35th Symposium of the European Society of **Nematologists**

Cordoba, Spain
15-19 April, 2024

Organize:

European
Society of
Nematologists

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Welcome

Dear colleagues,

It is a pleasure to announce the upcoming 35th International Symposium of the European Society of Nematologists, to be held in Córdoba (Spain) by 15-19 April 2024. A unique opportunity to exchange Nematology knowledge with the scientific and stakeholder communities.

Two years have passed since our last symposium in Antibes (France) and some changes have arisen since then. Next year we will have the opportunity to know how to face these challenges. Scientific sessions will include hot topics based on Nematology Research and applications around the world:

- Nematode Taxonomy, Systematics and Diagnosis
- Nematode Phylogeny, Phylogeography and Phylogenomic
- Biodiversity, Distribution and Ecology of PPN, EPN, and free-living
- Plant-nematode interactions and host response
- Nematode omics, metabolism and physiology
- Marine and Freshwater nematodes / Animal parasites
- Entomopathogenic nematodes
- Management of plant-parasitic nematodes
- Nematodes as bioindicators and nematode community assemblies
- Nematode effectors and parasitism genes
- Nematode interactions with other organisms: Complex diseases and Biocontrol
- New nematicidal products: Botanicals and Agrochemicals
- Cultural management of PPN: Cover crops, organic amendments, ASD
- Role of soil microbiome in nematode suppression
- Quarantine Nematodes: Diagnostics and management.

We look forward to seeing you all during the upcoming 35th International Symposium of the European Society of Nematologists in Cordoba, Spain. April 2024.

The Local Organizing Committee

Local Organizing Committee

- Antonio Archidona Yuste [IAS-CSIC]
- Juan Emilio Palomares Rius [IAS-CSIC]
- Lee Robertson [INIA-CSIC]
- Miguel Talavera Rubia [IFAPA]
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- Sergio Álvarez Ortega [Universidad Rey Juan Carlos]
- Soledad Verdejo Lucas [IFAPA]
- Win Wesemael [ILVO]

Monday, 15th April 2024

16:00-18:00 **ESN Governing Board**

17:00-19:00 **Registration**

19:00-22:00 **Welcome Reception**

Tuesday, 16th April 2024

08:00-10:30 **Registration**

09:00-09:30 **Official Opening Ceremony**

09:30-11:00 **Plenary session**
Chairs: Sara Sánchez Moreno and Juan Emilio Palomares-Rius

09:30-11:00 / **Plenary session 1**
Vegetable production in solar plastic-houses infested with root-knot nematodes
Speaker: Soledad Verdejo Lucas

10:00-10:30 / **Plenary session 2**
Custom low-cost robotics and AI: Plant-parasitic nematode phenotyping, at scale
Speaker: Sebastian Eves Van den Akker

10:30-11:00 / **Plenary session 3**
Vertical and Horizontal Transgenerational Epigenetic Inheritance of Learned Pathogen Avoidance in C. elegans
Speaker: Coleen Murphy

11:00-11:30 **COFFEE BREAK**

11:30-13:00 **Oral parallel sessions**

11:30-13:00 / **Oral parallel sessions 1 : Plan Nematode interactions and host response (I). Room: Plenary Room**
Chairs: Janice De Almeida Engler and Stephanie Jaubert.
Defence oral communication: from 4 to 9.

11:30 -13:00 / **Oral parallel session 1-2: Biodiversity, Distribution and Ecology of PPN, EPN, and free-living (I). Room 2.**
Chairs: Sergio Álvarez Ortega and Sofia Costa.
Defence oral communication: from 10 to 15.

11:30 -13:00 / **Oral parallel session 1-3:Entomopathogenicnematodes (I).Room 3**
Chairs: **Ana Morton and Ralph Udo-Ehlers.**
Defence oral communication: from 16 to 21.

13:00- 13:45 **Poster Session I**
Defence poster communication: from 186 to 239.

13:45- 15:15 **LUNCH**

16:00-17:00 **Poster Session I**
Defence poster communication: from 186 to 239.

16:00-17:00 **Oral parallel sessions**

16:00-17:00 / **Oral parallel session 2-1: Integrated management of PPN (I) Room: Plenary Room**
Chair: Sebastian Kiewnick and Lendert Molendijk.
Defence oral communication: from 22 to 25.

16:00-17:00 / **Oral parallel session 2-2: Nematode interactions with other organisms: Complex diseases and Biocontrol (I). Room 2.**
Chair: Alejandro Expósito Creo and Federico López Moya.
Defence oral communication: from 26 to 29.

