali Roshan-Bakhsh and Ebrahim Pourjam

Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

corresponding author: majid.pedram@modares.ac.ir, mj_pedram@yahoo.com

During an extensive survey on the occurrence of members of the family Trichodoridae in Iran, three populations of the rare species, *Trichodorus variabilis*, were recovered from northern Iran. Nematodes were extracted from soil using the tray method and transferred to pure glycerin. Males of the three recovered populations are similar to the males described in original description of the species and are characterised by having body length of 661-831, 511-866 and 614-869 μm , onchiostyle length of 58-69, 51-62 and 55-59 μm , spicules 43-48, 40-48 and 44-45 μm , two cervical papillae posterior to onchiostyle base and anterior to excretory pore, three precloacal supplements, the first supplement being located anterior to retracted spicules, the latter (spicules) with wide manubrium gradually merging with a slender and equally developed calomus and well developed gubernaculum. Few bristles were also observed in the middle of spicules of some males. Females are also similar to females described in original description, and are characterised by position of their excretory pore at the level of anterior end of pharyngeal bulb, location of lateral body pores behind the vulva and small triangular to rounded vaginal sclerotisations in lateral view. The phylogenetic analyses using partial sequences of 28S rDNA D2/D3 fragments of representatives of all three populations revealed they form a clade with an isolate of the species (JQ716463) from Europe with maximal clade support (1.00 Bayesian posterior probability) in Bayes phylogenetic tree. The three presently sequenced isolates and one previously sequenced European isolate have remarkable variation in sequences of this genomic fragment (28S rDNA D2/D3) and show the species is a complex taxon. The similarity percent of Iranian isolates with the European isolate of the species (JQ716463) was 96.7-97.3%. The similarity percent between Iranian isolates was 98.8-99.7%.

Keywords: 28S rDNA D2/D3; complex species; molecular; *Trichodorus*.

Description of *Lelenchus brevislitus* n. sp. (Nematoda: Tylenchidae) from Iran and its phylogenetic relations with other species in the family

Majid Pedram¹, Mahya Soleymanzadeh¹, Ebrahim Pourjam¹ and Sergio Álvarez-Ortega²

¹ Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

² Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus 'Las Lagunillas' s/n, Edificio B3, 23071-Jaén, Spain

corresponding author: majid.pedram@modares.ac.ir, mj_pedram@yahoo.com

Lelenchus brevislitus n. sp. is described and illustrated based on morphological, morphometric and molecular data. The new species is characterised by having females with slender and 452-488 μm long body, lateral field vestigial as a plain simple band under scanning electron microscopy (SEM) in vulva region and absent in proximal and distal body ends, cephalic region short, ca 2 μm high, conical and truncate in anterior end in lateral view under SEM, dorso-ventrally flattened, amphidial pouches pocket-shaped and large under light microscopy (LM), their apertures short (ca 1 μm long in SEM) longitudinal sinuous slits, remarkably wide near small oral disc, significantly narrowing posteriad, slender and 6.5-8.0 μm long stylet, developed median bulb with distinct valvular plates, monodelphic-prodelphic reproductive system having a short postvulval uterine sac (PUS), transverse vulva ($V = 47.5-51.7$) and bearing small lateral membranes at each side of vulval slit, long filiform female tail (154-170 μm , $c = 2.8-3.1$, $c' = 21.5-28.3$), and males unknown. The new species is morphologically and morphometrically very similar to *L. leptosoma*, but can be distinguished from it in its cephalic region morphology, amphidial openings characters, lateral field characters, and basic differences in sequences of nearly full length 18S and 28S rRNA genes. Morphological comparisons with other species of the genus are discussed. Phylogenetic studies were performed for further characterisation of the new species, and the results using nearly full length 18S rDNA data revealed the new species forming a clade with an isolate of *L. leptosoma*, both of which in a sister clade with an isolate of *Sakia*. In reconstructed phylogenetic tree using partial 28S rDNA sequences, the new species formed a monophyletic group again with *L. leptosoma*, and a sister clade to some *Malenchus* spp.

Keywords: 18S rDNA; 28S rDNA D2/D3; Gilan province; *Lelenchus leptosoma*.

Elements for a cladistic classification of the Dorylaimida: lip region and amphids

Reyes Peña-Santiago

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

corresponding author: rpena@ujaen.es

Leaving aside its primary subdivision into two suborders, Nygolaimina and Dorylaimina, the classification of the Dorylaimida shows many enigmas, with their superfamilies and most families probably being artificial (para- or polyphyletic) groups. Molecular data are providing fundamental information about the matter, but both the number of taxa and genes hitherto sequenced are not enough to elucidate the internal relationships of dorylaimid genera with accuracy. Cladistic principles and techniques using morphological characters have been applied to analyse the classification of particular taxa, for example the families Actinolaimidae and Longidoridae. However, no general cladistic study covering the whole order Dorylaimida is available yet. This contribution presents and discusses several features of the dorylaimid lip region and amphids that are susceptible of cladistic analysis. Eight of them concern the lip region: i) general demarcation (offset → continuous); ii) symmetry (hexaradial → biradial); iii) anterior margin (flat → distinctly depressed or sucker-like / elevated or cap-like); iv) expansion (not expanded → visibly expanded or disc-like); v) nature of lips (separated → fused); vi) oral field (wide → reduced); vii) perioral area (non-differentiated → differentiated); and viii) oral aperture (circular → transverse slit). Two of them refer to amphids: ix) position (postlabial → labial); and x) aperture (transverse slit → pore-like). The polarity (plesiomorphic → apomorphic state) of each character is established and its taxonomical weight to characterise species, genera and families is discussed.

Keywords: Dorylaims; features; morphology; polarity; systematics.

New isolate of *Meloidogyne enterolobii* from South Africa

Milad Rashidifard¹, Ebrahim Shokoohi¹, Hendrika Fourie¹, Mieke Daneel² and Mariette Marais³

¹ Unit for Environmental Sciences and Management, North West University, Potchefstroom, South Africa

² Agricultural Research Council - Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa

³ Nematology Unit, Biosystematic Division, Agricultural Research Council, Plant Protection Research Institute, (ARC-PPRI), Roodeplaat, Queenswood 0121, South Africa

corresponding author: 27216179@nwu.ac.za

During a survey on *Meloidogyne* in Nelspruit (South Africa), different isolates of *M. enterolobii* were studied using morphological and molecular characters. The morphological characters of those isolate of *M. enterolobii* fit well with those studied previously in term of perineal pattern. Molecular analysis of *M. enterolobii* based on sequences of the 28S rDNA placed them in a group (76% bootstrap values) with the same species from USA (KP901079) and China (JN005869; JN005868; KT354576). In addition, phylogenetic analysis based on the sequence of COI placed them together with *M. enterolobii* from China (JX683714; KM887151) and Kenya (KT936633) in a well-supported clade (99% bootstrap values).

Keywords: 28S rDNA; COI; guava; *Meloidogyne enterolobii*; South Africa.

Status of *Tylenchulus semipenetrans* in Iran

Milad Rashidifard, Ebrahim Shokoohi, Hendrika Fourie and Gerhard Du Preez

Unit for Environmental Sciences and Management, North West University, Potchefstroom, South Africa

corresponding author: 27216179@nwu.ac.za

Tylenchulus semipenetrans causes the most yield losses in citrus cultivated areas in Iran. To date, citrus nematode has been reported from southern and northern parts of Iran. During a study on plant-parasitic nematodes in the citrus orchards of southern part of Iran, *T. semipenetrans* was isolated from 90% of soil and root samples (1350 out of 1500 of soil samples). Molecular identification using D2-D3 segment of 28S rDNA showed that there was a difference of only six base pairs between Iranian populations and American population (JN112250; 98% identity) and a difference of five bp from the South Korean population (FJ969715; 99% identity). Phylogenetic analysis using Maximum Likelihood method, revealed that the Iranian populations of *T. semipenetrans* places in a well-supported group (89% bootstrap values) with other molecularly identified populations of the same species. Distribution map of *T. semipenetrans* in Iran is given.

Keywords: 28S rDNA; citrus; distribution; Iran; *Tylenchulus semipenetrans*.

Phylogenetic relationships of *Paratrichodorus teres* (Trichodoridae) as revealed by analyses of the 18S and 28S rDNA sequence data

Katarzyna Rybarczyk-Mydlowska¹, Krassimira Ilieva-Makulec², Tadeusz Malewski¹, Grażyna Winiszewska¹, Łukasz Flis¹, Anna Tereba¹ and Katarzyna Kowalewska¹

¹ Museum and Institute of Zoology Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

² Institute of Ecology and Bioethics, Kardynał Stefan Wyszyński University, Wóycickiego 1/3, 01-938 Warsaw, Poland

corresponding author: katarzynar@miiz.waw.pl

Due to its ability to transmit plant viruses *Paratrichodorus teres* is recognised as an economically important trichodorid species. Partial 18S and 28S rDNA fragments from the Polish *P. teres* representatives were acquired and analysed. Bayesian phylogenetic analysis based on the 18S rDNA sequences confirmed the close positioning of *P. teres*, *P. allius* and *P. porosus* and suggested a high relatedness of the Polish *P. teres* populations to those originating from The Netherlands. A comparison of the 28S rDNA data from the Polish populations with the only *P. teres* 28S rDNA sequence available (from Iran; GenBank), showed a significant sequence variability (9.3%). Although the variation between these sequences was higher than in case of many other pairs of Trichodoridae species, they grouped together in a well-supported cluster. Interestingly, *P. porosus* 28S rDNA sequence has not been positioned in this clade, whilst *P. allius* 28S rDNA data was not available. Relatedness of the Polish and Dutch *P. teres* populations and the detected differences between the Polish and Iranian *P. teres* populations have been confirmed in a detailed morphological study. The main divergences concern the shape and structure of the walls of *pars proximalis vaginae* and also the shape of the rectum.

Keywords: Iran; Poland; stubby-root nematodes; virus vector nematodes.

Phylogenetic position of *Helicotylenchus dihyстера* from South Africa using 28S rDNA

Ebrahim Shokoohi, Milad Rashidifard and Hendrika Fourie

Unit for Environmental Sciences and Management, North West University, Potchefstroom, South Africa

corresponding author: 27216179@nwu.ac.za

During a project on *Helicotylenchus* phylogeny in South Africa, *H. dihyстера* was identified morphologically and molecularly in Potchefstroom from the rhizosphere of *Cupressus* sp. In relation to the morphological characters, this population fits well with the other population studied previously. The D2-D3 region of 28S rDNA was amplified to study its phylogenetic position. Nblast result showed a close relationship with *H. dihyстера* from China (KF443217, KF486503; 98% identity). Phylogenetic analysis revealed that the population studied is placed together with *H. dihyстера* from USA (HM014244; HM014255). In addition, the phylogenetic tree based on Maximum Likelihood indicated that *H. dihyстера* forms a well-supported clade (99% bootstrap values).

Keywords: 28S rDNA; *Helicotylenchus dihyстера*; phylogeny; South Africa.

Distribution and identification of potato cyst nematodes from Ain Defla region, Algeria

Nadia Tirchi¹, Aissa Mokabli¹, Alberto Troccoli², Francesca De Luca² and Elena Fanelli²

¹ University Djilali Bounaama of Khemis Miliana, Theniet El Had Road, Ain Defla, Algeria

² Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche (CNR), Via Amendola 122/D, 70126 Bari, Italy

corresponding author: tirchin1977@yahoo.fr

Potato cyst nematodes (PCN) are the most economically damaging pest of potato crop worldwide. During 2013, a survey was carried out in Ain Defla region of Algeria. 81 soil samples collected from potato fields of 14 localities were submitted to nematological analysis, which revealed the presence of these nematodes in 22.22% of the prospected fields. Sixteen PCN populations from five localities were characterised by combination of features of the perineal regions of cysts and those of second-stage juveniles. The morphological identification has been confirmed by the analysis of the ITS-RFLP profiles, sequencing and phylogenetic analysis of the ITS region. The results revealed that the two species *Globodera rostochiensis* and *G. pallida* are present in this region occurring separately or in mixed populations. However, we noted a dominance of *G. pallida* since only 12.25% of the populations have been identified as *G. rostochiensis*, whereas 31.5% were *G. pallida* and 56.25% of the populations consisted of a mixture of the two species and among these mixed populations, 77.77% were dominated by *G. pallida*. The predominance of *G. pallida* has been noted in the sites of Ain Defla, El Amra, Mekhatria and Arib. *G. rostochiensis* was dominant in Rouina. Intraspecific variation was noted between populations of *G. rostochiensis* and *G. pallida*. Because of the high divergence among Algerian populations of *G. pallida* and *G. rostochiensis* it can be assumed that they were multi-introduced in Algeria. The most divergent population of *G. pallida*, that formed a well separated group with some populations from Chile and Peru, suggests a later or independent introduction of this population in Algeria.

Keywords: Ain Defla; Algeria; distribution; identification; potato cyst nematodes (PCN).

New insights in the characterisation of *Pratylenchus* species with description of two morphotypes of *P. bolivianus* Corbett, 1983 and their phylogenetic relationships with closely related species

Alberto Troccoli¹, Sergei A. Subbotin^{2,3}, John J. Chitambar², Toon Janssen⁴, Lieven Waeyenberge⁵, Jason D. Stanley⁶, Larry W. Duncan⁷, Paula Agudelo⁸, Gladis E. Múnera Uribe⁹, Javier Franco¹⁰ and Renato N. Inserra⁶

¹ CNR, Istituto per la Protezione Sostenibile delle Piante, Via G. Amendola 122/D, Bari 70126, Italy

² Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832, USA

³ Center of Parasitology, A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia

⁴ Ghent University, Department of Biology, Research Group Nematology, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

⁵ ILVO, Institute for Agricultural and Fisheries Research, Plant Crop Protection, Burg. Van Gansberghelaan 96,9820Merelbeke, Ghent, Belgium

⁶ Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, P.O. Box 147100 Gainesville, FL 32614-7100, USA

⁷ University of Florida, IFAS, Citrus Research and Education Center, Department of Entomology Lake Alfred, FL 33850-2299, USA

⁸ Department of Entomology, Soils and Plant Sciences, Clemson University, Clemson, SC 29634, USA

⁹ Corporación Colombiana de Investigación Agropecuaria – CORPOICA, km 7 Via Las Palmas, Vereda Llanogrande, Rionegro (Antioquia), Colombia

¹⁰ Fundación de Producción e Investigación de Productos Andinos (PROINPA), Proyecto MIP, Casilla Postal 4285, Cochabamba, Bolivia

corresponding author: alberto.troccoli@ips.cnr.it

Plant-parasitic nematodes are one of the major constraints to economic crop production. Their accurate identification is essential for diagnostic purposes and the implementation of appropriate control strategies. However, the conserved morphology of nematodes, as well as the discovery of cryptic species, hampers the reliability of species identification. Evidences of the occurrence of cryptic species, which complicates species identification, have been found in several nematode taxa, including plant-parasitic nematodes. Within the genus *Pratylenchus*, species complex have been ascertained for *P. hippeastri* and *P. coffeae*, two damaging species of regulatory relevance. The recent finding for the first time of two morphotypes in *P. bolivianus*, genetically identical, but differing morphologically and biologically, complicates further the separation of *Pratylenchus* species by morphological analyses. Amphimictic populations with numerous males occurring on declining ferns in Florida and Costa Rica are shown to be morphological variant of *P. bolivianus* toptype (parthenogenetic with males absent) from Bolivia. These populations differed not only in their reproductive habits, but also in having different morphological and morphometric traits, such as configuration of the lip patterns, body size, tail shape, and spermatheca dimension. Results of morphological and molecular analysis of these two morphotypes of *P. bolivianus* are presented and illustrated in detail, together with a phylogenetic study, which confirmed that they were genetically identical and formed a highly supported clade in the majority consensus trees.

Keywords: Molecular identification; morphometrics; phylogeny; *Pratylenchus* morphotypes.

Plant genetic background increasing the efficiency and durability of major resistance genes to root-knot nematodes can be resolved into a few resistance QTLs

Arnaud Barbary¹, Caroline Djian-Caporalino¹, Philippe Castagnone-Sereno¹,
Nathalie Marteu¹, Ariane Fazari¹, Bernard Caromel² and Anne-Marie Sage-Palloix²

¹ INRA, Université de Nice-Sophia Antipolis, CNRS, UMR1355-7254, Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

² INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, CS 60094, 84140 Montfavet Cedex, France

corresponding author: caporali@sophia.inra.fr

With the banning of most chemical nematicides, the control of root-knot nematodes (RKN) in vegetable crops is now based essentially on the deployment of single, major resistance genes (R-genes). However, these genes are rare and their efficacy is threatened by the capacity of RKN to adapt. In pepper, several dominant R-genes are effective against RKN, and their efficacy and durability have been shown to be greater in a partially resistant genetic background. However, the genetic determinants of this partial resistance were unknown. QTL analysis was performed on the F2:3 population from the cross between Yolo Wonder, an accession considered partially resistant or resistant, depending on the RKN species, and Doux Long des Landes, a susceptible access. A genetic linkage map was constructed from 130 F2 individuals, and the 130 F3 families were tested for resistance to the three main RKN species, *Meloidogyne incognita*, *M. arenaria* and *M. javanica*. For the first time in the pepper-RKN pathosystem, four major QTLs were identified and mapped to two clusters. The cluster on chromosome P1 includes three tightly linked QTLs with specific effects against individual RKN species. The fourth QTL, providing specific resistance to *M. javanica*, mapped to pepper chromosome P9, which is known to carry multiple NBS-LRR repeats, together with major R-genes for resistance to nematodes and other pathogens. The newly discovered cluster on chromosome P1 has a broad spectrum of action with major additive effects on resistance. These data highlight the role of host QTLs involved in plant-RKN interactions and provide innovative potential for the breeding of new pepper cultivars or rootstocks combining quantitative resistance and major R-genes, to increase both the efficacy and durability of RKN control by resistance genes.

Keywords: *Capsicum annuum*; major resistance; *Meloidogyne* spp.; quantitative resistance; resistance durability.

Preliminary data on genetics and mapping of grapevine resistance from a muscadine source to *Xiphinema index*

Guillaume Lalanne-Tisné¹, Jean-Pascal Tandonnet¹, Ulysse Portier², Cyril Van Ghelder², Maria Lafargue¹, Nathalie Ollat¹ and Daniel Esmenjaud²

¹ EGFV, Bordeaux Sciences Agro, INRA, Université de Bordeaux, 33140 Villenave d'Ornon, France

² ISA, INRA, Université de Nice-Sophia Antipolis, CNRS, 06900 Sophia Antipolis, France

corresponding author: daniel.esmenjaud@sophia.inra.fr

The dagger nematode *Xiphinema index* vectors Grapevine fanleaf nepovirus (GFLV), the first grapevine virus worldwide. Resistance to *X. index* for grape rootstock breeding is a promising research alternative to the removal of highly toxic chemical nematicides. In the muscadine species *Muscadinia rotundifolia*, accession NC184-4 is today one of the best sources for resistance (R) to *X. index*. Despite the difficult hybridisation between the genera *Vitis* and *Muscadinia*, a backcross progeny of over 50 individuals could be obtained between the F1 R source VRH8771 (= *V. vinifera* × NC184-4) and the *V. vinifera* cv. Cabernet-Sauvignon (CS). Inheritance of resistance to *X. index* (non-viruliferous individuals) is being evaluated using a one-year test for the selection of durable R factors. The approx. 7 R : 1 S segregation observed in this cross suggests the hypothesis of three dominant and independent R factors. From the genetic map in progress for the cross VRH8771 × CS, detection of markers linked to resistance at three chromosomal locations fits this hypothesis. This might be an example of polygenic inheritance conferred by major dominant genes towards a migratory ectoparasitic nematode species. If the genetic hypothesis is confirmed, future studies will aim at deciphering the putative effects of the combination/pyramiding of R factors on both the durability of resistance to *X. index* and the correlative resistance/delayed infection to GFLV.

Keywords: Grapevine fanleaf nepovirus (GFLV); *Muscadinia rotundifolia*; vector nematode; *Vitis vinifera*.

How the entomopathogenic nematode overcomes the insect defences?Duarte Toubarro and Nelson Simões

Centro de Biotecnologia dos Açores, Universidade dos Açores, 9501-855 Ponta Delgada, Portugal

corresponding author: duartetoubarro@uac.pt

Steinernema carpocapsae is an entomopathogenic nematode in a wide variety of insect species, used as biological control agent. This entomopathogen is known to invade the insect by natural openings, invade the insect haemocoel, where they deplete the insect's defence systems and produce numerous virulence factors. Our studies conducted in the Lepidoptera *Galleria mellonella* raise an interesting possibility that serine protease inhibitors produced by this nematode could take over key pathways in insect innate immunity. A BPTI-Kunitz family inhibitor (*sc-ku-4*) and a serpin-like inhibitor (*sc-srp-6*) are both up-regulated in the pathogen during invasion and installation; therefore, we presumed their role in the regulation of host serine proteases. Recombinants Sc-KU-4 and Sc-srp-6 were produced in *Escherichia coli* and biochemically characterised. Sc-KU-4 inhibited chymotrypsin and elastase activities and Sc-SRP-6 exhibited inhibitory activity against trypsin and α -chymotrypsin. Studies in the interaction with host proteins clarified the role of these inhibitors in parasitism. Sc-SRP-6 interact with insect humoral proteins apolipoprotein, hexamerin and trypsin-like, which are required for clotting, inhibiting the formation of hardened clots and impairing wound sealing and encapsulation of the parasite. Differently, the Sc-KU-4 targeted insect immune recognition proteins such as masquerade-like and serine protease-like protein homologues, inhibiting haemocyte aggregation and keeping the clot fibres sparsely distributed in the haemolymph, thus avoiding the entrapment of foreign bodies. Our studies help to broaden the understanding of insect defences and could provide new weapons for biological control of insects.

Keywords: Entomopathogenic nematode; host-pathogen interaction; insect defences.

Identification of avirulence genes in *Globodera pallida* as tools for assessing durability of resistance

Kyriakos Varypatakis¹, Vivian C. Blok¹, Peter J.A. Cock¹ and John T. Jones^{1,2}

¹ The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

² School of Biology, University of St Andrews, St Andrews KY19 9ST, UK

corresponding author: kyriakos.varypatakis@hutton.ac.uk

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are severe pests of potato causing damage valued at over £50 million per year in the United Kingdom (UK). Both species include different genotypes (*i.e.*, pathotypes) that have originated from different sites in South America where it is presumed that have been independently introduced from into Europe in the 19th century along with wild potato material. The major resistance gene *H1* has been used to control *G. rostochiensis* which has led to a predominance of *G. pallida* in the UK. Therefore, the development of efficient and sustainable control measures against *G. pallida* is crucial. Two wild potato species, *Solanum vernei* and *S. tuberosum* spp. *andigena* have been widely used in PCN resistance breeding programmes. Continuous rearing of *G. pallida* field populations on partially resistant potato lines allowed selection for increased reproduction. These studies therefore provided a series of nematode populations selected for enhanced virulence against these two resistance sources. The current project aims to identify avirulence (*Avr*) genes from *G. pallida* that are recognised by resistance genes located in quantitative trait loci (QTL) from these sources. The selected populations originate from the field populations Newton and Farcet (unselected). Screening of the Farcet unselected and selected populations on different potato lines with different resistance sources confirmed differences in their reproductive ability, and the selected sub-populations showed higher reproductive ability compared to the original unselected Farcet population. Another series of screening tests including the Newton and Farcet unselected and selected populations is underway (April 2016). Capture arrays, sequencing of the effector complements from the selected populations will follow in order to identify candidate avirulence effectors by relating polymorphisms to changes in virulence and these will be validated in functional assays.

Keywords: Effectors; potato cyst nematodes; selection pressure; virulence.

Assessment of uncertainty of qualitative PCR for *Globodera* spp. detection

Maria L. Inácio, Clara Fernandes and Eugénia Andrade

INIAV - Instituto de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras, Portugal

corresponding author: lurdes.inacio@iniav.pt

The analysis procedure to identify *Globodera pallida* and *G. rostochiensis* by a biomolecular approach, described under the PM 7/40(3) appendix 3A, can only be used on nematodes morphologically identified as *Globodera* spp. as some cross reactions may occur with *Heterodera* spp. cysts. This method was internally adapted to qualitative SYBRGreen real-time PCR in order to reduce the response time of the laboratory and to increase the sensitivity. SYBRGreen chemistry was chosen because it is also important to detect *Heterodera* nematodes in addition to *Globodera* spp., it is less expensive than TaqMan chemistry and allows for post-amplification verification of specificity. Method transferability directly generated quantitative results (Ct values and melting temperature of the amplicon) rather than qualitative, which were, indirectly, used to assign a qualitative answer (presence/absence/undetermined). The new method was validated determining the optimal primer concentration, PCR efficiency and coefficient of determination, sensitivity (LOD), repeatability and reproducibility. The latter could be calculated from the standard deviation of reproducibility and from the uncertainty. Uncertainty of the Ct values and of the T_{melting} were obtained from the reproducibility test. The new method was shown to be very useful to detect three species of cyst nematodes in one PCR. It saves time and reduces PCR-to-PCR variation.

Keywords: Detection; method validation; PCR uncertainty; potato cyst nematodes; SYBRGreen.

Molecular analyses for the identification of *Meloidogyne* species on strawberry in Korea

Heonil Kang¹, Namsook Park², Donggeun Kim² and Insoo Choi^{1,2}

¹ Department of Plant Bioscience, Pusan National University, Miryang, Korea

² Nematode Research Center, Life and Industry Convergence Research Institute, Pusan National University, Miryang, Korea

corresponding author: ichoi@pusan.ac.kr

This study has been conducted to identify root-knot nematode (RKN), *Meloidogyne* spp., from strawberry in Korea using molecular analyses. Strawberry plants showed localised stunting and galled roots. Molecular analyses of COII/lrRNA, 28S rDNA D2-D3 segment and internal transcribed spacer (ITS) region were employed. Polymerase Chain Reaction (PCR) amplification of COII/lrRNA region produced a single fragment ca 528 bp. Restriction digestion of the amplified PCR products with DraI enzyme produced two fragments at 200 and 250 bp, which indicated that this nematode is *Meloidogyne hapla*. 28S rDNA D2-D3 segment and ITS were cloned and sequenced. 28S rDNA D2-D3 segment and ITS region produced a single fragment of 1004bp and 560bp, respectively. In the comparison using BLAST search in Genbank, DNA sequences of this nematode accord with those of other known *M. hapla*. All of the RKN samples tested in this study were *M. hapla*. Our results suggest that the dominant species of RKN on strawberry in Korea is *M. hapla*.

Keywords: 28S rDNA D2-D3 segment; COII/lrRNA; internal transcribed spacer; root-knot nematode; strawberry.

DNA-barcoding of Swiss soil nematodes

Sebastian Kiewnick, Urs Büchler, Irma Roth and Juerg-Ernst Frey

Agroscope, National Competence Center for Nematology, 8820 Wädenswil, Switzerland

corresponding author: sebastian.kiewnick@agroscope.admin.ch

Barcoding has grown into an important tool for reliable taxon identification and for research in biodiversity and molecular ecology. A detailed analysis of nematode assemblages and biodiversity at the species level provides a solid base for developing specific nematode bioindicators that enable identification of anthropogenic stress factors for soil health. This project aimed at establishing baseline data originating from a single field site to i) analyse the nematode diversity in a soil with a high species diversity and ii) establish the SSU rDNA and mitochondrial COI barcode sequence for the most abundant species. Soil nematodes were extracted and from the obtained nematode suspension, 300 specimen were hand-picked and high-resolution multifocal images were generated followed by DNA extraction. The second half of the suspension was subdivided into samples of approx. 5000 specimen each, followed by DNA extraction. For individual specimen, Sanger sequencing was used for the amplified SSU and COI regions. For the complex DNA sample, 454 pyrosequencing technology was used to perform SSU rDNA amplicon sequencing to identify all nematode species or genera which occur at a frequency of above 0.1% in the respective sample. Overall, 1220 sequences were generated resulting in 198 COI and 63 SSU sequences that fulfill the criteria for submission to the BOLD database. Species allocation was done by comparison to available databases, but only for SSU could species be assigned due to available reference sequences. COI sequence data revealed a higher number of potential species due to higher PCR success rate. Pyrosequencing of the short SSU fragment (approx. 400bp) generated 65000 reads with sufficient quality, which were assembled to unique sequences (682 contigs) representing potential species. Of these 682 contigs, 261 SSU sequences with the desired length and quality could be used for species assignment showing a higher diversity compared to single specimen sequencing.

Keywords: Biodiversity; diagnostic; pyrosequencing; species.

***Xiphinema brevicolle*: identification and notes on its distribution**

Stela Lazarova¹, Claudio M.G. Oliveira², Thomas Prior³, Shesh Kumari⁴ and Vlada K. Peneva¹

¹ Institute of Biodiversity and Ecosystem Research - BAS, 2, Y. Gagarin Street, 1113 Sofia, Bulgaria

² Instituto Biológico, Caixa Postal 70, CEP 13001-970, Campinas, SP, Brazil

³ Plant Pest & Disease Programme, O2FA05, Fera, Sand Hutton, York, YO41 1LZ, UK

⁴ Division of Plant Health, Crop Research Institute, Drnovská 507, Ruzyně, 16106 Prague 6, Czech Republic

corresponding author: stela.lazarova@gmail.com

Xiphinema americanum - group consists of plant-parasitic nematode species, which have a global distribution, some of them known to transmit nepoviruses that cause substantial economic damage to a wide range of crops. The identification of species belonging to this group is difficult even for experienced nematologists and the taxonomic status of many species is still controversial. Their morphology is very conservative, morphometric characters often overlap, and the existing keys do not always allow species differentiation and identification. During the last decade, the wide application of DNA sequencing revealed the existence of a number of cryptic species with few studies relating the genetic diversity with detailed morphological data. In this study the variability of selected ribosomal and mitochondrial DNA markers from populations of *X. taylori* - subgroup from Bulgaria, the Czech Republic and Slovakia was studied and compared with genetic data from the type population of *X. brevicolle* from Brazil, together with morphological characterisation of both species. They have very similar morphometrics and some workers consider *X. taylori* as a junior synonym of *X. brevicolle*, whilst others recognise its validity. The presence of *X. brevicolle* in Europe is discussed. Acknowledgements: The authors are grateful to MZe-RO0414, ANIDIV2 and STACCATO projects for supporting this presentation.

Keywords: Cryptic species; mitochondrial DNA; ribosomal DNA; *Xiphinema taylori*.

Direct determination and quantification of the sugar beet cyst nematode *Heterodera schachtii* in soil samples using TaqMan real-time PCR

Marie Maňasová¹, Miloslav Zouhar¹, Jana Wenzlová¹ and Ondřej Douša²

¹Czech University of Life Sciences, Faculty of Agrobiolgy, Food and Natural Resources, Department of Plant Protection, Kamycka 129, 165 21, Prague 6, Czech Republic

²Crop Research Institute, Drnovská 507/73, 161 06 Prague 6, Czech Republic

corresponding author: marie.manasova1@gmail.com

The sugar beet cyst nematode (BCN), *Heterodera schachtii*, belongs to the group of cyst forming nematodes. The extraction of cysts from a soil sample is usually the first step in diagnostic process. The soil sample has to be dried before the extraction, because it is essential for rapid cyst floating and easy sedimentation of soil particles in the Fenwick can. The most common methods for BCN identification are morphometric techniques which are time-consuming and require an expert nematologist. Extracted cysts can be also determined by conventional PCR. In this work we focused on direct detection of BCN in soil samples without prior cyst extraction. The application of this technique significantly decreases the time of analysis and also increases the accuracy of diagnostics. The main important step is the homogenisation of samples. We optimised this step using an oscillation mill and liquid nitrogen freezing. The total DNA was extracted from soil samples using a commercial isolation kit which makes the technique ideal for commercial use. The sequence of heat shock protein was used for design of species-specific primers and double-labelled TaqMan probes. The specificity of reaction was tested on non-target organisms that are known to be present in soil samples originating from sugar beet cultivation fields. This technique can be widely used, because its specificity and sensitivity can bring fruitful results in short time and farmers can easily decide which variety of sugar beet will be suitable for particular field. We have to remember that the actual information about nematode infestation and infectious pressure is required for profitable produce of sugar beet. Acknowledgment: This work was supported by project of Technology Agency of the Czech Republic no. TA04021117.

Keywords: *Heterodera schachtii*; quantification; real-time PCR; specific primers; sugar beet cyst nematode; TaqMan probe.

Sedentary endoparasitic nematodes in the Czech Republic – detection survey

Kateřina Mikuřková, Vladimír Gaar, Kateřina Tománková and Václav Čermák

Central Institute for Supervising and Testing in Agriculture, Division of Diagnostics, Šlechtitelů 773/23, 779 00 Olomouc, Czech Republic

corresponding author: katerina.mikuskoval@ukzuz.cz

Plant-parasitic nematodes, especially cyst- and root-knot nematodes, are a major problem in modern agriculture and cause diseases in all crops of economic importance. The presence of sedentary endoparasitic nematodes was also proved in the Czech Republic, where six genera - *Heterodera*, *Globodera*, *Meloidodera*, *Meloidogyne*, *Punctodera* and *Sphaeronema* were confirmed. Cyst-forming nematodes are represented by the genera *Heterodera*, *Globodera* and *Punctodera*. The genus *Heterodera* has been represented by the following four species - *H. avenae*, *H. latipons*, *H. schachtii* and *H. humuli*, which were all officially identified in the Czech Republic by 2013. In our detection survey of plant-parasitic nematodes (2013 - 2016) two species of cyst nematodes new to the region were found - *H. ripae* and *H. trifolii*. The occurrence of species belonging to the genus *Globodera* has been represented up to now by two species only – the quarantine potato cyst nematodes *G. pallida* and *G. rostochiensis*. *Punctodera punctata*, the grass nematode, was detected in meadows in the southern part of the Czech Republic. *Sphaeronema alni* was found on the roots of *Alnus glutinosa* in several locations throughout the Czech Republic, unlike *Meloidodera alni*, which has been found only in one location in the northern part of our republic, also on the roots of *A. glutinosa*. The majority of *Meloidogyne* species live in tropical regions; however, some of them are spread worldwide or are native to temperate zones. Two species, *M. incognita* and *M. hapla*, have been known in the Czech Republic well before 2014. *M. incognita* has been found only in glasshouses, unlike *M. hapla* which has been detected in several vegetable fields as well as in glasshouses throughout the country. The third species of root-knot nematodes, *M. naasi*, was detected during the identification of an unknown pest in the turf of golf courses in 2015.

Keywords: Cyst nematodes; economic importance; meadow; root-knot nematodes; turf.

First record of *Rotylenchus fragaricus* (Tylenchida: Hoplolaimidae) in Serbia

Violeta Oro, Nenad Milovanovic and Violeta Petrovic

Institute of Plant Protection and Environment, 11000 Belgrade, Serbia

corresponding author: viooro@yahoo.com

The spiral plant-parasitic nematode genus *Rotylenchus* contains a cosmopolitan group of nematodes that is predominantly found in temperate regions. They parasitise a wide-range of hosts, including vegetables, ornamentals, and fruit and forest trees. *Rotylenchus robustus* was reported as pathogenic to spruce, pine, olives, peas, carrots and lettuce. Little is known about the damage of other species of the same genus. In 2014, a strawberry producer from Belasica, the central part of Serbia, was concerned over sudden wilting of strawberry plants in his field. The soil samples together with plants were taken and investigated in the Nematology laboratory of the Institute of Plant Protection and Environment. The root system of strawberry plants as a whole was poorly developed and mostly necrotised. The samples revealed, beside other nematode species, a monosexual spiral nematode population. Morphologically, the nematodes had cylindrical body that sometimes formed a spiral shape even alive in water suspension, a truncate head with massive stylet and large basal knobs. Oesophageal glands overlapped intestine on dorsal side and a short tail was wider than long. The morphometrics of females fitted well with the original description of *R. fragaricus*. Genomic DNA was extracted from female nematodes and the internal transcribed spacers (ITS) region was amplified with TW81 and AB28. Performing a BLAST search, the obtained sequence of the ITS region (GenBank Accession No. KR002682) confirmed species identification with 98% homology with the only *R. fragaricus* available in the database. This is to our knowledge, the first record of *R. fragaricus* on strawberries in Serbia. However, beside the *R. fragaricus* in the soil samples, there were other plant-parasitic nematode species (*Meloidogyne* sp., *Pratylenchus* sp.) that could create synergistic disease complex and large-scale damage.

Keywords: Monosexual; spiral nematode; strawberries.