16:00-17:00 **Oral parallel session 2-3: Animal parasites and new methods in Nematology. Room 3.**
Chair: Alfonso Navas Sánchez.
Defence oral communication: from 31 to 33.

17:00 -17:30 **COFFEE BREAK**

17:30-19:00 **Oral parallel sessions**

17:30-19:00 / **Oral parallel session 3-1: Plan nematode interactions and host response (II). Room: Plenary Room**
Chairs: Carolina Escobar and Jose Domingo Domínguez.
Defence oral communication: from 34 to 39.

17:30-19:00 / **Oral parallel session 3-2: New nematicidal products: Botanicals and Agrochemicals (I). Room 2.**
Chairs: MariFe Andrés and Pier Luigi Caboni.
Defence oral communication: from 40 to 45.

17:30-19:00 / **Oral parallel session 3-3: Biodiversity, Distribution and Ecology of PPN, EPN, and free-living (II). Room 3.**
Chairs: Sergio Alvarez Ortega and Sergei Subbotin.
Defence oral communication: from 46 to 50.

Wednesday, 17th April 2024

08:30-09:30 **Plenary sessions**
Chairs: Raquel Campos Herrera and Miguel Talavera

08:30-09:00 / **Plenary session 4**
Advancing Research on Entomopathogenic Nematodes:
A Career Retrospect and Prospects for the Future
Speaker: Patricia Stock

09:00-09:30 / **Plenary session 5**
Going Bananas? a paper trail to a nematode's nightmare!
Speaker: Danny Coyne

09:30-11:00 **Oral parallel sessions**

09:30-11:00 / **Oral parallel session 4-1: Workshop: Advanced technologies and potential impact on nematode management: State-of-the-Art and future potential. Room: Plenary Room**
Chair: Richard Sikora

09:30-11:00 / **Oral parallel session 4-2: Nematode resitance genes and virulence I. Room 2**
Chairs: Aska Goverse and Lida Derevnina.
Defence oral communication: from 55 to 60.

09:30-11:00 / **Oral parallel session 4-3: Quarantine Nematodes: Diagnostics and management / Legal and regulatory aspects. Room 3**
Chairs: Lee Robertson and Maria Grossi de Sa.
Defence oral communication: from 61 to 66.

11:00-11:30 **COFFEE BREAK**

11:30-13:00 **Oral parallel sessions**

11:30-13:00 / **Oral parallel session 5-1: Plant nematode interactions and host response (III). Room: Plenary Room**
Chairs: Carolina Escobar and John Jones.
Defence oral communication: from 67 to 72.

11:30-13:00 / **Oral parallel session 5-2: Cultural management of PPN: Cover crops, organic amendments, ASD (I). Room 2**
Chairs: Jose Antonio López Pérez and Caridad Ros Ibañez.
Defence oral communication: from 73 to 78.

11:30-13:00 / **Oral parallel session 5-3: Biodiversity, Distribution and Ecology of PPN, EPN, and free-living (III). Room 3.**
Chairs: Antonio Archidona Yuste and Inmaculada Criado Navarro.
Defence oral communication: from 79 to 84.

13:00-13:45 **Short oral presentations I**
Chair: Miguel Talavera and Antonio Archidona Yuste.
Defence short oral communication: from 85 to 92.

13:45-15:15 **LUNCH**

16:00-20:00 **Symposium Tour**

Thursday, 18th April 2024

08:30-09:30 **Plenary sessions**
Chairs: Juan Emilio Palomares-Rius and Sara Sánchez Moreno

08:30-09:00 / **Plenary session 6**
How do plants fight against parasitic nematode attack? And can we help them?
Speaker: Tina Kyndt

09:00-09:30 / **Plenary session 7**
Nematode ecology: insights and applications
Speaker: Thomais Kakouli-Duarte

09:30-11:00 **Oral parallel sessions**

09:30-11:00 / **Oral parallel session 6-1: Nematodes as bioindicators and nematode community assemblies. Room: Plenary Room**
Chairs: Sara Sánchez Moreno and Thomaé Kakouli.
Defence oral communication: from 95 to 99.

09:30-11:00 / **Oral parallel session 6-2:Entomopathogenic nematodes(II).Room 2.** Chairs: Rubén Blanco Pérez and Antoinette Malan.
Defence oral communication: from 100 to 106.

09:30-11:00 / **Oral parallel session 6-3: Nematode interactions with other organisms: Complex diseases and Biocontrol (II). Room 3**
Chairs: Luis Vicente López-Llorca and Laura Rosso.
Defence oral communication: from 107 to 112.