Distribution, identification and molecular characterisation of *Xiphinema index*, *X. pachtaicum* and *X. italiae* (Nematoda: Longidoridae) occurring in vineyards of Thrace Region, Turkey

Lerzan Ozturk¹, Gurkan Guvenc Avci¹ and Ibrahim Halil Elekcioğlu²

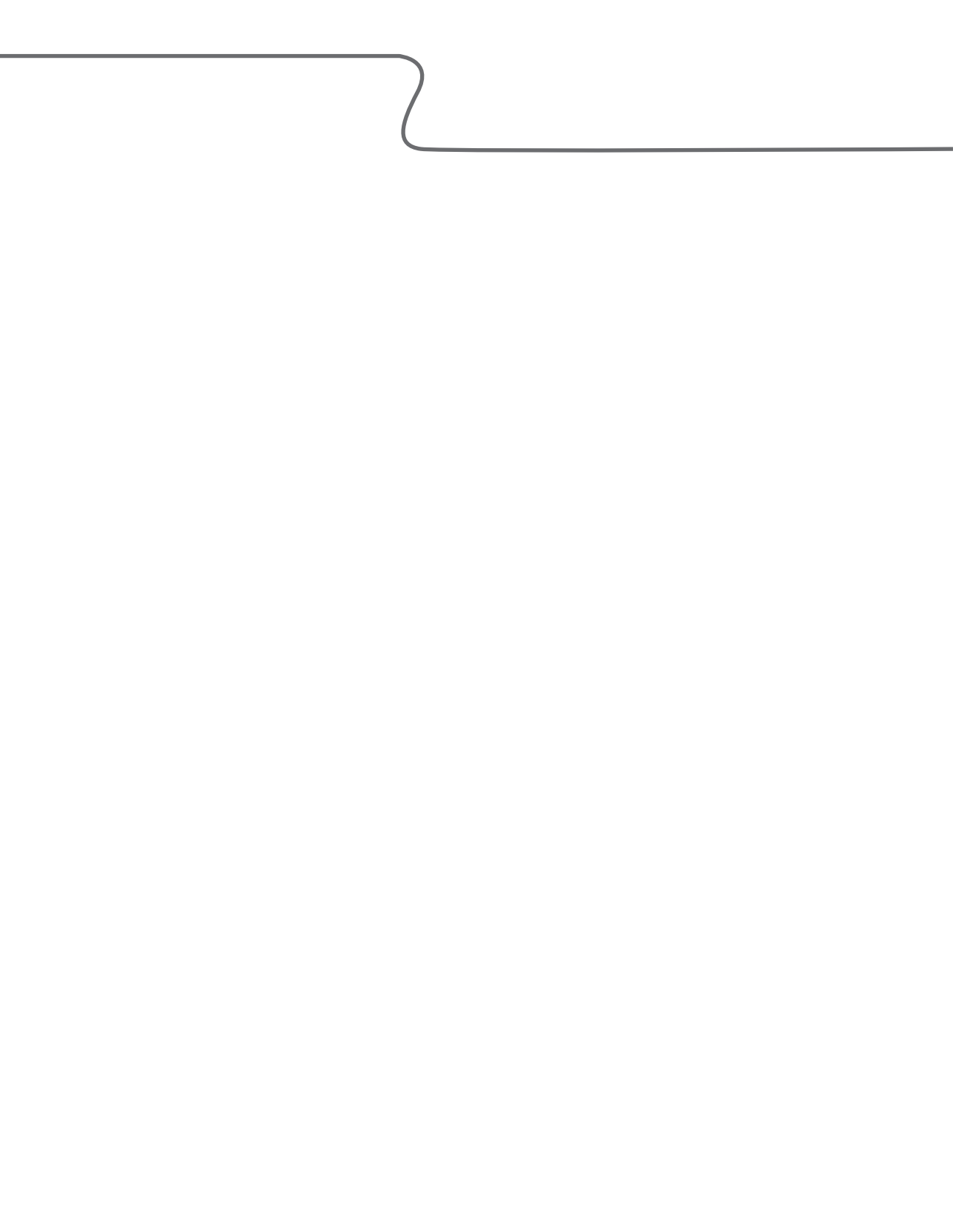
¹ Viticulture Research Station, Tekirdag, 59100 Suleymanpasa, Tekirdag, Turkey

² Plant Protection Department, Faculty of Agriculture, Cukurova University, 01330 Balcali, Saricam, Adana, Turkey

corresponding author: lerzanzoturk@hotmail.com

Xiphinema pachtaicum, *X. index* and *X. italiae* were highly distributed nematode Longidorid species in cultivated areas in the world. Both *X. index* and *X. italiae* are vectors of Grapevine Fanleaf Virus (GFLV) nepovirus, an important virus disease of vineyards. These nematode species were found in various plantations in different parts of Turkey, but very little is known about the occurrence and distribution of these longidorid nematodes in viticultural areas of northwestern Thrace Region. A survey was carried out in vineyards of Edirne, Kirklareli and Tekirdağ districts in northwestern part of Turkey to evaluate the occurrence and distribution of *Xiphinema* species in grape growing areas. A total of 249 soil samples were collected from soils near rhizosphere of grapevines at two different soil depths (0-30, 30-60 cm). Populations of *X. index*, *X. italiae* and *X. pachtaicum* collected from vineyards were described morphologically and molecularly. PCR reactions were established with the following primers: ITS1-S, P28S (Vrain *et al.*, 1992); A-ITS1, I27, ITA26 (Wang *et al.*, 2003) that amplify the complete ITS region (ITS1, 5.8S and ITS2). In addition, three primer sets amplifying 18S, ITS1 and 28S regions were designed (XindexR/XindexR; XpachF/XpachR; XitaF/XitaR) and used in diagnostic tests. Among *X. spp.* *X. pachtaicum* was most frequent, present in 80% of the sampled area and the density of nematode up to 0-30 cm and 30-60 cm soil depth was recorded as 30 and >30 specimens (200 g soil)⁻¹, respectively. Distribution rate of virus vector nematodes *X. index* and *X. italiae* were found as 12% and 1.2% with a lower density (1-10 specimens (200 g soil)⁻¹ at 30 cm soil depth). PCR diagnostic tests using published and designed primers and DNA from a single female nematodes of each species yielded amplification products with expected lengths. PCR and specific primers enabled successful identification of *X. index*, *X. Italiae* and *X. pachtaicum* isolates from the region.

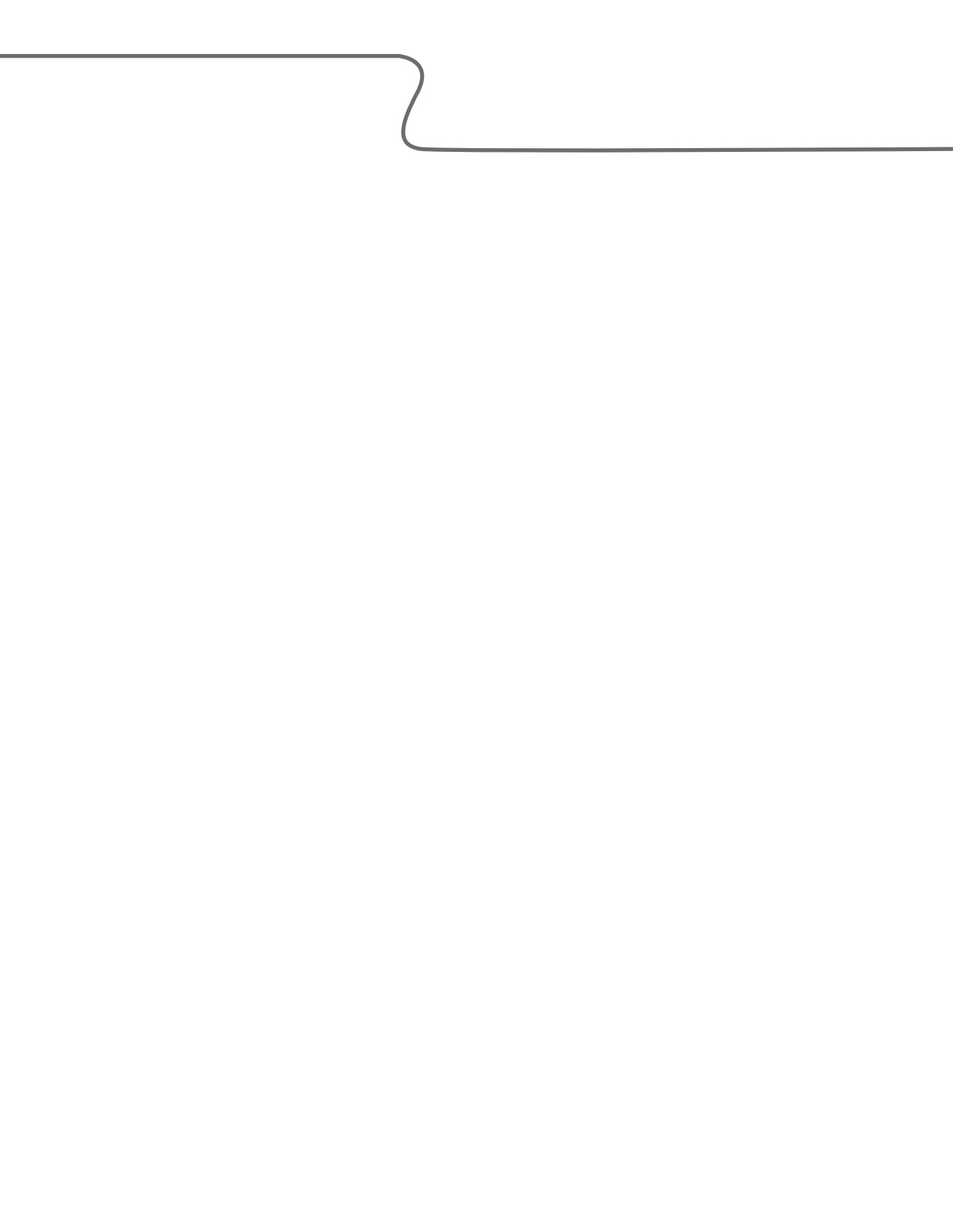
Keywords: Grapevine; longidorids; soil depths; species-specific primers.





Rua do Souto and Chafariz
do Largo do Paço (1723)

AUTHOR INDEX AND LIST OF PARTICIPANTS



A

Aardt, Willie J. van	207
Abad, Pierre	43, 62, 141, 174, 327
Abdel-Razek, Atef S.	259
Abelleira, Adela	183, 184, 274
Abelleira-Sanmartin, Andrea	183, 184, 274
Abolafia, Joaquín	169, 242
Abrantes, Isabel	29, 30, 153, 185, 187, 188, 208, 220, 230, 254, 258, 285, 348, 349, 350
AbuJaleel, Gharam	246
Adamtey, Noah	269
Affokpon, Antoine	83
Agudelo, Paula	361
Aguin, Olga	274
Ahmadinejad, Nahal	142
Ahmed, Mohammed	176
Ahrens, Christian	328
Akar, Zeki Mutlu	282
Akashi, Ryo	227
Albar, Juan Pablo	148
Albarrán, Mar	112
Albuquerque, Erika V. S.	327
Ali, Nadine	321
Alkader, Mariam Alabed	71
Almeida, Ana M. S. F. de	348
Almeida, Maria Teresa	295, 310
Almeida-Engler, Janice de	62, 121, 172, 237
Altop, Emine Kaya	339
Álvarez-Ortega, Sergio	169, 354
Amora, Deisy	121
Anaraki, Fahime Taheri	92
Anderson, Eric	96
Andrade, Eugénia	366
Andres, Maria Fe	238, 247
Anjam, Muhammad Shahzad	126, 216, 292, 296
Anjo, Sandra I.	30
Anwer, Muhammad Arslan	126
Archidona-Yuste, Antonio	138

Arcos, Susana C.	29, 148, 301
Arefin, Badrul	76
Arita, Tetsuya	52
Ashrafi, Samad	35
Ater, Mohamed	321
Atkinson, Howard J.	129
Aury, Jean-Marc.	141
Avci, Gurkan Guvenc.	239, 373
Aydınlı, Gökhan.	86, 105, 159, 248, 334, 338, 339
Ayora, Mario	340, 341

B

Back, Matthew	176, 336
Baimey, Hugues.	121
Bakker, Jaap	61, 115, 124, 127
Baldwin, James G.	85, 168
Balestrini, Raffaella	235, 330
Banaay, Grace.	50
Banakar, Prakash.	144, 147, 312
Banna, Luma Al.	246
Barbary, Arnaud	362
Barbosa, Pedro M.	190
Barcala, Marta.	43, 54, 238
Bardou-Valette, Sylvie.	84
Barg, Mike.	77, 78, 265
Barreto, Carolina.	192
Barroso, José G.	190
Baum, Thomas	320
Becker, Ole	73, 82
Beek, Nikolai Van	270
Been, Thomas	98, 103, 298
Begum, Farhana	275
Behmand, Tohid	229
Bekker, Suria	81, 267
Belda, Jose E.	197, 198
Bell, Christopher A.	129
Beltman, Marieke.	180
Beneventi, Magda	121

Berg, Wim v.d.	117
Berger, Jens	229
Bert, Wim	114, 161, 162, 163, 165, 166, 271, 337, 345
Besnard, Guillaume	321
Besteiro, Celia	322, 323, 340, 341
Bileva, Tanya	308
Binnemans, Isabelle	114
Bird, David	66, 120
Blanc-Matthieu, Romain	141
Blanco-Pérez, Rubén	224, 260
Blaxter, Mark	64, 143, 145, 148, 150
Bleyaert, Peter	283
Blok, Vivian	88, 143, 145, 150, 336, 365
Bode, Helge	23
Bolaños, Patricia	247
Bonifácio, Luís	48, 222
Bonkowski, Michael	136
Boubaker, Hassan	38
Boyes, Ian	145
Bozbuga, Refik	60, 299
Braeckman, Bart	20
Braga, Mara	220, 254
Braga, Roberto	70
Braun-Kiewnick, Andrea	328
Bravo, Maria Antonia	170, 243
Brazil, Dina	261
Bredenbruch, Sandra	249
Bretaudeau, Anthony	175
Brito, L. Miguel	208, 335
Brodeur, Jacques	171
Brown, Tom	116
Bruening, George	41
Bruggen, Anne Sophie van	151
Brus, Dick J.	115
Büchler, Urs	368
Bucki, Patricia	36
Bueno-Pallero, Francisco A.	191, 224, 225, 260

C

Cabrera, Javier	43, 54, 238
Calahorra, Fernando	211
Calvão, Teresa	188
Campos-Herrera, Raquel	132, 224, 260
Cantalapiedra-Navarrete, Carolina	138
Capitão, João	335
Cardoso, Joana M.S.	30, 153, 185, 187, 350
Careche, Mercedes	148
Cares, Juvenil E.	168
Carneiro, Regina M.	153, 327
Caromel, Bernard	362
Carreira, Diego	340, 341
Carrera, Alejandra Centurión	78
Carvalho, Luiz Henrique	322
Carvalho, Marisa	33
Castagnone-Sereno, Philippe	29, 94, 141, 174, 327, 362
Castillo, Pablo	138
Castrillon, Jose Maria Guitian	152
Castro, Yoel	183, 184, 274
Cathcart, Ashley	209
Cazareth, Julie	141
Čepulytė-Rakauskienė, Rasa	41
Čermák, Václav	221, 371
Cha, Soyeon	120
Chang, Kuei-Fu	168
Chappé, Anne-Marie	223
Chapuis, Elodie	321
Chen, Changlong	317
Chen, Shulong	294
Cheng, Xinyue	186
Chim, Rita	254
Chitambar, John J.	165, 361
Chitambo, Oliver	42, 268
Chitwood, David J.	319
Chizhov, Vladimir N.	165
Chochola, Jaromír	194
Choi, Insoo	367

Choi, Young Hwa	189, 290
Chopra, Divykriti	42, 217
Chronis, Demosthenis	44
Chuche, Julien	277
Ciancio, Aurelio	235, 330
Ciordia, Sergio	29, 148
Clarbout, Jolien	283
Clarke, Jihong Liu	315
Claudius-Cole, Abiodun O.	337
Close, Timothy J.	173
Cock, Peter J.A.	32, 123, 145, 150, 365
Coelho, Luísa	191, 225
Coelho, Roberta Ramos	62
Coke, Mirela C.	123
Colagiero, Mariantonietta	235, 330
Cole, Yvonne.	96
Conceição, Isabel Luci	102, 208, 285
Condemarín, Carlos	192
Correia, Otília	188
Cortesero, Anne-Marie.	21
Costa, Sofia R.	119, 208, 295, 310, 335, 349
Cotton, James A	145
Couloux, Arnaud	141
Couvreur, Marjolein	163
Cox, Deborah	39, 209
Coyne, Daniel L.	80, 161, 271, 337
Cranenbrouck, Sylvie	71
Cui, Jiangkuan	106, 315, 329
Cunha, Maria José	153, 285
Curtis, Rosane H.C.	208, 258
Curto, Giovanna	152

D

Dababat, Abdelfattah A.	35, 157, 232, 273, 299
Dalzell, Johnathan.	39, 80, 209, 213, 215
Danchin, Etienne	141, 145, 174, 327
Dandurand, Louise-Marie	228, 313
Daneel, Mieke	81, 267, 281, 302, 356

Daniell, Tim J.	116
Danquah, Wiseborn B.	41
Davie, Kim	96, 154
Davies, Keith	34, 89, 200, 202, 204
Davis, Eric L.	56
De Meester, Nele	113, 130
De Waele, Dirk	50
Declerck, Stephan	71
Decombel, An	283
Decraemer, Wilfrida	66, 131
Deeren, Anne-Marie	155
Deka, Pawel	180
Demeulemeester, Kürt	155
Demissie, Shiferaw.	74
Dereeper, Alexis.	172
Dervišević, Marina M.	278
Derycke, Sofie	113, 130, 131
Desaeger, Johan	71
Desta, Temesgen Addis	74
Devran, Zübeyir.	86, 284
Díaz, Carmen Elisa	247
Díaz-Granados, Amalia	127
Díaz-Manzano, Fernando E.	238
Dìgennaro, Peter	120
Dillon, James.	211
Dìng, Zhong	250
Dionisio, Lidia	224
Divsalar, Nafise	342
Djian-Caporalino, Caroline	94, 362
Dìloughy, Milan	300
Dmowska, Ewa	321
Dobeš, Pavel	76
Dobrev, Petre.	59
Dong, Ke.	165
Doonan, James.	25
Dörfler, Verena	77, 78, 265
Douda, Ondřej	193, 194, 300, 370
Duarte, Aida	313
Duarte, Paulo	192

Dubey, Mukesh	199
Duceppe, Marc-Olivier	145
Dufils, Arnaud	94
Duman, Nagihan	232
Duncan, Larry Wayne	266, 361
Dupont, Pascal	155
Dupuy, Lionel X.	70
Dybal-Lima, Katarzyna J.	336

E

Edwards, Simon G.	336
Egan, Aoife	195, 303
Egas, Conceição	30, 185
Eger, Barbara	59
Ehlers, Ralf-Udo.	74, 77, 78, 93, 265, 277
Eisenback, Jonathan D.	240, 320
Ekesi, Sunday	268
El Mousadik, Abdelhamid.	38, 321
El Oualkadi, Aïcha.	321
Elashry, Abdelnaser	128, 142, 273, 328
Elbakkali, Ahmed.	321
El-Borai, Fahiem E.	266
Elekcioglu, İbrahim Halil	229, 232, 239, 289, 299, 373
Elshishka, Milka T.	179, 308, 343
Engler, Gilbert	62, 237
Eoche-Bosy, Delphine.	175
Erb, Matthias	51
Erginbas-Orakci, Gul.	157, 273
Escobar, Carolina	43, 54, 238
Escobar, Elisabeth	231
Esmenjaud, Daniel.	363
Espada, Margarida	32
Esquibet, Magali	145, 175
Esteves, Ivânia	153, 220, 230, 254, 285
Eugênio, Wilma dos Santos	323
Eves-van den Akker, Sebastian	32, 88, 125, 145
Evlice, Emre.	152
Ezra, David	36

- F**
- Fabiyi, Oluwatoyin A. 196
- Faktorova, Lucie 75
- Fanelli, Elena. 235, 314, 324, 326, 330, 360
- Fang, Yiwu 163, 352
- Faria, Jorge M.S. 190
- Favery, Bruno. 43
- Fazari, Ariane 94, 362
- Feist, Emily 211
- Fenoll, Carmen 43, 54, 238
- Ferji, Zahra 38
- Fernandes, Clara. 366
- Fernandez, Diana 121, 172
- Ferreira, António 335
- Fiaboe, Komi. 268, 269
- Figueiredo, A. Cristina 190
- Filipecki, Marcin. 63
- Filipiak, Anna 219
- Finckh, Maria R. 35
- Finkers-Tomczak, Anna. 61, 127, 145
- Firmino, Paulo N. 188
- Fleming, Colin. 215
- Flier, Wilbert 71
- Flis, Łukasz 358
- Flot, Jean-François. 141
- Flot, Jean-Luc 223
- Foda, Mohamed A. 259
- Foit, Jiří 221
- Folcher, Laurent. 137, 177, 223
- Fonseca, Luís. 30, 185, 187, 188, 220, 254, 350
- Forest, Isabelle. 94
- Fossati, Odile 38, 321
- Fosu-Nyarko, John. 149, 275
- Fourie, Hendrika 81, 245, 267, 302, 342, 356, 357, 359
- Fournet, Sylvain 21, 67, 84, 175
- Fouville, Didier 177
- Francisco, Romeu 33
- Franco, Javier 361
- Freire, Erika. 121