11:00-11:30 **COFFEE BREAK**

11:30-13:00 **Oral parallel sessions**

11:30-13:00 / **Oral parallel session 7-1: Integrated Management of PPN (II). Room: Plenary Room**
Chairs: Emmanuel Tzortzakakis and Tim Thoden.
Defence oral communication: from 113 to 118.

11:30-13:00 / **Oral parallel session 7-2: Entomopathogenic nematodes (III). Room 2**
Chairs: Raquel Campos and Bart Vandenbossche.
Defence oral communication: from 119 to 124 and 139.

11:30-13:00 / **Oral parallel session 7-3: Nematode interactions with other organisms: Complex diseases and Biocontrol (III). Room 3**
Chairs: Federico López Moya and Rosa Manzanilla.
Defence oral communication: from 125 to 130.

13:00-13:45 **Poster Session II**
Defence poster communication: from 240 to 300.

13:45-15:15 **LUNCH**

15:15-16:00 **Poster Session II**
Defence poster communication: from 240 to 300.

16:00-17:00 **Oral parallel sessions**

16:00-17:00 / **Oral parallel session 8-1 Plant-nematode interactions and host response (IV). Room: Plenary Room**
Chairs: Shahid Siddique and Jap-Jaan Willig.
Defence oral communication: from 135 to 138.

16:00-17:00 / **Oral parallel session 8-2: Cultural management of PPN: Cover crops, organic amendments, ASD (II). Room 2**
Chairs: Matthew Back and Johan Desaeger.
Defence oral communication: from 131 to 134.

16:00-17:00 / **Oral parallel session 8-3: Nematode Taxonomy, Systematics and Diagnosis. Room 3**
Chairs: Joaquín Abolafia and Oleksander Holovachov.
Defence oral communication: from 139 to 142.

17:00-17:30 **COFFEE BREAK**

17:30 -19:00 **ESN General Meeting**

20:30-24:00 **GALA DINNER**

Friday, 19th April 2024

09:00-09:30 **Plenary session**
Chairs: Pablo Castillo and Joaquin Abolafia

09:00-09:30 / **Plenary session 8**
Phylogenomics of Nematoda – thinking beyond the trees
Speaker: Oleksandr Holovachov

09:30-11:00 **Oral parallel sessions**

09:30-11:00 / **Oral parallel session 9-1 Integrated Management of PPN (III). Room: Plenary Room**
Chairs: Juan Emilio Palomares-Rius and Wim Wesemael.
Defence oral communication: 144, 145, 146, 147, 149 and 175.

09:30-11:00 / **Oral parallel session 9-2: Nematode resistance genes and virulence (II). Room 2**
Chairs: Xavier Sorribas and Soledad Verdejo-Lucas.
Defence oral communication: from 172 to 174, 176 and 177.

09:30-11:00 / **Oral parallel session 9-3: New nematicidal products: Botanicals and Agrochemicals (II). Room 3**
Chairs: Yuki Oka and Ioannis Giannakou.
Defence oral communication: 148,156,157,159 and 160.

11:00-11:30 **COFFEE BREAK**

11:30-13:00 **Oral parallel sessions**

11:30-13:00 / **Oral parallel session 10-1: Plant nematode interactions and host response (IV). Room: Plenary Room**
Chairs: Catherine Lilley and Sophie Mantelin.
Defence oral communication: from 161 to 166.

11:30-13:00 / **Oral parallel session 10-2: Nematodode Phylogeny, Philogeography and Phylogenomic. Room 2**
Chairs: **Pablo Castillo and Wim Bert**.
Defence oral communication: from 167 to 171.

11:30-13:00 / **Oral parallel session 10-3: Role of soil microbiome in nematode suppression. Room 3**
Chairs: **Ariadna Giné and Olivera Topalovic**.
Defence oral communication: from 150 to 155.

13:00-13:45 **Short oral presentations II**
Chair: **Miguel Talavera and Antonio Archidona Yuste**.
Defence short oral communication: from 178 to 180 and from 182 to 185.

13:45-15:15 **LUNCH**

ORAL PLENARY COMMUNICATIONS

1. Vegetable production in solar plastic-houses infested with root-knot nematodes. *Soledad Verdejo-Lucas and Miguel Talavera*

2. Custom low-cost robotics and AI: Plant-parasitic nematode phenotyping, at scale. *Sebastian Eves-van den Akker*

3. Vertical and horizontal transgenerational epigenetic inheritance of learned pathogen avoidance in *C. elegans*. *Coleen T. Murphy*

52. Advancing research on Entomopathogenic Nematodes: A career retrospect and prospects for the future. *S. Patricia Stock*

53. Going Bananas? A paper trail to a nematode's nightmare! *Danny Coyne, Laura Cortada and Solveig Haukeland*

93. How do plants fight against parasitic nematode attack? And can we help them? *Tina Kyndt*

94. Nematode Ecology: Insights and applications. *Thomais Kakouli-Duarte*

143. Phylogenomics of Nematoda - Thinking beyond the trees. *Oleksandr Holovachov, Erna King and Qing Xue*

1. Vegetable production in solar plastic-houses infested with root-knot nematodes

Soledad Verdejo-Lucas[1], Miguel Talavera[2]
1 IFAPA Centro La Mojonera, Junta de Andalucia, Almería, Spain
2 IFAPA Centro Alameda del Obispo, Junta de Andalucia, Córdoba, Spain

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: root-knot nematodes, solar plastic-houses, management

ABSTRACT:

Solar plastic-houses use the sunlight directly to facilitate crop cultivation by enhancing plant growth and productivity. Solanaceous crops (tomato, sweet pepper, aubergine) are grown in monoculture or in double cropping systems with Cucurbits (cucumber, melon, zucchini, watermelon). In Spain, *Meloidogyne incognita* and *M. javanica* are the most prevalent species of root-knot nematodes (RKN). Cucurbit crops are all hosts to RKN but differ in suitability level and response to RKN species. Thus, zucchini and watermelon are poorer hosts than cucumber and melon. *Meloidogyne incognita* showed lesser reproductive fitness but greater pathogenic potential than *M. javanica* on zucchini. On watermelon, the reproductive fitness of *M. javanica* was lesser than *M. incognita* whereas both RKN species reproduce similarly on cucumber. The estimated tolerance limits (nematode density below which no measurable yield loss occurs) were 0.01, 20, and 402 M. *javanica* /100 cm3 soil for cucumber, watermelon and zucchini, respectively. Maximum yield losses were estimated to be 88% in cucumber, 53% in zucchini and 35% in watermelon. Nematode build-up and yield losses are affected by the growing season so that lower tolerance limit and higher yield losses occurred in autumn than spring plantings of tomato and zucchini. Soil fumigants have the highest efficacy (78-87%) against RKN followed by non-fumigant nematicides (51-64%) and natural products and essential oils (41-48%). Most nematicidal treatments managed RKN infestation levels of about 2 juveniles/cm3 soil and maintained profitability of all vegetable crops in solar-plastic houses. Levels above 3.5 to 4 juveniles/cm3 soil were only managed by treatments with efficacies superior to 70%. Nonetheless, final populations are usually similar in treated and untreated plots with inconsistent results in yield responses. Solarization in the summer months is a routine practice in RKN infested soils in Southeastern Spain. Grafting exploits specific resistance genes or non-host resistance mechanisms. Most tomato rootstocks show partial RKN resistance, they reduce root galling providing tolerance to the nematode. Virulent populations are rapidly selected after repeated cultivation of some rootstocks. Watermelon is commonly grafted onto squash hybrids, all susceptible to RKN which show higher root galling and reproduction than un-grafted watermelon. Grafting onto watermelon rootstocks such as *Citrullus amarus* reduced root galling and reproduction compared to un-grafted watermelon. Management decisions will depend on RKN species, initial populations, crop susceptibility and growing season.

2. Custom low-cost robotics and AI: Plant-parasitic nematode phenotyping, at scale

Sebastian Eves-van den Akker[1]
1 The University of Cambridge, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: plant-parasitic nematodes, AI, forward genetics

ABSTRACT:

Plant-parasitic nematodes are a major threat, and in some crops the dominant threat, to food security throughout the world. A central tenet of the discipline is to advance our understanding of plant-parasitic nematode biology in sufficient detail to alleviate their threat to food security. There is an expectation that the development of functional genetic tools will accelerate the progress of research on plant-parasitic nematodes, and thereby the development of novel control solutions.

This talk will describe both recent and rapid progress in developing low-cost, AI-powered, phenotyping using 3D printing and Raspberry Pi computers. In developing this technology to address a bottle neck in our research, something more important emerged - the ability to be truly holistic (i.e. phenotyping every individual nematode on the whole plant) and dynamic (i.e. across two complete life cycles of the nematode) in our analysis of host infection. We think this approach sets a precedent for pathology in general and will allow genetic dissection of entirely new aspects of plant-nematode interactions.