Freitas, Helena	119
Freitas, Vânia Moreira de	210
Frey, Juerg-Ernst	368
Frey, Jürg	328
Fridrich, Pavel	194
Frouz, Jan	309
Fukuda, Kenji	31
Futami, Ricardo	148

G

Gaar, Vladimír	221, 371
Galeano, Magda	197, 198
Galhano, Cristina I.C.	253, 255
Gamel, Sylvie	177
Garcia, Alejandra	238
Garcia, Nathan	137
García-Villarrubia, Carlos	109
Gauthier, Mathieu	175
Gazengel, Kevin	21
Genc, Nimet	286
Gesare, Janet	269
Ghelder, Cyril Van	363
Gheysen, Godelieve	50, 156, 172
Glazer, Itamar	277
Gleason, Cynthia A.	122
Goeminne, Marc	155
Goillon, Claire	94
Gomaa, Ahmed Elhady	287
Gonçalves, Elsa V.	188
Gonçalves, Maria A.	191, 225
González, María Rosa	29
González, Rosa	301
González-Coloma, Azucena	247
González-Muñoz, Micuel	148
Gort, Gerrit	115
Gouzy, Jérôme	141
Goverse, Aska	61, 124, 127
Gracianne, Cécile	67, 84
Graf, Alexander	231

Granados, Amalia Diaz	124
Graora, Draga Đ.	278
Grass, Gregor	47
Grenier, Eric	84, 87, 88, 137, 145, 175, 177
Griffin, Christine	277
Griffiths, Scott	180
Gronseth, Jenni	111
Grossi-de-Sa, Maira	121
Grossi-de-Sa, Maria Fatima	62, 121, 327
Grousset, Fabienne	152
Grove, Ivan G	336
Groza, Mariana B.	179
Grujic, Nikola S.	278
Grundler, Florian M. W.	42, 44, 57, 58, 59, 100, 126, 128, 142, 216, 217, 231, 249, 268, 273, 292, 296
Gu, Jianfeng	352
Guilini, Katja	113
Guillerm-Erckelboudt, Anne-Yvonne	21
Guo, Quanxin	317
Gurung, Reenu	249
Gutiérrez, Savina	192
Gutiérrez-Gutiérrez, Carlos	170, 243
Guy, Julie	141

H

Habash, Samer	128, 142, 231
Haddadi, Fatima	293
Haegeman, Annelies	311
Haegerbaeumer, Arne	304
Haidar, Rana	172
Hallmann, Johannes	206, 287
Hamamouch, Noureddine	56
Hamza, Mohamed Aït	38, 321
Harandi, Majid Fasihi.	90
Harkes, Paula	134
Harnett, Margaret M.	25
Harnett, William	25
Hartley, Sue E.	49
Hasan, Shamim.	42, 217

Hashiguti, Masatugu	227
Haukeland, Solveig	268, 269, 270, 279
Hein, Ingo	24
Heininger, Peter	304
Helder, Johannes	66, 115, 127, 134
Henrissat, Bernard	145
Herren, Gisele L.	114
Heuer, Holger	206, 287
Hodgetts, Jennifer	333
Hofmann, Julia	59
Höfte, Monica	50, 156, 283
Holbein, Julia	57, 58
Holden-Dye, Lindy	40, 211
Holgado, Ricardo	95, 294
Holroyd, Nancy E.	145
Holterman, Martijn	66
Höss, Sebastian	304
Hoysted, Grace A.	49
Hristov, Petar I.	343
Huang, Wenkun.	106, 250, 315, 319
Hudson, Adam	211
Hurley, Mary Jo	261
Hussein, Mona A.	259
Huynh, Bao-Lam	173
Hyršl, Pavel	76

I

Ida, Takanori	227
Ilieva-Makulec, Krassimira	358
Ilyas, Muhammad	231
İmren, Mustafa	232, 289, 299
Inácio, Maria L.	48, 222, 344, 366
Inserra, Renato N.	361
Iqbal, Mudassir	199
Ishida, Takashi.	227

J

Jamâa, Mohamed L. Ben	187, 350
James, Christopher	40

Jan, Pierre-Loup	84
Janssen, Toon	161, 163, 345, 361
Jaubert-Possamai, Stéphanie	43
Jayaswal, Pawan	147
Jensen, Dan F.	199
Jeong, Mungi	233
Jian, Heng.	315, 317
Jiang, Daohong.	319
Jiménez-Ruiz, Yolanda	148, 301
Jin, Soyeong	276
Jones, John T.	32, 123, 125, 145, 212, 365
Jones, Laura M.	108, 145
Jones, Michael.	69, 149, 275
Joos, Lisa	114
Joseph, Soumi	143
Junior, José Dijair A. de S.	237
K	
Kabir, Faisal.	233, 290
Kahraman, Abdullah	229
Kakimoto, Tatsuo	44
Kakouli-Duarte, Thomae	110, 195, 261, 303, 305
Kalinkina, Daryia S.	107, 139, 306, 325
Kamali, Shokoofeh.	92
Kamerhofer, Nina	59
Kamo, Kathryn.	240, 320
Kamra, Anju	200
Kang, Heonil	367
Kanzaki, Natsumi.	31, 135, 346
Kapp, Caro	118
Kapranas, Apostolos	277
Karaca, Mehmet Said.	288
Karanja, Edward	269
Karegar, Akbar	66
Karima, Sabri	236
Karimi, Javad	90, 92
Kariuki, George.	268, 269, 270
Karlsson, Magnus	199
Karssen, Gerrit	66, 151, 152, 161, 163, 176, 345

Kasapoğlu, Ece Börteçine	229
Kasapoğlu, Ece Ece Börteçine	289
Kaushik, Sandeep	147
Keiser, Andreas	252
Kelly, Eileen	305
Kepenekci, İlker	91, 251, 256
Kergunteuil, Alan	53
Kersaudy, Emmanuel	223
Keyser, Ellen De	157
Khadari, Bouchaib	321
Khan, Saad	83
Khot, Sameer	149
Kiewnick, Sebastian	201, 252, 328, 368
Kikuchi, Taisei	31, 133
Kilcrease, James	240
Kim, Donggeun	367
Kim, Jeongeun	233, 290
Kim, Jin Ho	189
Kim, Jiyeon	65, 276, 347
Kim, Taeho	65, 276, 347
Kiriga, Agnes W.	270
Kitagami, Yudai	135
Knox, J Paul	60
Knudsen, Randi	95
Kolombia, Yao Adjiguita	271, 337
Kong, Ligan	315, 319
Kong, WenKun Huang Ling'an	329
Koppenhöfer, Albrecht	92
Korthals, Gerard W.	117
Koutsovoulos, Georgios	64, 145
Kowalewska, Katarzyna	358
Kozlowski, Djampa	141
Kröel-Dulay, György	68
Kruger, Meike	346
Kübler, Daniel P.	263
Kublik, Susanne	33
Kučerová, Lucie	76
Kumar, Kommu Kiran	202
Kumar, Lava P.	271, 337

Kumari, Shesh	179, 369
Kuroda, Ryo.	52
Kwak, Haena.	276, 347
Kwon, OhGyeong	189
Kwon, Soon-Bae	291
Kyndt, Tina	50, 156, 172

L

Labdelli, Fatiha	293
Lachaise, Tom	21
Laetsch, Dominik	143
Laetsch, Dominik R.	145, 150
Lafargue, Maria.	363
Lafond-Lapalme, Joël.	87, 145, 171
Lakhtar, Hicham	38
Lalanne-Tisné, Guillaume.	363
Landa, Blanca B.	138
Landeweert, Renske	180
Lang, Christian	100
Lanterbecq, Déborah	155
Lavrova, Victoria V.	97, 107, 234, 325
Lawson, Rebecca	332, 333
Lazarova, Stela	179, 308, 343, 369
Laznik, Žiga.	262
Lebreton, Lionel.	21
Lee, Chae Min	189
Lee, Dong-Woon	189, 291
Lee, DongWoon.	233, 290
Lee, Jae-Kook	290, 291
Lee, Nam-Gil	291
Lee, Sang Myeong	189
Leelarasamee, Nathanon	122
Lefevre, Amélie	94
Legeai, Fabrice	175
Lennon, John W.H..	108
Leocádio, João C.S.	253, 255
León, Gustavo	192
Leon, Jens	273

Letort, Aude	177
Li, Chunying	56
Li, Hongmei	294, 352
Liao, Jinling	294
Liarzi, Orna	36
Lilley, Catherine	49, 60, 108, 125, 129, 145
Lindo, David	192
Lingan, Kong	106
Linglin, Juliette	21
Link, Peggy	71
Lira, Felipe	301
Lišková, Jana	300
Liu, Jianxi	250
Liu, Jing	315
Liu, Pei	317
Liu, Qian	317
Liu, Rentao	307
Llorens, Carlos	148
Long, Haibo	316
López-Gómez, Manuel	158
Lopez-Llorca, Luis	37
Lopez-Perez, Antonio	109
Losada, Maria Teresa	340, 341
Lozano-Torres, Jose	61, 127
Luambano, Nessie	80
Luca, Francesca De	235, 314, 324, 326, 330, 360
Luimes, Jan	226
Lumb, Felicity E.	25
Lunt, David H.	143
Luo, Lilian	319

M

MacFarlane, Stuart A.	70
Machado, Andressa	327
Machado, Ricardo A.R.	51
Mackiewicz, Jasmina	263
Macleod, Bill	275
Maes, Martine	311

Magliano, Marc	43
Magnusson, Christer	152
Máguas, Cristina	188
Mahlitz, Sina-Valerie	292
Maier, Thomas	320
Maier, Wolfgang	35
Majić, Ivana	203
Malan, Antoinette	118, 281
Maleita, Carla	153, 185, 220, 230, 254, 348, 349
Malewski, Tadeusz	358
Malinouski, Mikalai	228
Manadas, Bruno	30
Mañasová, Marie	300, 370
Mangusson, Christer	95
Mansilla, Pedro	183, 184, 274
Mantelin, Sophie	123, 145
Manzanares-Dauleux, Maria	21
Marais, Mariette	356
Maria, Munawar	352
Markus, Robert	76
Marques, Eder	327
Marteu, Nathalie	94, 362
Martínez, Isabel	238
Martínez, José Luis	301
Martin-Jimenez, Cristina	141
Martín-Lammerding, Diana	112
Martins, Ana	119
Marvin, Jessica M.C.	145
Massawe, Cornel	80
Mateille, Thierry	38, 94, 321, 326
Matera, Christiane	42, 216, 217
Mathes, Pascale	45
Matsuoka, Koichi	249
Matthews, William C.	173
Matuszkiewicz, Mateusz	63
Matveeva, Elizaveta M.	97, 107, 139, 234, 306
Maule, Aaron	213, 215
Maule, Aaron G.	39
Maung, ZinThuZar	111

Mbiro, Alexander	104
McCarthy, James	129
Medina, Clémence	43
Mejri, Manel	187, 350
Melakeberhan, Haddish	111
Melillo, Maria Teresa	218, 235, 330
Mellage, Karina	249
Meller, Roi	307
Mena, María del Carmen	29
Mendy, Badou	57, 58, 231
Mennan, Hüsrev	339
Mennan, Sevilhan	86, 105, 111, 159, 248, 334, 338, 339
Ment, Dana	277
Mezzalira, Itamara	121
Mialhe, Eric	192
Miché, Lucie	38
Mijušković, Nevena	74
Mikušková, Kateřina	371
Miloud, Hammache	236
Milovanovic, Nenad	372
Mimee, Benjamin	87, 145, 171
Mitchum, Melissa G	44
Mitreva, Makedonka	26
Miyara, Sigal Braun	36
Mo, Aisu	244
Mobaseri, Mahyar	351
Moens, Maurice	157, 214
Moens, Tom	113, 130, 131
Mohan, Sharad	89, 200, 202, 204
Mokabli, Aissa	236, 293, 360
Molendijk, Leendert P.G.	99, 103, 117
Molina, Carlos	77, 78, 265, 277
Mollov, Dimitre	165
Monge, Gerardo Alcides Sánchez	163
Montarry, Josselin	21, 84, 145, 175
Monteiro, Luana da Costa	113
Montes-Borrego, Miguel	138
Mooijman, Paul	66, 115
Moon, Yun-Gi	291

Morais, Paula V.	33, 47
Morais, Paula Vasconcelos	102
Moreno, Jesús	197, 198
Morgounov, Alexei	157, 273
Morris, Robert	39
Mota, Manuel	29, 32, 170, 190, 243, 344
Mougel, Christophe	21
Moukhli, Abdelmajid	38, 321
Moura, Luísa	208, 335
Mourão, Isabel	208, 295, 335
Mowery, Joseph	240
Mracek, Zdenek	75
Mulder, Winfried	180
Mulet, Karine	174
Mundo-Ocampo, Manuel	168
Munoz-Amatriain, Maria	173
Musyoka, Martha	269
Mwamula, Abraham O.	290

N

Nadler, Steven A.	65
Nagy, Péter	68
Nahar, Kamrun	156
Nameghi, Hussein Sadeghi	90
Nascimento, Danila C. do	237
Navarrete, Mireille	94
Navas, Alfonso	29, 148, 301
Navas-Cortes, Juan A.	138
Naves, Pedro	48, 222
Naz, Fareeha	69
Ndeve, Arsenio	173
Neilsen, Dahlia	120
Neilson, Roy	70, 116
Nel, Andre	81, 267
Nermut, Jiri	75
Neto, Luis	260
Nguyen, Chau N.	168
Nguyen, Duong Thi Anh.	136
Nguyen, Duong Thi Anh.	169

Nicol, Julie M.	157
Niere, Björn	160
Nijs, Loes J.M.F. den	151, 152, 331
Niu, Junhai	317
Nježić, Branimir M.	203, 278
Nóbrega, Filomena	222, 344
Novákc, Ondrej	44
Nunez, Joe	82
Nyarko, John Fosu	69

O

O'Callaghan, Felicity	70
O'Connor, Vincent	40, 211
O'Tuama, Pdraig	277
Öcal, Atilla	256, 299
Ogbonnaya, Francis	157
Oka, Yuji	72, 272
Okumura, Etsuko	264
Olatunji, Gabriel A.	196
Oliveira, Claudio M.G.	369
Oliveira, Daniel Apolônio Silva de	131
Oliveira, Margarida	180
Ollat, Nathalie	363
Olmo, Rocío	238
Ónodi, Gábor	68
Oota, Morihito	55, 227
Opperman, Charles	66, 83
Oreste, Monica	324
Orlando, Valeria	165, 345
Oro, Violeta	372
Osunlola, Oluremi S.	196
Overall, Andrew D.J.	84
Overmars, Hein	124, 145
Ozcariz, M. Virginia	255
Ozturk, Lerzan	239, 373

P

Palloix, Alain	94
Palomares-Rius, Juan E.	138

Panahi, Hadi	241
Papolu, Pradeep	318
Pares, Laure.	94
Pariyar, Shree	273
Park, Joong-Ki	65, 276, 347
Park, Namsook	367
Patel, Vikas	318
Patil, Jagadeesh.	280
Patten, Cheryl	215
Pawar, Swapnil	200
Pedram, Majid	164, 351, 353, 354
Pedro, Luis G.	190
Peña-Santiago, Reyes	136, 169, 242, 355
Peneva, Vlada	308
Peneva, Vlada K.	179, 343, 369
Peng, Deliang	106, 250, 257, 294, 315, 316, 319, 329
Peng, Huan	106, 315, 319, 329
Pen-Mouratov, Stanislav	307
Pentimone, Isabella	235, 330
Pereira, André R.	255
Pereira, Tiago Jose.	85, 168
Perfus-Barbeoch, Laetitia	141, 174
Perpétuo, Soraia	102
Petit, Eric	67, 84
Petitot, Anne-Sophie.	121, 172
Petrovic, Violeta	372
Phillips, Wendy	145
Pickup, Jon	96, 154
Pimentel, Carla S.	188
Pineda, Miguel A.	25
Piñón, Pilar	183, 184, 274
Pires, David	295
Ploeg, Antoon	73, 82
Poinsot, Denis	21
Porte, Catherine.	21, 67
Portier, Ulysse	363
Postma, Wiebe.	124
Pourjam, Ebrahim	164, 351, 353, 354
Prado, Alicia	183, 184, 274

Preez, Gerhard Du	245, 302, 357
Price, James A.	212
Prigigallo, Maria Isabella	218
Prior, Thomas	152, 176, 332, 333, 369
Proença, Diogo Neves	33, 47, 102
Proft, Michel De	155
Puza, Vladimir	75

Q

Qi, Jiaguo	111
Qiao, Fen	319
Qing, Xue	166, 168
Qiu, Zhuo Q.	244
Quimi, Juan	192
Quist, Casper	66, 115

R

Radakovic, Zoran S.	44, 57, 58, 59, 231, 292, 296
Radicci, Vincenzo	326
Radochová, Petra	309
Radoslavov, Georgi A.	343
Ragsdale, Erik J.	168
Ramireddy, Eswarayy	44
Ramos, Ricardo	148
Rancurel, Corinne	141, 145
Rangasamy, Vijayakumar	280
Rao, Uma	144, 147, 312, 318
Rashidifard, Milad	245, 356, 357, 359
Rasmann, Sergio	53
Reeb, Pablo D.	111
Regis, Mary	59
Reid, Alex	88
Reis, Mário M.	191, 225
Reuther, Marie	100
Riek, Jan De	157
Righi, Kada	293
Robert, Christelle A.M.	51
Roberts, Adrian M. I.	154
Roberts, David M.	116

Roberts, Philip A.	173
Robertson, Lee	29, 148, 301
Rocha, Martine Da.	43, 141, 145, 174
Rödelsperger, Christian	140
Rodgers, David	25
Rodrigues, Ana M.	190
Rodrigues, J. Raul	335
Rodrigues, José M.	48, 222
Rodriguez, Julian	198
Rodriguez, María Victoria	198
Roshan-Bakhsh, Ali	353
Rosso, Laura	235, 330
Roth, Irma	368
Rott, Michael	145
Roussos, Sebastianos	38
Rowley, Jenny	228
Rozanskae, Elzbieta	44
Ruiz, Alicia.	198
Rupnik, Maja	205
Rybarczyk-Mydlowska, Katarzyna	358

S

Sa, Maria Eugênia Lisei de	121
Sabeh, Michael	145
Sage-Palloix, Anne-Marie	94, 362
Sağlam, Hayriye Didem	91, 251, 256
Sajnani, Manisha.	144, 147
Salaheddine, Doumandji	236
Salari, Elham.	90
Salazar, Laura	143
Sallet, Erika	141
Sanadhya, Payal	36
Sánchez-Moreno, Sara.	109, 112
Sannemann, Wiebke	273
Santiago, Luis	198
Santos, Giovanni A.P. dos.	131
Santos, Jansen.	173
Santos, Margarida Teixeira	170, 243

Santos, Maria Clara Vieira dos	208, 258
Sapp, Melanie	176
Sarajlić, Ankica	203
Šarić, Gabriella Kanižai	203
Sarniguet, Corinne.	223
Sawa, Shinichiro	52, 55, 227
Scarcia, Gianluca	324
Schaik, Casper	127
Schausberger, Peter	59
Schiex, Thomas	141
Schleker, Sylvia	249
Schlöter, Michael	33
Schmülling, Thomas.	44
Schöler, Anne	33
Schomaker, Corrie.	98, 103, 298
Schoof, Heiko	142
Schots, Arjen	127
Šeker, Semiha	338
Seres, Anikó	68
Serra, Maria Clara A.	46
Settele, Josef	308
Shah, Syed Jehangir	126, 231
Sharma, Shashi	275
Shehata, Ibrahim E.	259
Shi, Hongli	297
Shimaoka, Chie.	55, 227
Shin, Jin Hee	189
Shokoohi, Ebrahim	241, 245, 342, 356, 357, 359
Siddique, Shahid	42, 44, 57, 58, 59, 126, 128, 216, 217, 231, 273, 292, 296
Sikder, Maniruzzaman	50
Sikora, Richard A.	79
Silva, Ana Cláudia.	32, 54, 238
Silva, Corinne Da	141
Silva, Luena N. da	255
Silva, Maria C.M..	327
Simões, Nelson	22, 277, 364
Şimşek, Şeyda	248

Singh, Divya	318
Širca, Saša	86, 205
Siť, Tim	83
Skrzecz, Iwona	277
Slabbert, Retha	281
Slootweg, Erik	124
Slos, Dieter	162
Smaha, Djamel	293
Smant, Geert	61, 66, 124, 127, 145
Smet, Francis	228
Smith, Ailsa	123
Smith, Terry K.	212
Smucker, Alvin J.M.	111
Sobczak, Mirosław	44, 63, 128, 216
Sommer, Ralf J.	140
Somvanshi, Vishal	144, 147
Sonmezoglu, Ozlem	282, 286, 288
Sousa, Edmundo R.	48, 222
Sousa, Hermínio C. de	220, 254
Srivastava, Arohi	204
Stanley, Jason D.	361
Stare, Barbara Gerič	86, 205
Steel, Hanne	114
Steinberger, Yosef	307
Stejskal, Václav	300
Sterken, Mark	61
Steyn, Willem	281
St-Marseille, Anne-Frédérique Gendron	171
Stoddard, Scott	82
Storelli, Alan	252
Storey, Sheila G.	118
Strauch, Olaf	74, 77, 78, 265, 277
Strnad, Mirosław	44
Sturrock, Matthew	39, 213
Subbotin, Sergei A.	165, 361
Suckling, Colin J.	25
Sugi, Takuma	264
Sumaya, Nanette H Nellas	77, 78, 265
Sun, Fengcheng	167

Sun, Yanfang	316
Sun, Yuhong	257
Sushchuk, Anna A.	139, 306, 325
Susič, Nik	205
Sutter, Nancy de	311
Suzuki, Reira	55, 227
Szakálas, Judit	68
Szitenberg, Amir	143, 145

T

Takeuchi-Kaneko, Yuko	31, 264
Talavera, Miguel	158
Tan, Jo-Anne	149
Tanaka, Ryusei	31, 133
Tanaka, Suguru E.	31
Tandonnet, Jean-Pascal	363
Tarasco, Eustachio	324
Tavares, Daniela	119
Tavoillot, Johannes.	94, 321
Tazi, Hafssa	38
Tchamitchian, Marc	94
Teixeira, Andreia	310
Teklu, Misghina Goitom	98, 298
Temmerman, Pieter-Jan De	104
Tenorio, José L.	112
Tenuta, Mario	167
Tereba, Anna.	358
Teshome, Asmamaw	74
Thakur, Prasoon.	147
Theopold, Ulrich	76
Thoden, Tim	71
Thorpe, Peter	123, 145
Tian, Zhongling	297
Tirchi, Nadia	293, 360
Tkach, Nadia.	272
Toksöz, Şeyma	339
Toktay, Halil.	232, 299
Tománková, Kateřina	371
Topalović, Olivera	206

Torre, Carola M. De La	44
Toubarro, Duarte	277, 364
Toumi, Fateh	157
Traunspurger, Walter	113, 304
Trdan, Stanislav	262
Trisciuzzi, Nicola	324
Troccoli, Alberto	314, 324, 326, 360, 361
Tsai, Allen Yi-Lun	52
Túllume, César	192

U

Udalova, Zhanna V.	234
Umar, Ajeka. K.	196
Upadhyay, Ackanksha	89
Urek, Gregor.	86, 205
Uribe, Gladis E. Múnera	361
Urwin, Peter E.	49, 60, 108, 123, 125, 129, 145

V

Vafeiadou, Anna-Maria	113
van den Elsen, Sven	66, 115
van Schaik, Casper	61
Vandenbossche, Bart	77, 78, 265, 277
Vandevelde, Isabel.	283
Vankova, Radomira	59
Varypatakis, Kyriakos	365
Vaz, Francisco	335
Vazquez, Yirina Valdes	214
Védie, Hélène	94
Venneman, Sofie	283
Vera Cruz, Casiana	50
Verbeek, Ruben	50
Verdejo-Lucas, Soledad	158
Verghese, Abraham	280
Verhaeven, Myrtle	161
Veríssimo, Paula C.	253
Veronico, Pasqua	218, 235, 330
Véronneau, Pierre-Yves	87, 171

Vervoort, Mariette T.W.	66
Vestergaard, Gisle	33
Viaene, Nicole	104, 155, 157, 214, 271, 283, 311, 337
Vieira, Paulo	29, 62, 170, 240, 243, 320, 344
Viketoft, Maria	199
Visser, Johnny H.M.	99, 117
Volckaert, Anneleen	283
von Reuss, Stephan	19
Vovlas, Alessio	324
Vovlas, Nicola	326, 348

W

Waeyenberge, Lieven	157, 311, 361
Wanamaker, Steve	173
Wang, Guo Liang	315
Wang, Meng	186
Wang, Xuan	352
Wang, Zhi	76
Wang'ombe, Mary Wanjiku	57, 58
Warmerdam, Sonja	61, 127
Warnock, Neil	39, 213, 215
Weili, Shazi	316
Wen, Yanhua	257
Wenzlová, Jana	370
Wepener, Victor	302
Wesemael, Wim	104, 152
Wieczorek, Krzysztof	59
Wilbers, Ruud	127
Wiles, John	71
Williamson, Valerie	41, 120, 143
Wilson, Leonie	39, 209, 213, 215
Wincker, Patrick	141
Winiszewska, Grażyna	321, 358
Winkel, Brenda S. J.	56
Wright, Kathryn M.	123
Wu, Hai Y.	244
Wu, Sheng-Yen	266

X

- Xie, Bingyan 186
Xu, Liangxiong 257

Y

- Yavuzaslanoglu, Elif 101, 282, 286, 288
Yimer, Henok Zemene 156
Yosuke, Matsuda 135
Yu, Qing 167
Yusup, Hazijah B. 125, 145

Z

- Zamora, Paula 29
Zasada, Inga A. 320
Zhang, Xi 51
Zheng, Jingwu 294, 297
Zhou, Hong 178
Zhu, Hongxue 297
Zinovieva, Svetlana V. 234
Zipfel, Cyril 57, 58
Zouhar, Miloslav 193, 194, 300, 370
Zurovcova, Martina 75
Žurovec, Michal 76

Abad, Pierre

INRA
Université Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
06903 Sophia Antipolis
France

email: Pierre.abad@sophia.inra.fr

Abdel-Razek, Atef

National Research Centre
Department of Plant Protection
12622 Cairo
Egypt

email: abdelrazek820@yahoo.com

Abelleira, Adela

Estación Fitopatolóxica Areeiro
Deputación de Pontevedra
36153 Pontevedra
Spain

email: adela.abelleira@depo.es

Abelleira-Sanmartín, Andrea

Estación Fitopatolóxica Areeiro
Deputación de Pontevedra
36153 Pontevedra
Spain

email: andrea.abelleira@depo.es

Abolafia, Joaquín

Departamento de Biología Animal, Biología Vegetal y Ecología
Universidad de Jaén
23071 Jaén
Spain

email: abolafia@ujaen.es

Abrantes, Isabel

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: isabel.abrantes@uc.pt

AbuJaleel, Gharam

Department of Plant Protection
School of Agriculture
University of Jordan
11942 Amman
Jordan

email: gharamabujaleel@yahoo.com

Ahmed, Mohammed

Harper Adams University
Newport, TF10 8NB
UK

email: mahmed@harper-adams.ac.uk

Ait Hamza, Mohamed

Université Ibn Zohr
Faculté des Sciences d'Agadir
Laboratoire Biotechnologie et Valorisation des Ressources Naturelles
BP 8106
80000 Agadir
Morocco

email: mohamed.aithamza@edu.uiz.ac.ma

Akanwari, Jerry

Ghent University
9000 Ghent
Belgium

email: jakanwari@gmail.com

Akar, Zeki Mutlu

Karamanoglu Mehmetbey University
Institute of Science
70001 Karaman
Turkey

email: zekimutluakar@gmail.com

Ali Khan, Rifat

Ghent University
9000 Ghent
Belgium

email: rifatali644@yahoo.com

Almeida, Maria Teresa

Centre of Molecular and Environmental Biology (CBMA)
Department of Biology
University of Minho
4710 – 057 Braga
Portugal

email: mtalmeida@bio.uminho.pt

Amand, Olivier

SESVanderHAVE N.V.
3300 Tienen
Belgium

email: olivier.amand@sesvanderhave.com

Ambaw Mequannt, Gebermedihin

Ghent University
9000 Ghent
Belgium

email: gebre87@gmail.com

Andrés, María Fe

Instituto Ciencias Agrarias
Consejo Superior de Investigaciones Científicas
28006 Madrid
Spain

email: mafay@ica.csic.es

Anjam, Muhammad Shahzad

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: shahzad.anjam@uni-bonn.de

Anthoine, Geraldine

ANSES – Plant Health Laboratory
49044 Angers
France

email: geraldine.anthoine@anses.fr

Aparecida Consoli, Erika

Ghent University
9000 Ghent
Belgium

email: erika.consoli@usp.br

LIST OF PARTICIPANTS

Ashrafi, Samad

Julius Kühn-Institut
Institute for Epidemiology and Pathogen Diagnostics
38104 Braunschweig
Germany

email: samad.ashrafi@julius-kuehn.de

Ayamoh Enow, Elsie

Ghent University
9000 Ghent
Belgium

email: ayamelsie@yahoo.com

Aydınlı, Gökhan

Ondokuz Mayıs University
Vocational High School of Bafra
55040 Samsun
Turkey

email: gokhanay@omu.edu.tr

Ayora, Mario

Department of Zoology and Physical Anthropology
Faculty of Veterinary
University of Santiago de Compostela
27002 Lugo
Spain

email: mario.ayora@usc.es

Back, Matthew

Crop and Environment Sciences Department
Harper Adams University
Newport
Shropshire, TF10 8NB
UK

email: mback@harper-adams.ac.uk

Baldwin, James

Department of Nematology
University of California
Riverside, CA 92521
USA

email: james.baldwin@ucr.edu

Baum, Thomas

Department of Plant Pathology
Iowa State University
Ames, 50011
USA

email: trmaier@iastate.edu

Been, Thomas

Plant Research International
Wageningen University
6700 AA Wageningen
The Netherlands

email: thomas.been@wur.nl

Begum, Farhana

Plant Biotechnology Research Group,
WA State Agricultural Biotechnology Centre
School of Veterinary and Life Sciences
Murdoch University
Perth, WA 6150
Australia

email: farhanakbd@gmail.com

Behmand, Tohid

Cukurova University
Faculty of Agriculture
Department of Plant Protection
01330 Balcalı, Adana
Turkey

email: tohid.behmand63@gmail.com

Bell, Christopher

Centre for Plant Sciences
University of Leeds
Leeds, LS2 9JT
UK

email: bscb@leeds.ac.uk

Bert, Wim

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: wim.bert@ugent.be

Besteiro, Celia

Department of Zoology and Physical Anthropology
Faculty of Veterinary
University of Santiago de Compostela
27002 Lugo
Spain

email: celia.besteiro@usc.es

Bird, David

Department of Entomology and Plant Pathology
NC State University
Raleigh NC 27695
USA

email: bird@ncsu.edu

Blanco-Pérez, Rubén

MeditBio University of Algarve
8005-139 Faro
Portugal

email: rbperez@ualg.pt

Blaxter, Mark

Institute of Evolutionary Biology
University of Edinburgh
Edinburgh, EH9 3TF
UK

email: mark.blaxter@ed.ac.uk

Blok, Vivian

The James Hutton Institute
Cell and Molecular Sciences
Invergowrie
Dundee, DD2 5DA
Scotland, UK

email: vivian.blok@hutton.ac.uk

Bode, Helge

Department of Biosciences and Buchmann
Institute for Molecular Life Sciences (BMLS)
Goethe University Frankfurt
60438 Frankfurt am Main
Germany

email: h.bode@bio.uni-frankfurt.de

Bozbuga, Refik

Centre for Plant Sciences
Faculty of Biological Sciences
University of Leeds
Leeds, LS2 9JT, West Yorkshire
UK

email: bsrb@leeds.ac.uk

Braeckman, Bart

Biology Department
Ghent University
B-9000 Ghent
Belgium

email: Bart.Braeckman@UGent.be

Braun Miyara, Sigal

Department of Entomology and the Nematology and Chemistry units
ARO, Volcani Center
50250 Rishon Letzion
Israel

email: sigalhor@volcani.agri.gov.il

Bueno-Pallero, Francisco A.

MeditBio University of Algarve
8005-139 Faro
Portugal

email: fapallero@ualg.pt

Cabrera, Javier

Facultad de Ciencias Ambientales y Bioquímica
Universidad de Castilla La Mancha
45071 Toledo
Spain

email: javier.cabrerachaves@uclm.es

Campos-Herrera, Raquel

MeditBio
University of Algarve
8005-139 Faro
Portugal

email: rcherrera@ualg.pt

Cardoso, Joana

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: joanasa_cardoso@hotmail.com

Castagnone-Sereno, Philippe

INRA
Université de Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
06903 Sophia Antipolis
France

email: philippe.castagnone@sophia.inra.fr

Castillo, Pablo

Institute for Sustainable Agriculture
Department of Plant Protection
Consejo Superior de Investigaciones Científicas (CSIC)
14004 Córdoba
Spain

email: p.castillo@csic.es

Chen, Shulong

Institute of Plant Protection
Hebei Academy of Agricultural and Forestry Sciences
Baoding 071000
China

email: chenshulong65@163.com

Cheng, Xinyue

College of Life Sciences
Beijing Normal University
Beijing 100875
China

email: chengxy@bnu.edu.cn

Chitambo, Oliver

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-Universität Bonn
53229 Bonn
Germany

email: s7olchit@uni-bonn.de

Choi, Insoo

Nematode Research Center
Life and Industry Convergence Research Institute
Pusan National University
50463 Miryang
South Korea

email: ichoi@pusan.ac.kr

Chopra, Divykriti

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-Universität Bonn
D-53115 Bonn
Germany

email: dchopra@uni-bonn.de

Ciancio, Aurelio

CNR - Istituto per la Protezione Sostenibile delle Piante (IPSP)
70126 Bari
Italy

email: aurelio.ciancio@ipsp.cnr.it

Claerbout, Jolien

ILVO (Institute for Agricultural and Fisheries Research)
9820 Merelbeke
Belgium

email: jolien.claerbout@ilvo.vlaanderen.be

Cobacho Arcos, Susana

Museo Nacional de Ciencias Naturales, CSIC
Madrid 28006
Spain

email: scobacho@mncn.csic.es

Coelho, Luísa I.

University of Algarve
8005-191 Faro
Portugal

email: meditbio@ualg.pt

Conceição, Isabel Luci

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: isabelluci@gmail.com

Condemarin, Carlos

Biotecoop
24001 Tumbes
Peru

email: ccondemarin@gmail.com

Costa, Sofia

Centre of Molecular and Environmental Biology (CBMA)
Department of Biology
University of Minho
4710-057 Braga
Portugal

email: sofia.costa@bio.uminho.pt

Cox, Deborah

Medical Biology Centre
Queen's University Belfast
Belfast, BT9 7BL
Northern Ireland
UK

email: d.cox@qub.ac.uk

Coyne, Daniel

International Institute of Tropical Agriculture (IITA)
Kasarani
P.O. Box 30772-00100 Nairobi
Kenya

email: d.coyne@cgiar.org

Cunha, Maria José

CERNAS
Department of Agronomic Sciences
High School of Agriculture
Bencanta
3045-601 Coimbra
Portugal

email: mjcunha@esac.pt

Dalzell, Jonathan

Medical Biology Centre
Queen's University Belfast
Belfast, BT9 7BL
Northern Ireland
UK

email: j.dalzell@qub.ac.uk

Damijonaitis, Arunas Jonas

Bayer CropScience
40789 Monheim
Germany

email: arunas.damijonaitis@bayer.com

Danchin, Etienne G. J.

INRA
Université Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
06903 Sophia Antipolis
France

email: etienne.danchin@sophia.inra.fr

Dandurand, Louise-Marie

Department of Plant, Soil and Entomological Sciences
University of Idaho
83844 Moscow
USA

email: lmd@uidaho.edu

Daneel, Mieke

Agricultural Research Council
Institute for Tropical and Subtropical Crops
Nelspruit, 1200
South Africa

email: Miele@arc.agric.za

Davie, Kim

Science and Advice for Scottish Agriculture
Edinburgh, EH12 9FJ
UK

email: kim.davie@sasa.gsi.gov.uk

Davies, Keith

Department of Biological and Environmental Sciences
University of Hertfordshire
Hatfield, AL10 9AB
UK

email: k.davies@herts.ac.uk

de Almeida-Engler, Janice

INRA, Institut National de la Recherche Agronomique
Université Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
6903 Sophia Antipolis
France

email: janice.almeida-engler@sophia.inra.fr

De Luca, Francesca

CNR - Istituto per la Protezione Sostenibile delle Piante (IPSP)
70126 Bari
Italy

email: francesca.deluca@ipsp.cnr.it

Decraemer, Wilfrida

Department of Biology
Ghent University
B-9000 Ghent
Belgium

email: wilfrida.decraemer@UGent.be

den Nijs, Loes

NPPO
6706 EA Wageningen
The Netherlands

email: l.j.m.f.dennijs@nvwa.nl

Derycke, Sofie

Royal Belgian Institute of Natural Sciences
1000 Brussels
Belgium

email: sofie.derycke@naturalsciences.be

Destá, Temesgen Addis

e-nema
24223 Schwentimental
Germany

email: t.addis@e-nema.de

Devran, Zübeyir

Department of Plant Protection
Faculty of Agriculture
Akdeniz University
07058 Antalya
Turkey

email: zdevran@akdeniz.edu.tr

Diaz Granados, Amalia

Department of Nematology
Wageningen University
6708 PB Wageningen
The Netherlands

email: amalia.diazgranadosmunoz@wur.nl

DiGennaro, Peter

University of Florida
94607 Oakland
USA

email: pdigenn@gmail.com

Di Rubbo, Pasquale
European Commission Directorate
Health and Food Safety
Unit G1 - Plant Health
149 Brussels
Belgium

e-mail: pasquale.di-rubbo@ec.europa.eu

Ding, Zhong

College of Plant Protection,
Hunan Agricultural University
410128 Changsha
China

email: dingzh@hunau.net

Divsalar, Nafiseh

Department of Plant Protection
College of Agriculture
Shahid Bahonar University
Kerman
Iran

email: Nadivsalar@gmail.com

Djian-Caporalino, Caroline

INRA
Université de Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
06903 Sophia Antipolis
France

email: caroline.caporalino@sophia.inra.fr

Douda, Ondřej

Crop Research Institute
Division of Plant Health
16106 Prague
Czech Republic

email: douda@vurv.cz

LIST OF PARTICIPANTS

Du Preez, Gerhard

Unit for Environmental Sciences and Management
North-West University
2531 Potchefstroom
South Africa

email: gc.dpreez@gmail.com

Duarte, Aida

University of Idaho
83844-2339 Moscow
USA

email: aida.damaso@hotmail.com

Dumitru, Mariana

National Phytosanitary Authority
77190 Voluntari
Romania

email: mariana_radoi@yahoo.com

Duncan, Larry

University of Florida
Citrus Research and Education Center (CREC)
Lake Alfred FL 33850
USA

email: Lwduncan@ufl.edu

Dybal-Lima, Katarzyna

Crop and Environment Sciences Department
Harper Adams University
Newport, TF10 7QJ
UK

email: kdybal-lima@harper-adams.ac.uk

Ebrahim, Awol Seid

Ghent University
9000 Ghent
Belgium

email: awolseid07@gmail.com

Egan, Aoife

Molecular Ecology and Nematode Research Group
enviroCORE
Department of Science and Health,
Institute of Technology Carlow
Carlow
Ireland

email: aoife.egan@itcarlow.ie

Ehlers, Ralf-Udo

e-nema GmbH
24223 Schwentimental
Germany

email: ehlers@e-nema.de

Elashry, Abdelnaser

INRES
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-Universität Bonn
Bonn 53115
Germany

email: elashry@uni-bonn.de

EL-Borai, Fahiem

University of Florida
Citrus Research and Education Center (CREC)
Lake Alfred FL 33850
USA

email: Fahiem@ufl.edu

Elekcioglu, Ibrahim Halil

University of Cukurova
Faculty of Agriculture
Department of Plant Protection
01330 Adana
Turkey

email: halile@cu.edu.tr

Elshishka, Milka

Institute of Biodiversity and Ecosystem Research
Bulgarian Academy of Sciences
1113 Sofia
Bulgaria

email: melshishka@gmail.com

Erb, Matthias

Institute of Plant Sciences
University of Bern
3013 Bern
Switzerland

email: matthias.erb@ips.unibe.ch

Escobar, Carolina

Facultad de Ciencias Ambientales y Bioquímica
Universidad de Castilla La Mancha
45071 Toledo
Spain

email: carolina.escobar@uclm.es

Esmenjaud, Daniel

INRA
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
Université de Nice-Sophia Antipolis
6903 Sophia Antipolis
France

email: daniel.esmenjaud@sophia.inra.fr

Espada, Margarida

NemaLab, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)
Universidade de Évora
Herdade da Mitra
7002-554 Évora
Portugal

email: mespada@uevora.pt

Esteves, Ivânia

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: iesteves@uc.pt

Estévez Geffriaud, Virginia

University of Barcelona
8130 Barcelona
Spain

email: geffriaudestevezv@live.com

Evans, Adrian

Imperial College
Apple Tree Cottage, Wellington Avenue
Virginia Water, GU25 4HU
UK

email: a.a.f.evans@imperial.ac.uk

Everaarts, Tjarda

HLB Research and Consultancy in Agriculture
9418 PD Wijster
The Netherlands

email: t.everaarts@hlbbv.nl

Eves-van den Akker, Sebastian

Division of Plant Sciences
College of Life Sciences
University of Dundee
Dundee, DD2 5DA
UK

email: s.evesvandenakker@dundee.ac.uk

Fabiyi, Oluwatoyin

Faculty of Agriculture
Dept. of Crop protection
University of Ilorin
18 Ilorin
Nigeria

email: fabiyitoyinike@hotmail.com

Fanelli, Elena

CNR - Istituto per la Protezione Sostenibile delle Piante (IPSP)
70126 Bari
Italy

email: elena.fanelli@ipsp.cnr.it

Fernandez, Diana

UMR IPME, Institut de Recherche pour le Développement (IRD)
34394 Montpellier
France

email: diana.fernandez@ird.fr

Filipiak, Anna

Institute of Plant Protection - National Research Institute
Department of Biological Pest Control
60-318 Poznan
Poland

email: a.filipiak@iorpib.poznan.pl

Fonseca, Luís

Centre for Functional Ecology (CFE)
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: luisbidarra@gmail.com

Fourie, Hendrika

Unit for Environmental Sciences and Management
North-West University
2520 Potchefstroom
South Africa

email: driekie.fourie@nwu.ac.za

Fournet, Sylvain

INRA – Institut de Génétique Environnement et Protection des Plantes
35653 Le Rheu
France

email: sylvain.fournet@rennes.inra.fr

Freire, Erika

Embrapa Recursos Genéticos e Biotecnologia
70770-917 Brasília
Brazil

email: erika.freire@sophia.inra.fr

Gaar, Vladimír

Central Institute for Supervising and Testing in Agriculture
Division of Diagnostics
779 00 Olomouc
Czech Republic

email: vladimir.gaar@ukzuz.cz

Galeano, Magda

Koppert España, S.L. R&D Departament
04745 La Mojonera (Almería)
Spain

email: mgaleano@koppert.es

Galhano, Cristina

Department of Environmental Sciences
Coimbra Agriculture School
Polytechnic of Coimbra
Bencanta
3045-601 Coimbra
Portugal

email: cicgalhano@esac.pt

Garcia, Nathan

ANSES - Laboratoire de la Santé des Végétaux
35653 Le Rheu
France

email: nathan.garcia@anses.fr

Gelena Kelbessa, Bekele

Ghent University
9000 Ghent
Belgium

email: bekelegelena20@gmail.com

Genc, Nimet

Karamanoglu Mehmetbey University
Institute of Science
70001 Karaman
Turkey

email: nimet_genc91@outlook.com

Gendron St-Marseille, Anne-Frédérique

Université de Montréal
Agriculture et Agroalimentaire Canada
H3L3M9 Montréal
Canada

email: anne-frederique.gendron@outlook.com

Gheysen, Godelieve

Department of Molecular Biotechnology
Ghent University
9000 Ghent
Belgium

email: godelieve.gheysen@ugent.be

Glazer, Itamar

ARO, Volcani Center
Rishon LeZion 7528809
Israel

email: glazerit@agri.gov.il

Gleason, Cynthia

Washington State University
Department of Plant Pathology
Pullman WA 99164
USA

email: cynthia.gleason@wsu.edu

Goelen, Silke

Ghent University
9000 Ghent
Belgium

email: Silke.Goelen@UGent.be

Gomaa, Ahmed

Julius Kühn Institute
Institute for Epidemiology and Pathogen Diagnostics
38104 Braunschweig
Germany

email: ahmed.elhady@fagr.bu.edu.eg

Goverse, Aska

Laboratory of Nematology
Wageningen University
6708 PB Wageningen
The Netherlands

email: aska.goverse@wur.nl

Grenier, Eric

INRA - Institut de Génétique Environnement et Protection des Plantes
35653 Le Rheu
France

email: eric.grenier@rennes.inra.fr

Grossi-de-Sá, Maria de Fátima

Embrapa Recursos Genéticos e Biotecnologia
70770-917 Brasília
Brazil

email: fatima.grossi@embrapa.br

Groza, Mariana

National Phytosanitary Authority
77190 Voluntari
Romania

email: mariana_bonta@yahoo.com

Grujić, Nikola

University of Belgrade
Faculty of Agriculture
11000 Belgrade
Serbia

email: grujic@agrif.bg.ac.rs

Gullotta, Luciano

OMEX Agrifluids Ltd
Norfolk, PE34 3JA
UK

email: lucianog@omex.com

Gutiérrez Gutiérrez, Carlos

NemaLab, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)
Departamento de Biologia
Universidade de Évora
Herdade da Mitra
7002-554 Évora
Portugal

email: carlosg@uevora.pt

Habash, Samer

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: Habash.samer@yahoo.com

Haegerbaeumer, Arne

University Bielefeld
33615 Bielefeld
Germany

email: a.haegerbaeumer@uni-bielefeld.de

Hafez, Saad

University of Idaho
83660 Parma
USA

email: shafez@uidaho.edu

Hamamouch, Noureddine

Polydisciplinary Faculty
University Sultan Moulay Slimane
23030 Beni-Mellal
Morocco

email: hamamouch@yahoo.com

Hammache, Miloud

Ecole Nationale Supérieure Agronomique
16200 El Harrach
Algeria

email: m.hammache@ensa.dz

LIST OF PARTICIPANTS

Harkes, Paula

Laboratory of Nematology
Graduate School of Experimental Plant Sciences
Wageningen University
6702 AB Wageningen
The Netherlands

email: paula.harkes@gmail.com

Harnett, William

Strathclyde Institute of Pharmacy and Biomedical Sciences
University of Strathclyde
Glasgow, G4 0RE
UK

email: w.harnett@strath.ac.uk

Hasan, Md. Shamim

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: s7mdhasa@uni-bonn.de

Haukeland, Solveig

ICIPE - International Centre for Insect Physiology and Ecology
Plant Health Division
00100 Nairobi
Kenya

email: shaukeland@icipe.org

Hein, Ingo

The James Hutton Institute
Invergowrie
Dundee, DD2 5DA
Scotland, UK

email: Ingo.Hein@hutton.ac.uk

Helder, Johannes

Wageningen University
Laboratory of Nematology
6708 PB Wageningen
The Netherlands

email: Hans.Helder@wur.nl

Herren, Gisele

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: gisele.herren@ugent.be

Holden-Dye, Lindy

Centre for Biological Sciences
University of Southampton
Southampton SO17 1BJ
UK

email: lmhd@soton.ac.uk

Holgado, Ricardo

Norwegian Institute of Bioeconomy Research
NO-1431 Ås
Norway

email: ricardo.holgado@nibio.no

Hoysted, Grace Anna

Centre for Plant Sciences
University of Leeds
Leeds, LS2 9JT
UK

email: bs11gah@leeds.ac.uk

Huang, Wenkun

Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Beijing 100193
China

email: wkhuang2002@163.com

Hurley, Mary Jo

Institute of Technology Carlow
R93V960 Carlow
Ireland

email: maryjo.hurley@itcarlow.ie

Hyršl, Pavel

Department of Animal Physiology and Immunology
Institute of Experimental Biology
Masaryk University
611 37 Brno
Czech Republic

email: hyrsl@sci.muni.cz

Ilyas, Muhammad

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: ilyas@mpipz.mpg.de

Imren, Mustafa

Abant İzzet Baysal University
Faculty of Agriculture and Natural Science
Department of Plant Protection
14280 Bolu
Turkey

email: m.imren37@gmail.com

Inácio, Maria Lurdes

INIAV - Instituto de Investigação Agrária e Veterinária.
2780-159 Oeiras
Portugal

email: lurdes.inacio@iniav.pt

Iqbal, Mudassir

Department of Forest Mycology and Plant Pathology
Swedish University of Agricultural Sciences
75007 Uppsala
Sweden

Email:mudassir.iqbal@slu.se

James, Christopher

School of Engineering
University of Warwick
Coventry, CV4 7AL
UK

email: C.James@warwick.ac.uk

Janssen, Toon

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: toon.janssen@ugent.be

Jaubert-Possamai, Stephanie

INRA
Université Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
6903 Sophia Antipolis
France

email: stephanie.jaubert@sophia.inra.fr

Jeongeun, Kim

Department of Ecological Science
Kyungpook National University
Sangju
Gyeongsangbukdo 37224
South Korea

email: kjeqaz6715@naver.com

Jian, Heng

Department of Plant Pathology
China Agricultural University
Beijing 100193
China

email: hengjian@cau.edu.cn

Jones, John

Cell and Molecular Sciences Group
The James Hutton Institute
Invergowrie
Dundee, DD2 5DA
UK

email: john.jones@hutton.ac.uk

Jones, Michael

Plant Biotechnology Research Group
WA State Agricultural Biotechnology Centre
School of Veterinary and Life Sciences
Murdoch University
Perth, WA 6150
Australia

email: m.jones@murdoch.edu.au

Kabir, Md. Faisal

Department of Ecological Science,
Kyungpook National University
Sangju
Gyeongsangbukdo 37224
South Korea

email: faisalmfk@yahoo.com

Kakouli-Duarte, Thomaé

Molecular Ecology and Nematode Research Group
enviroCORE
Institute of Technology Carlow
Carlow
Ireland

email: thomae.kakouli@itcarlow.ie

Kalinkina, Daryia

Institute of Biology of Karelian Research Centre
Russian Academy of Sciences
185910 Petrozavodsk
Russia

email: kalinkinads@gmail.com

Kamali, Shokoofeh

Biocontrol and Insect Pathology Laboratory
Department of Plant Protection
School of Agriculture
Ferdowsi University of Mashhad
9177948974 Mashhad
Iran

email: Shokoofeh.Kamali@gmail.com

Kanzaki, Natsumi

Forestry and Forest Products Research Institute
Tsukuba
305-8687 Ibaraki
Japan

email: nkanzaki@affrc.go.jp

Kapp, Caro

Department of Conservation Ecology and Entomology
Stellenbosch University
Stellenbosch, 7602
South Africa

email: ckapp@sun.ac.za

Karimi, Javad

Biocontrol and Insect Pathology Laboratory
Department of Plant Protection
Faculty of Agriculture
Ferdowsi University of Mashhad
9177948974 Mashhad
Iran

email: jkb@um.ac.ir

Kasapoglu, Ece Bortecine

Cukurova University
Faculty of Agriculture
Department of Plant Protection
1330 Saricam, Adana
Turkey

email: ecekasapoglu@gmail.com

Kato, Tetsuro

Nippon Soda Ltd.
250-0280 Kanagawa
Japan

email: tet.kato@nippon-soda.co.jp

Kergunteuil, Alan

Laboratory of Functional Ecology
Institute of Biology
University of Neuchâtel
100875 Neuchâtel
Switzerland

email: alan.kergunteuil@unine.ch

Khosa, Mbokota Candy

Agricultural Research Council-ITSC
1200 Nelspruit
South Africa

email: mbokota@arc.agric.za

Kiewnick, Sebastian

Agroscope
National Competence Center for Nematology
8820 Waedenswil
Switzerland

email: sebastian.kiewnick@agroscope.admin.ch

Kigozi, Andrew

Ghent University
9000 Ghent
Belgium

email: apolloalexandria1@gmail.com

Kim, Taeho

Division of Environmental Science and Ecological Engineering
College of Life Sciences and Biotechnology
Korea University
Seoul 02841
South Korea

email: godspeedtaeho@gmail.com

Kitagami, Yudai

Laboratory of Forest Mycology
Graduate School of Bioresources
Mie University
Tsu, Mie, 514-8507
Japan

email: 515M109@m.mie-u.ac.jp

Kolombia, Yao Adjiguita

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: kolombia3@hotmail.com

Korthals, Gerard

Wageningen University
Netherlands Institute of Ecology
6708 PB Wageningen
The Netherlands

email: gerard.korthals@wur.nl

Kwag, Haina

Ewha Womans University
Seoul 3760
South Korea

email: gosk1020@gmail.com

Kwon, Soon-Bae

Gangwondo Agricultural Research & Extension Service
Chuncheonsi, 200-150
South Korea

email: snbkwon@korea.kr

Laetsch, Dominik

Institute of Evolutionary Biology
University of Edinburgh
Edinburgh, EH9 3FL
UK

email: dominik.laetsch@gmail.com

Landeweert, Renske

ClearDetections
6709 PA Wageningen
The Netherlands

email: renske.landeweert@cleardetections.com

Lavrova, Victoria

Institute of Biology
Karelian Research Centre
Russian Academy of Sciences
185910 Petrozavodsk
Russia

email: victoria.v.lavrova@gmail.com

Lawson, Rebecca

Fera Science Ltd
National Agri-Innovation Campus
YO41 1LZ York
UK

email: bex.lawson@fera.co.uk

Lazarova, Stela

Institute of Biodiversity and Ecosystem Research
Bulgarian Academy of Sciences
1113 Sofia
Bulgaria

email: stela.lazarova@gmail.com

Lee, DongWoon

Department of Ecological Science
Kyungpook National University
Sangju
Gyeongsangbukdo 37224
South Korea

email: whitegrub@knu.ac.kr

Lennon, John

Faculty of Biological Sciences
University of Leeds
Leeds, LS2 9JT
UK

email: bs09j2l@leeds.ac.uk

Li, Hongmei

Department of Plant Pathology
Nanjing Agricultural University
Nanjing 210095
China

email: lihm@njau.edu.cn

Li, Huixia

Gansu Agricultural University
730070 Lanzhou
China

email: lihx@gsau.edu.cn

Lilley, Catherine

Centre for Plant Sciences
University of Leeds
Leeds, LS2 9JT
UK

email: c.j.lilley@leeds.ac.uk

Long, Haibo

Environmental and Plant Protection Institute
Chinese Academy of Tropical Agricultural Sciences
571101 Haikou
China

email: hb_long@yeah.net

Lopez-Llorca, Luis

Laboratory of Plant Pathology
Department of Marine Sciences and Applied Biology
Multidisciplinary Institute for Environmental Studies (MIES) Ramon Margalef
University of Alicante
E-03080 Alicante
Spain

email: lv.lopez@ua.es

López-Pérez, Antonio

Centro Agrario Marchamalo
Instituto Regional Agrario Investigaciones Agroalimentarias y Forestales (IRIAF)
19180 Marchamalo, Guadalajara
Spain

email: jalopezp@jccm.es

Lozano Torres, Jose

Laboratory of Nematology
Wageningen University
6708 PB Wageningen
The Netherlands

email: jose.lozano@wur.nl

Lueth, Peter

Bayer CropScience Biologics GmbH
23970 Wismar
Germany

email: peter.lueth@peterlueth.de

Luimes, Jan

Dutch General Inspection Service (NAK)
8304 AS Emmeloord
The Netherlands

email: jluimes@nak.nl

Lunt, Dave

School of Environmental Sciences
University of Hull
Hull, HU6 7RX
UK

email: dave.lunt@gmail.com

Machado, Ricardo

Institute of Plant Sciences
University of Bern
3013 Bern
Switzerland

email: ricardo.machado@ips.unibe.ch

Mackiewicz, Jasmina

Institute of Environmental Science
Jagiellonian University
30-387 Krakow
Poland

email: jasmina.mackiewicz@uj.edu.pl

Magnusson, Christer

NIBIO
1431 Aas
Norway

christer.magnusson@nibio.no

Mahlitz, Sina-Valerie

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: s7simahl@uni-bonn.de

Maier, Thomas

3Department of Plant Pathology
Iowa State University
Ames 50011
USA

email: trmaier@iastate.edu

Majić, Ivana

Faculty of Agriculture in Osijek
University of Osijek
HR 31000 Osijek
Croatia

email: Croatiaimajic@pfos.hr

Maleita, Carla

CIEPQPF - Chemical Process Engineering and Forest Products Research Centre
Department of Chemical Engineering
University of Coimbra
3030-790 Coimbra
Portugal

email: carlamnmaleita@hotmail.com

Maňasová, Marie

Czech University of Life Sciences
Faculty of Agrobiolgy, Food and Natural Resources
Department of Plant Protection
16521 Prague 6
Czech Republic

email: marie.manasova1@gmail.com

Mantelin, Sophie

Cell and Molecular Sciences Group
The James Hutton Institute
Invergowrie
Dundee, DD2 5DA
UK

email: sophie.mantelin@hutton.ac.uk

Martins, Ana

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: anafdmartins@gmail.com

Mateille, Thierry

Institut de Recherche pour le Développement (IRD)
UMR CBGP (INRA/IRD/CIRAD/Montpellier SupAgro)
34988 Montferrier-Sur-Lez
France

email: thierry.mateille@ird.fr

Mathes, Pascale

European Commission
Directorate General Health and Food Safety
Directorate F - Health and food audits and analysis
Grange, Dunsany, Co Meath
Ireland

email: Pascale.MATHES@ec.europa.eu

Matuszkiewicz, Mateusz

Department of Plant Genetics Breeding and Biotechnology
Warsaw University of Life Sciences
02-787 Warsaw
Poland

email: mateusz_matuszkiewicz@sggw.pl

Matveeva, Elizaveta

Institute of Biology
Karelian Research Centre
Russian Academy of Sciences
185910 Petrozavodsk
Russia

email: elizaveta.matveeva@gmail.com

Mayda, Umma

Ghent University
9000 Ghent
Belgium

email: ummamayda@yahoo.com

Medina, Clémence

INRA
Université Nice Sophia Antipolis
CNRS, UMR 1355-7254 Institut Sophia Agrobiotech
06903 Sophia Antipolis
France

email: clemence.medina@sophia.inra.fr

Melakeberhan, Haddish

Agricultural Nematology Laboratory
Department of Horticulture
Michigan State University
East Lansing, MI 48824
USA

email: melakebe@msu.edu

Mendy, Badou

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: s7bamend@uni-bonn.de

Mennan, Hüsrev

Ondokuz Mayıs University
Faculty of Agricultural
Department of Plant Protection
55139 Samsun
Turkey

email: hmennan@omu.edu.tr

Mennan, Sevilhan

Ondokuz Mayıs University
Faculty of Agricultural
Department of Plant Protection
55139 Samsun
Turkey

email: smennan@omu.edu.tr

Miglino, Roberto

HZPC Holland B.V. R&D
9123 JB Metslawier
The Netherlands

email: Roberto.Miglino@hzpc.nl

Mikušková, Kateřina

Central Institute for Supervising and Testing in Agriculture
Division of Diagnostics
779 00 Olomouc Czech Republic

email: katerina.mikuskova@ukzuz.cz

Mimee, Benjamin

Agriculture and Agri-Food Canada
St-Jean-sur-Richelieu Research and Development Centre
St-Jean-sur-Richelieu, QC, J3B 3E6
Canada

email: benjamin.mimee@agr.gc.ca

Mitreva, Makedonka

McDonnell Genome Institute and Department of Medicine
Washington University School of Medicine
St. Louis, MO 63108
USA

email: mmitreva@genome.wustl.edu

Moens, Tom

Ghent University
Biology Department
Marine Biology Lab
9000 Ghent
Belgium

email: tom.moens@ugent.be

Mohan, Sharad

Division of Nematology
ICAR - Indian Agricultural Research Institute
New Delhi 110012
India

email: sharad@iari.res.in

Molendijk, Leendert

Applied Plant Research
Wageningen UR
8219 PH Lelystad
The Netherlands

email: leendert.molendijk@wur.nl

Molina, Carlos

e-nema GmbH
24223 Schwentinental
Germany

email: c.molina@e-nema.de

Montarry, Josselin

INRA - Institut de Génétique Environnement et Protection des Plantes
UMR 1349 IGEPP
35653 Le Rheu
France

email: josselin.montarry@rennes.inra.fr

Morais, Paula

Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: pvmorais@ci.uc.pt

Morris, Robert

Medical Biology Centre
Queen's University Belfast
Belfast, BT12 5PS
Northern Ireland
UK

email: rmorris10@qub.ac.uk

Mota, Manuel

NemaLab, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM)
Universidade de Évora
Herdade da Mitra
7002-554 Évora
Portugal

email: mmota@uevora.pt

Mougel, Christophe

INRA- Institut de Génétique Environnement et Protection des Plantes
UMR 1349
35653 Le Rheu
France

email: christophe.mougel@rennes.inra.fr

Moura Silva, Daniel

Ghent University
9000 Ghent
Belgium

email: dmoura.ufpe@gmail.com

Muringai, Nancy

Ghent University
9000 Ghent
Belgium

email: nancymuringai@gmail.com

Nascimento, Danila

Institut National de la Recherche Agronomique (INRA)
UMR 1355 ISA/Centre National de la Recherche Scientifique
UMR 7254 ISA/Université de Nice-Sophia Antipolis
Université Nice Sophia Antipolis
6903 Sophia Antipolis
France

email: danila.cabraln@gmail.com

Navas, Alfonso

Museo Nacional de Ciencias Naturales, CSIC
Madrid 28006
Spain

email: anavas@mncn.csic.es

Naz, Fareeha

Plant Biotechnology Research Group
WA State Agricultural Biotechnology Centre
School of Veterinary and Life Sciences
Murdoch University
Perth, WA 6150
Australia

email: fareehajaved@hotmail.com

Neilson, Roy

Ecological Sciences
The James Hutton Institute
Dundee, DD2 5DA
Scotland
UK

email: roy.neilson@hutton.ac.uk

Neuskens, Lander

Ghent University
9000 Ghent
Belgium

email: landerneuskens@hotmail.com

Nguyen, Duong Thi Anh

Institute of Zoology
Department of Terrestrial Ecology
University of Cologne
D-50674 Köln
Germany

email: nad2807@yahoo.com

Niere, Björn

Julius Kühn-Institut
Institute for Plant Protection in Field Crops and Grassland
38102 Braunschweig
Germany

email: bjoern.niere@julius-kuehn.de

Nježić, Branimir

University of Banja Luka
Faculty of Agriculture
78000 Banja Luka
Bosnia and Herzegovina

email: branimir.njezic@agrofabl.org

O'Callaghan, Felicity

The James Hutton Institute
Invergowrie
Dundee, DD2 5DA
Scotland
UK

email: rong@online.de

O'Connor, Vincent

Centre for Biological Sciences
University of Southampton
Southampton, SO17 1BX
UK

email: voconno@soton.ac.uk

Oka, Yuji

Gilat Research Center
Agricultural Research Organization
M.P. Negev 8528000
Negev
Israel

email: okayuji@volcani.agri.gov.il

Okumura, Etsuko

Graduate School of Agriculture
Kyoto University
606-8502 Kyoto
Japan

email: etsuko8okumura@gmail.com

Olajide Olorunfemi, Emmanuel

Ghent University
9000 Ghent
Belgium

email: olajide.o.emmanuel@gmail.com

Oliveira, Daniel

Department of Biology
Ghent University
9000 Ghent
Belgium

email: apoloniobio@gmail.com

Oota, Morihiko

Kumamoto University
Kumamoto-shi, 860-0851
Japan

email: 126s6006.kumadai@gmail.com

Oro, Violeta

Institute of Plant Protection and Environment
11000 Belgrade
Serbia

email: viooro@yahoo.com

Ozturk, Lerzan

Viticulture Research Station
59100 Suleymanpasa
Tekirdag
Turkey

email: lerzanzoturk@hotmail.com

Palomares Rius, Juan

Instituto de Agricultura Sostenible (IAS)
Consejo Superior de Investigaciones Científicas (CSIC)
14004 Córdoba
Spain

email: palomaresje@ias.csic.es

Pariyar, Shree

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53129 Bonn
Germany

email: pariyar@uni-bonn.de

Park, Joong-Ki

Division of EcoScience
Ewha Womans University
Seoul 03760
South Korea

email: jkpark@ewha.ac.kr

Patil, Jagadeesh

ICAR-National Bureau of Agricultural Insect Resources
Bengaluru 560 024
Karnataka
India

email: patiljaggi@gmail.com

Paul, Grace

St. Pious X Degree College for Women
Osmania University
500047 Hyderabad
India

email: gracebpaul@rediffmail.com

Pedram, Majid

Department of Plant Pathology
Faculty of Agriculture
Tarbiat Modares University
98 Tehran
Iran

email: Mj_pedram@yahoo.com

Peña-Santiago, Reyes

Departamento de Biología Animal, Biología Vegetal y Ecología
Universidad de Jaén
23071 Jaén
Spain

email: rpena@ujaen.es

Peneva, Vlada

Institute of Biodiversity and Ecosystem Research
Bulgarian Academy of Sciences
1113 Sofia
Bulgaria

email: vpeneva@ecolab.bas.bg

Peng, Deliang

State Key Laboratory for Biology of Plant Diseases and Insect Pests
Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Beijing 100193
China

email: dlpeng@ippcaas.cn

Peng, Huan

State Key Laboratory for Biology of Plant Diseases and Insect Pests
Institute of Plant Protection
Chinese Academy of Agricultural Sciences
Beijing 100193
China

email: hpeng@ippcaas.cn

Pen-Mouratov, Stanislav

The Steinhardt Museum of Natural History and Israel National Center for Biodiversity Studies
Department of Zoology
George S. Wise Faculty of Life Sciences
Tel Aviv University
Ramat Aviv 6997801
Israel

email: stanislavpm@post.tau.ac.il

Pereira, Tiago

Department of Nematology
University of California, Riverside
Riverside, CA 92521
USA

email: tiagojp@ucr.edu

Perpétuo, Laura Soraia

CFE - Centre for Functional Ecology
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: soraiaperpetuo@gmail.com

LIST OF PARTICIPANTS

Perry, Roland

University of Hertfordshire
Hatfield
UK

email: r.perry2@herts.ac.uk

Pickup, Jon

Science and Advice for Scottish Agriculture
Edinburgh EH12 9FJ
UK

email: jon.pickup@sasa.gsi.gov.uk

Pires, David

Centre of Molecular and Environmental Biology (CBMA)
Department of Biology
University of Minho
4710-057 Braga
Portugal

email: david.pires89@gmail.com

Pitos, Abraham

Ghent University
9000 Ghent
Belgium

email: abraham.pitos27@gmail.com

Ploeg, Antoon

Department of Nematology
UC Riverside
Riverside CA 92521
USA

email: antoon.ploeg@ucr.edu

Poot, Natasja

Eurofins Agro
6708 BL Wageningen
The Netherlands

email: natasjapoot@hotmail.com

Pougheishangbam, Rolish

Ghent University
9000 Ghent
Belgium

email: rolishsingh@gmail.com

Prado, Alicia

Estación Fitopatológica Areeiro
36071 Pontevedra
Spain

email: alicia.prado@depo.es

Price, James

Biomedical Sciences Research Complex
University of St Andrews
St Andrews KY16 9ST
UK

email: jp203@st-andrews.ac.uk

Prior, Thomas

Fera Science Ltd
National Agri-Food Innovation Campus
York YO41 1LZ
UK

email: thomas.prior@fera.co.uk

Proença, Diogo

Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: diogo.proenca@gmail.com

Puza, Vladimir

Institute of Entomology, Biology Centre CAS
37005 Ceske Budejovice
Czech Republic

email: vpuza@seznam.cz

Quist, Casper

Laboratory of Nematology
Wageningen University
6707 GJ Wageningen
The Netherlands

email: casperwquist@gmail.com

Radakovic, Zoran

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: s7zorada@uni-bonn.de

LIST OF PARTICIPANTS

Radochová, Petra

Institute for Environmental Studies
Faculty of Science
Charles University in Prague
12843 Prague 2
Czech Republic

email: radochovap@gmail.com

Rao, Uma

Division of Nematology
ICAR - Indian Agricultural Research Institute
New Delhi, 110012
India

email: umarao@iari.res.in

Rashidifard, Milad

Unit of Environmental Sciences and Management
North West University
2520 Potchefstroom
South Africa

email: 27216179@nwu.ac.za

Reuther, Marie

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-Universität Bonn
53115 Bonn
Germany

email: reuther@ruebe.info

Ripa, Linda

Syngenta Seeds AB
26123 Landskrona
Sweden

email: linda.ripa@syngenta.com

Rist, Marc

Bayer CropScience AG
40789 Monheim am Rhein
Germany

email: marc.rist@bayer.com

Roberts, Philip

Department of Nematology
University of California
Riverside, CA 92521
United States of America

email: philip.roberts@ucr.edu

Robertson, Lee

Museo Nacional de Ciencias Naturales, CSIC
Madrid 28006
Spain

email: lee.r@mncn.csic.es

Rybarczyk-Mydlowska, Katarzyna

Museum and Institute of Zoology
Polish Academy of Sciences
00-679 Warsaw
Poland

email: katarzynar@miiz.waw.pl

Sağlam, Hayriye Didem

Ahi Evran University
Faculty of Agriculture
Department of Plant Protection
40200 Kırşehir
Turkey

email: saglamhds@gmail.com

Sánchez-Monge, Gerardo Alcides

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: alcides.sanchez@gmail.com

Sánchez-Moreno, Sara

Plant Protection Products Unit (DTEVPPF)
National Institute for Agriculture and Food Science and Technology
28040 Madrid
Spain

email: sarasm@inia.es

Santos, Jansen

Department of Nematology
University of California
Riverside, CA 92521
USA

email: jansenps@ucr.edu

LIST OF PARTICIPANTS

Sarniguet, Corinne

ANSES- Laboratoire de la Santé des Végétaux
French Agency for Food, Environmental and Occupational Health and Safety
Plant Health Laboratory – Nematology Unit
35653 Le Rheu
France

email: corinne.sarniguet@anses.fr

Sawa, Shinichiro

Kumamoto University
860-8555 Kumamoto
Japan

email: sawa@sci.kumamoto-u.ac.jp

Schleker, Sylvia

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: sylvia.schleker@uni-bonn.de

Serra, Maria Clara

Direção Geral Alimentação e Veterinária (DGAV)
Tapada da Ajuda - Edifício 1
1349-018 Lisboa
Portugal

email: cserra@dgav.pt

Shah, Syed Jehangir

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-Universität Bonn
53115 Bonn
Germany

email: jehangir@uni-bonn.de

Shokoohi, Ebrahim

Unit for Environmental Sciences and Management
North West University
2520 Potchefstroom
South Africa

email: ebrahim.shokoohi@nwu.ac.za

Sibbel, Lia

Eurofins Agro
6709 PD Wageningen
The Netherlands

email: lia.sibbel@eurofins-agro.com

Siddique, Shahid

INRES, Institute of Crop Science and Resource Conservation
Department of Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53115 Bonn
Germany

email: siddique@uni-bonn.de

Sikora, Richard

INRES-Phytopathology
University of Bonn
53127 Bonn
Germany

email: rsikora@uni-bonn.de

Silva, Ana Cláudia

Àrea de Fisiología Vegetal
Facultad de Ciencias Ambientales y Bioquímica
Universidad de Castilla La Mancha
45071 Toledo
Spain

email: anaclaudia.pereira@uclm.es

Simões, Nelson

Centro de Biotecnologia dos Açores
Universidade dos Açores
9500-801 Ponta Delgada
Portugal

email: nelson.jo.simoes@azores.gov.pt

Slaats, Brigitte

Syngenta Crop Protection AG
4332 Stein
Switzerland

email: brigitte.slaats@syngenta.com

LIST OF PARTICIPANTS

Slos, Dieter

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: dieterg.slos@ugent.be

Smant, Geert

Wageningen University
Laboratory of Nematology
6708 PB Wageningen
The Netherlands

email: geert.smant@wur.nl

Smet, Francis

Union Biometrica, Inc.
Holliston, MA 1746
USA

email: fsmet@unionbio.com

Sommer, Ralf J.

Max-Planck Institute for Developmental Biology
72076 Tuebingen
Germany

email: ralf.sommer@tuebingen.mpg.de

Somvanshi, Vishal

Division of Nematology
ICAR - Indian Agricultural Research Institute
New Delhi 110012
India

email: vssomvanshi@iari.res.in

Sousa, Edmundo

INIAV - Instituto Nacional de Investigação Agrária e Veterinária
2780-159 Oeiras
Portugal

email: edmundo.sousa@iniav.pt

Spiegel, Yitzhak

ARO, TheVolcani Center
50250 Bet Dagan
Israel

email: spiegely@agri.gov.il

Srivastava, Arohi

Department of Biological and Environmental Sciences
University of Hertfordshire
Hatfield AL10 9AB
UK

email: a.srivastava@herts.ac.uk

Stare, Barbara

Agricultural Institute of Slovenia
Plant Protection Department
SI-1000 Ljubljana
Slovenia

email: barbara.geric@kis.si

Steel, Hanne

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: hanne.steel@ugent.be

Steyn, Willem

Agricultural Research Council - Institute for Tropical and Subtropical Crops
Nelspruit 1200
South Africa

email: willems@arc.agric.za

Strauch, Olaf

e-nema GmbH
24223 Schwentinental
Germany

email: o.strauch@e-nema.de

Sturrock, Matthew

Queen's University Belfast
Belfast, BT9 7BL
UK

email: msturrock01@qub.ac.uk

Subbotin, Sergei

Plant Pest Diagnostic Center
California Department of Food and Agriculture
Sacramento, CA 95832-1448
USA

email: sergei.a.subbotin@gmail.com

Sumaya, Nanette Hope

e-nema GmbH
24223 Schwentimental
Germany

email: nanettehope.sumaya@gmail.com

Susič, Nik

Agricultural Institute of Slovenia
Plant Protection Department
1000 Ljubljana
Slovenia

email: nik.susic@kis.si

Suzuki, Reira

Kumamoto University
860-8555 Kumamoto-shi
Japan

email: mukozizi320@yahoo.co.jp

Szakálas, Judit

Department of Zoology and Animal Ecology
Szent István University
2100 Gödöllő
Hungary

email: szakalaszjudit@gmail.com

Tanaka, Ryusei

Division of Parasitology
University of Miyazaki
5200 Kihara
Miyazaki
Japan

email: caenorhabditisjaponica@gmail.com

Tanaka, Suguru

Laboratory of Forest Botany
The University of Tokyo
113-8685 Tokyo
Japan

email: sgr.tnk.021101@fr.a.u-tokyo.ac.jp

Taylor, Victoria

Harper Adams
Liverpool L3 8BB
UK

email: vtaylor@harper-adams.ac.uk

Teixeira, Andreia

Centre of Molecular and Environmental Biology (CBMA)
Department of Biology
University of Minho
4710-057 Braga
Portugal

email: andrea_se_teixeira@hotmail.com

Teklu, Misghina

Laboratory of Nematology
Wageningen University
6708 PB Wageningen
The Netherlands

email: Misghina.goitomteklu@wur.nl

Thapa, Srijana

Ghent University
9000 Ghent
Belgium

email: agstd123@gmail.com

Thoden, Tim

DuPont de Nemours (France) S.A.S.
European Research Center
68740 Nambenheim
France

email: tim.thoden@dupont.com

Topalovic, Olivera

Julius Kühn Institute
38104 Braunschweig
Germany

email: olivera.topalovic.best@gmail.com

Toubarro, Duarte

Centro de Biotecnologia dos Açores
Universidade dos Açores
9501-855 Ponta Delgada
Portugal

email: duartetoubarro@uac.pt

Toumi, Fateh

Faculty of Bioscience engineering
Ghent University
9000 Ghent
Belgium

email: fateh.toumi@ugent.be

Trichard, Louis

LTLA Landscape Studios
1240 White River
South Africa

email: louis.studios@ltladevelopment.co.za

Trinh, Quang Phap

Department of Nematology
Institute of Ecology and Biological Resources
Vietnam Academy of Science and Technology
10000 HaNoi
Vietnam

email: tqphap@yahoo.com

Troccoli, Alberto

CNR - Istituto per la Protezione Sostenibile delle Piante (IPSP)
70126 Bari
Italy

email: alberto.troccoli@ipsp.cnr.it

Tsai, Allen Yi-Lun

Graduate School of Science and Technology
Kumamoto University
Kumamoto city
860-8555 Kumamoto
Japan

email: allen@sci.kumamoto-u.ac.jp

Urwin, Peter

Centre for Plant Sciences
Faculty of Biological Sciences
University of Leeds
Leeds LS2 9JT
UK

email: p.e.urwin@leeds.ac.uk

Valdes, Yirina

Universidade Federal de Pernambuco (UFPE)
Centro Acadêmico de Vitória
55608-680 Vitória de Santo Antão - Pernambuco
Brazil

email: yirina80@yahoo.es

Van Aalst, Eveline

RijkZwaan Breeding B.V.
2678 KX De Lier
The Netherlands

email: p.barendse@rijkszwaan.nl

Van Aardt, Willie

North-West University
2520 Potchefstroom
South Africa

email: willie.vanaardt@nwu.ac.za

Van Beers, Thea

Agrifirm Plant
7325AW Apeldoorn
Netherlands

email: t.vanbeers@agrifirm.com

Van Bruggen, Anne Sophie

National Plant Protection Organization (NPPO)
6706 EA Wageningen
The Netherlands

email: a.s.vanbruggen@nvwa.nl

Van Zwol, Hille Jan

EnzaZaden R&D B.V.
1602 DB Enkhuizen
The Netherlands

email: J.vondenSteinen@enzazaden.nl

Vandenbossche, Bart

e-nema GmbH
24223 Schwentinental
Germany

email: b.vandenbossche@e-nema.de

Varypatakis, Kyriakos

The James Hutton Institute
University of St Andrews
Dundee, DD2 1AF
UK

email: ak.varypatakis@gmail.com

Verbeek, Ruben

Department of Molecular Biotechnology
Faculty of Bioscience Engineering
Ghent University
9000 Ghent
Belgium

email: ruben.verbeek@ugent.be

Verdejo-Lucas, Soledad

IFAPA, Centro La Mojonera
4745 Almería
Spain

email: soledad.verdejo@juntadeandalucia.es

Verhage, Adriaan

RijkZwaan Breeding B.V.
2678 KX De Lier
The Netherlands

email: p.barendse@rijkszwaan.nl

Verhoeven, Ava

Wageningen University
6708 PB Wageningen
The Netherlands

email: ava.verhoeven@wur.nl

Veronico, Pasqua

CNR, Istituto per la Protezione Sostenibile delle Piante (IPSP)
70126 Bari
Italy

email: pasqua.veronico@ipsp.cnr.it

Viaene, Nicole

Institute for Agricultural and Fisheries Research (ILVO)
B-9820 Merelbeke
Belgium

email: nicole.viaene@ilvo.vlaanderen.be

Vieira, Paulo

Virginia Tech
Department of Plant Pathology, Physiology and Weed Science
VA 24061 Blacksburg
USA

email: pvieira@uevora.pt

Vieira dos Santos, Maria Clara

Centre for Functional Ecology (CFE)
Department of Life Sciences
University of Coimbra
3000-456 Coimbra
Portugal

email: mcvs@sapo.pt

Viketoft, Maria

Department of Ecology
Swedish University of Agricultural Sciences
SE-75007 Uppsala
Sweden

email: maria.viketoft@slu.se

von Reuss, Stephan

Max Planck Institute for Chemical Ecology
Department of Bioorganic Chemistry
7745 Jena
Germany

email: svonreuss@ice.mpg.de

Waeyenberge, Lieven

ILVO
Institute for Agricultural and Fisheries Research
9820 Merelbeke
Belgium

email: lieven.waeyenberge@ilvo.vlaanderen.be

Wang'ombe, Mary Wanjiku

INRES, Institute of Crop Science and Resource Conservation
Molecular Phytomedicine
Rheinische Friedrich-Wilhelms-University of Bonn
53129 Bonn
Germany

email: s7mawamg@uni-bonn.de

Warmerdam, Sonja

Laboratory of Nematology
Wageningen University
6708 PB Wageningen
The Netherlands

email: sonja.warmerdam@wur.nl

Warnock, Neil

Queen's University Belfast
Medical Biology Centre
Belfast BT9 7BL
Northern Ireland
UK

email: n.warnock@qub.ac.uk

Webster, John

Simon Fraser University
Burnaby, BC V5A 1S6
Canada

email: jwebster@sfu.ca

Wen, Yanhua

College of Agriculture
South China Agricultural University (SCAU)
510642 Guangzhou City
China

email: yhwen@scau.edu.cn

Wesemael, Wim

Institute for Agricultural and Fisheries Research (ILVO)
B-9820 Merelbeke
Belgium

email: wim.wesemael@ilvo.vlaanderen.be

Wieczorek, Krzysztof

Division of Plant Protection
Department of Crop Sciences
University of Natural Resources and Life Sciences
3430 Tulln
Austria

email: krzysztof.wieczorek@boku.ac.at

Williamson, Valerie

Department of Plant Pathology
University of California
Davis, CA 95616
USA

email: vmwilliamson@ucdavis.edu

Wilson, Leonie

Medical Biology Centre
Queen's University Belfast
Belfast, BT9 7BL
Northern Ireland
UK

email: lwilson61@qub.ac.uk

Winkler, Nils

Omex
Kings Lynn PE34 3JA
UK

email: nilsw@omex.com

Wu, Duqing

University of Leeds
Leeds LS2 9JT
UK

email: bswd@leeds.ac.uk

Wu, Hai

Agricultural College of Guangxi University
Nanning 530004
China

email: wuhy@gxu.edu.cn

Xue, Qing

Nematology Research Unit
Department of Biology
Ghent University
9000 Ghent
Belgium

email: xueqing4083@gmail.com

Yavuzaslanoglu, Elif

Karamanoglu Mehmetbey University
Technical Sciences Vocational School
Plant and Animal Production Department
70001 Karaman
Turkey

email: eyavuzaslanoglu@kmu.edu.tr

Yimer, Henok

Department of Molecular Biotechnology Coupure
University of Ghent
9000 Ghent
Belgium

email: HenokZemene.Yimer@UGent.be

Yu, Qing

Ottawa Research and Development Centre
Agriculture and Agri-Food Canada
Ottawa, K1A 0C6
Ontario
Canada

email: Qing.yu@agr.gc.ca

Zheng, Jingwu

Institute of Biotechnology
College of Agriculture and Biotechnology
Zhejiang University
Hangzhou 310058
China

email: jwzheng@zju.edu.cn

Zhou, Hong

College of Marine Life Science
Ocean University of China
266003 Qingdao
China

email: hzhou@ouc.edu.cn

Zouhar, Miloslav

Czech University of Life Sciences in Prague
Faculty of Agrobiological, Food and Natural Resources
Department of Plant Protection
Prague 6, 16521
Czech Republic

email: zouharmiloslav@seznam.cz

NOTES

Suppression of *Meloidogyne incognita* by the entomopathogenic fungus *Lecanicillium muscarium*

Manzoor Hussain, Miloslav Zouhar and Pavel Ryšánek

Czech University of Life Sciences, Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Plant Protection, Kamýcká 961/129, 165 00 Praha 6-Suchbát, Czech Republic

corresponding author: hussain@af.czu.cz

The entomopathogenic fungus, *Lecanicillium muscarium* (Petch) Zare and Gamsis, is currently being developed as a biocontrol agent against insect pests, as well as some plant pathogenic fungi and bacteria. Data about its activity against plant-parasitic nematodes exist, but are relatively limited. To expand this understanding, we investigated the biocontrol efficiency of three isolates of *L. muscarium* (Lm) against the root-knot nematode, *Meloidogyne incognita*, in both *in vitro* and *in vivo* conditions. The isolate Lm1 was designated as the best biocontrol agent against nematode eggs as well as second-stage juveniles (J2). It showed the highest colonisation of eggs and significantly decreased hatching. The results from two additional isolates, Lm2 and Lm3, were also significant ($P = 0.05$) but less pronounced than those observed with Lm1. In our experiments, the maximum number of nematode eggs, J2, females and egg masses that were parasitised were quantified after 72 h exposure to the fungus. *Lecanicillium muscarium* treatments had significant ($P = 0.05$) positive effects on plant shoot and root growth compared to the growth of control plants. These results suggest the effectiveness of the fungus may be either to infect eggs and J2, or to produce secondary metabolites that induced plant defence mechanisms and lead to systemic resistance. Our study demonstrates that *L. muscarium* could be used as a potential biocontrol agent against root-knot nematodes.

Keywords: Biocontrol; entomopathogenic; juveniles; parasitism; root-knot nematodes.

Screening to identify genes that are involved in *Meloidogyne incognita*-induced root-knot formation processes in *Arabidopsis thaliana*

Reira Suzuki, Chika Ejima, Tomomi Sagara, Satoru Nakagami, Morihiro Oota, Chie Shimaoka, Takashi Ishida and Shinichiro Sawa

Kumamoto University, Kurokami, 2-39-1, Kumamoto, 860-8555, Japan

corresponding author: mukozizi320@yahoo.co.jp

Root-knot nematodes (*Meloidogyne incognita*) are phytoparasitic nematodes that significantly damage crop plants all over the world. Recent studies have showed that *M. incognita* disrupts various cellular processes to initiate specialised feeding sites and induce gall formation. Here, we established an *M. incognita* infection assay to analyse the infection process. To decipher the molecular mechanism underlying the infection process, we conducted mutant screens using the model plant *Arabidopsis thaliana* to search for *M. incognita*-resistant mutants. We identified 87 such mutants with various resistance levels. Resistance to *M. incognita* from different genes can be combined in higher-order mutants, and this information can be utilised to improve *M. incognita* control in agriculture. Further, we also examined the expression patterns of these *M. incognita*-resistance genes during nematode infection. Here we discuss our recent results of the mutant screens and the gene expression patterns for some of these resistance genes.

Keywords: *Arabidopsis*; feeding site; infection steps; nematode resistance; *Meloidogyne incognita*.

Resistance screening of clones and commercial tomato cultivars to *Meloidogyne incognita* and *M. javanica* populations from Ethiopia

Awol Seid^{1,3*}, Chemada Fininsa¹, Tesfamariam Mekete², Wilfrieda Decraemer^{3,4} and Wim M. L. Wesemael^{3,5,6}

¹ Haramaya University, College of Agriculture and Environmental Sciences, School of Plant Sciences -Crop Protection Unit, P. O. Box 138, Dire Dawa, Ethiopia

² University of Florida, Entomology and Nematology Department, Bldg.970, Natural Area Drive, Gainesville, FL 32611, USA

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 Institute for Agricultural and Fisheries Research (ILVO), Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium

⁶ Ghent University, Faculty of Bioscience engineering, Laboratory for Agrozoology, Coupure links 653, B-9000 Ghent, Belgium.

corresponding author: awolseid07@gmail.com

Soil and root samples were collected from major tomato growing areas of Ethiopia during the 2012/2013 growing season to identify root-knot nematode problems. DNA based and isozyme (esterase and malate dehydrogenase) techniques revealed that *Meloidogyne incognita* and *M. javanica* were the predominant *Meloidogyne* species across the sampled areas. The aggressiveness of the different populations of these two species was assessed on two susceptible tomato cultivars (Marmande and Moneymaker). The two most aggressive populations of each species were further used to screen 23 commercial tomato cultivars and 10 clones from Ethiopia. The resistance screening was done by inoculating 30 individual seedlings (4-leaf stage) of each cultivar with 100 freshly hatched (< 24 h) second-stage juveniles (J2). Plants were grown in plastic tubes (66 ml volume) and kept in a growth chamber in a completely randomised design. Eight weeks after inoculation the number of egg masses produced on each cultivar was assessed. Lower numbers of egg masses of both populations of *M. incognita* were produced on the cultivars Bridget40, Galilea, Assila, Eden, IRMA and Tisey. Lower numbers of egg masses of both populations of *M. javanica* were produced on the cultivars and clones Assila, Eden, Galilea, Mersa, Tisey, VL-642, 2366A, 2366B and 2366C. On several plants of these cultivars no egg masses were found, indicating possible resistance. The resistance mechanism (penetration and development) among the populations tested on selected tomato cultivars and clones was examined. Fewer J2 penetrated and development into adult females seemed delayed. Even though complete resistance was not found, our study showed that some cultivars and clones performed well and this is important information for local breeding programmes. Promising cultivars and clones were further tested in a field trial to assess their tolerance.

Keywords: Resistance; root-knot nematodes; screening; *Solanum lycopersicum*.

Field application of entomopathogenic nematodes against plum sawfliesBranimir Nježić¹ and Ralf-Udo Ehlers²¹ University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Petra Bojovića 1A, 78000 Banja Luka, Bosnia and Herzegovina² e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germanycorresponding author: branimirnjezic@yahoo.co.uk

Plum sawflies *Hoplocampa minuta* L. (Black) and *H. flava* H. (Yellow). are the main pests of European plum (*Prunus domestica*). During the course of feeding, larvae can damage up to six fruits. The management tactic is based on use of broad spectrum insecticides pyrethroides and neonicotenoides in countries where these products are still registered. Farmers in organic production do not have tools to control these pests and can face up to 100% damage. Commercial strains of the entomopathogenic nematodes (EPN), *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*, were applied against larvae. Laboratory tests were performed in Petri dishes filled with sterile sandy soils and 500, 1000 and 2000 infective juveniles (IJ) were applied against 10 sawfly larvae per Petri dish. Age of larvae was considering by testing larvae 1 day after they exited infested fruit, and 10, 20 and 40 days older larvae. In treatments with larvae 1 day after exited mortality was 92-100% depending on dosage but not species. No mortality was observed for older larvae. Field application was done before the anticipated time when the first larval stages dropped to the soil to pupate. Since a portion of larvae remain in diapause for 2 years, the experiment was repeated the following year in the same place. Evaluation of results was in the year following application by placing insect proof nets over the treated soil and placing white sticky plates under it. The number of adults on plates was counted. The highest reduction of adults after the first year was in the treatment with *H. bacteriophora*. To set up field test against adult sawflies, plum trees were covered by insect proof net. The same EPN species were applied to soil surface at the dosage of 0.5 million IJ m⁻² before the anticipated day of adult emergence. In 2013 reduction in fruit infestation was 90-98%, whereas in 2014 the reduction was 30-90%, with *S. feltiae* as the most efficient. The lower efficacy in 2014 is explained by late application of EPN. An open field trial against adults was performed with *S. feltiae*. The nematodes were applied before the anticipated time of first adult emergence with standard rate of 0.5 million m⁻² and half dose rate. There was a reduction in the number of deposited sawflies eggs in the trial with standard and half dose rates of 67.8% and 41.1%. respectively.

Keywords: Biological control; *Heterorhabditis bacteriophora*; plum sawflies; *Steinernema feltiae*; *Steinernema carpocapsae*.

Are positively-selected effectors of the root-knot nematode *Meloidogyne incognita* involved in plant resistance?

Ava Verhoeven, Pjotr Prins, Anna Finkers Tomczak, Koen Varossieau, Debbie van Raaij, Rikus Pomp, Aska Goverse and Geert Smant

Laboratory of Nematology, Department of Plant Sciences, Wageningen University, PO Box 8123, 6700 ES Wageningen, The Netherlands

corresponding author: ava.verhoeven@wur.nl

The tropical root-knot nematode *Meloidogyne incognita* is a global problem in tomato and many other vegetable crops. The obligate endoparasitic *M. incognita* can cause about 5% crop loss annually. Chemical pesticides can no longer be used to control *M. incognita* because of environmental and public health concerns. Meanwhile, the most frequently used Mi-1 resistance in tomato is increasingly being broken by virulent populations of *M. incognita*. Virulent root knot nematodes are thought to have acquired effectors that enable them to avoid recognition by resistance proteins in plants. Therefore, insight into the repertoire of effectors of *M. incognita* can help to identify new sources of resistance to which the field populations of nematodes in tomato-producing areas have not yet adapted. To identify positively-selected effector families in the genome sequence of *M. incognita*, a bioinformatics pipeline centred on the algorithms PRANK, CodeMl and Phobius was constructed. In total, 77 positively-selected clusters of paralogous genes were identified in the genome of *M. incognita*, which encode a total of 254 secretory proteins. The effectiveness of the pipeline was demonstrated by the fact that several positively-selected clusters of paralogous genes have been identified as effectors of *M. incognita* in earlier studies. Highly ranking candidate effectors from the pipeline are currently being functionally characterised in plants using transient and stable overexpression and using host-delivered RNA-interference in nematodes.

Keywords: Bioinformatics; overexpression; secretory proteins.

Commission's ongoing activities on pinewood nematode

Pasquale Di Rubbo

European Commission, Directorate General Health and Food Safety, Directorate G – Crisis management in Food, Animals and Plants, Brussels, Belgium

corresponding author: Pasquale.DI-RUBBO@ec.europa.eu

Pinewood nematode (PWN) is a regulated quarantine organism under the general EU Plant Health Directive 29/2000/EC, whose introduction into, and spread within, all EU Member States shall be banned. Import of susceptible coniferous plant species from non-European countries is prohibited. Strict requirements apply to wood of conifers (Coniferales) imported from countries where PWN is known to occur (Canada, China, Japan, Republic of Korea, Mexico, Taiwan and USA), while EU Member States are obliged to take all necessary measures to eradicate, or if that is impossible, inhibit the spread of PWN in the EU once detected in their territory. Since the first occurrence of PWN in the Union territory, reported by Portugal in 1999, emergency measures have been in place to prevent its further spread in the rest of the Union territory. The current measures are laid down under Commission Implementing Decision (EU) 2012/535/EU and are applicable to all EU Member States. The Decision requires each Member State to carry out annual survey activities for the presence of PWN in its territory, and have contingency plans in place in case of any outbreak. Detailed requirements are in place to demarcate the area once PWN is confirmed, including detailed containment and eradication measures applicable so far in Portugal and in a few isolated cases in Spain. Strict requirements are also applicable on movement of susceptible plants, wood and bark from the infected zone into the surrounding buffer zone and from a buffer zone outside the demarcated area. Following the recommendations of the Commission's Task Force on PWN, the Commission will now review some elements of the Commission Implementing Decision (EU) 2012/535/EU. Furthermore, as support to the Portuguese Authorities, the Commission has recently financed the application of remote sensing tools for the acquisition and processing of large scale images of coniferous trees. The project, implemented by the Commission's Joint Research Centre, intends to survey the entire buffer zone of Portugal by 2017 with the aim to identify declining trees. This should help Portugal in its task to remove all such trees because they can be attractant of the insect vector of PWN. Lastly, EU co-financing is foreseen for the implementation of EU emergency measures in Portugal and Spain, as well as survey activities in all EU Member States.

Keywords: European Commission; legislation; pinewood nematode.