3. Vertical and horizontal transgenerational epigenetic inheritance of learned pathogen avoidance in *C. elegans*.

Coleen T, Murphy[1]
1 Princeton University, USA

Area:
Type: Oral
Keywords:

52. Advancing research on Entomopathogenic nematodes: A career retrospect and prospects for the future

S. Patricia Stock[1]
1 College of Agriculture, California State University, Chico, CA, USA

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: entomopathogenic nematodes, bacterial symbionts, mutualism, model systems

ABSTRACT:

The year 2023 marked the 100th anniversary of the discovery of the first entomopathogenic nematode (EPN) species, *Steinernema kraussei*. The field of entomopathogenic nematology has grown exponentially over the past decades, branching research into diverse basic and applied fields. Early on, EPN were solely viewed and as alternatives to chemical pesticides, and most of the research efforts were placed on the discovery of new species and strains, as well as in the understanding of the biotic and abiotic parameters that enhance their performance in insect pest management. However, as we entered the 21st century, and with the advent of molecular biology, we gained a deeper understanding of EPN and their bacterial symbionts, specifically in relation to their complex symbiotic relationship. Furthermore, recognition of their experimental tractability awarded them the status of model systems for the study of interactions between microbes and their eukaryotic hosts. In this presentation, I will summarize research contributions in the field of entomopathogenic nematology, with emphasis on my scientific trajectory of 30 years. I will also discuss prospects for future research and applications.

53. Going Bananas? A paper trail to a nematode’s nightmare!

Danny Coyne[1,2], Laura Cortada[2,3], Solveig Haukeland[4,5]
1 Institute of Tropical Agriculture (IITA), Nairobi, Kenya
2 Nematology Research Unit, Department of Biology, Ghent University, Belgium
3 International Plant Biotechnology Outreach - Vlaams Instituut voor Biotechnologie (VIB-IPBO), Ghent, Belgium
4 International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
5 Norwegian Institute for Bioeconomy Research, As, Norway

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: bio-circular economy, biodegradable, climate smart, ligno-cellulose matrix, sustainable crop protection

ABSTRACT:

Banana fibre may not at first appear special - but it is! The particular combination of cellulose, hemicellulose and lignin creates a special matrix, with properties that enable it to tightly bond to certain chemical compounds. This was discovered when assessing various biodegradable materials that could be used as a novel delivery mechanism for abamectin. Banana fibre paper supported impregnation with the lowest (ultra-low) effective dosages of abamectin and provided the longest shelf life. It also results in a slow release of abamectin as the bonds gradually deteriorate in the ground. Controlled studies then provided a glimpse of the potential impact this could have for environmentally sensitive nematode management. Initially, vegetatively propagated root and tuber crops, such as yam, were the focus. The aim was to reduce losses due to nematodes on key food crops in smallholder cropping systems in sub-Saharan Africa. Seed material wrapped in the impregnated paper would be protected during the critical, early stages of root development. Further studies on potato against potato cyst nematodes gave rise to remarkable, if not unbelievable, results. A key to this appears to lie in the ability of the paper to adsorb semiochemical attractants from root exudates, disrupting nematode host location. So, even without impregnation with a nematicide, the paper is still effective, the nematicide provides additional knockdown. Furthermore, the paper is effective as a carrier for biologically based products, such as biocontrol agents. Moving onto other crops and other pest targets is currently being looked into. Meanwhile efforts are underway to develop more farmer-usable products in Africa. Follow the trail, from discovery, through farmer validation, to implementation. Is this real? Or are we going bananas? You be the judge!

93. How do plants fight against parasitic nematode attack? And can we help them?

Tina Kyndt[1]
1 Ghent University, Campus Proeftuin, Proeftuinstraat 86, building N1, Ghent, Belgium

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: induced resistance, plant immunity

ABSTRACT:

Plants dominate most ecosystems on our planet, where they encounter herbivores, pathogens, and parasites. Although immobility prevents plants from running away from their potential attackers, disease is rather the exception than the rule in natural ecosystems. A specialized plant immune system is at play to ward off enemies, showing parallels but also many differences with animal immunity. For example, there are remarkable similarities in the cellular responses triggered by pathogen-associated molecular patterns. However, in contrast to animal immunity, plant immunity is not based on specialized circulating cells and antibodies but on morphological adaptations and chemicals that are synthesized continuously or activated upon stress. This inducible plant immunity can be triggered by pathogen attack, but also by deliberate external stimulation with compounds, leading to a phenotype called ‘Induced resistance (IR)’. IR increases the efficiency of the plant’s immune system and enables it to be more competent in fighting off future challenges. During my talk, I will illustrate how work from my research team and many others has uncovered the potential of IR in plant protection against parasitic nematodes. I will demonstrate how studying plant responses to infestation, and nematode interference with plant immunity, has supported the identification of bioprotectant compounds that effectively induce resistance against plant-parasitic nematodes. I will describe the current knowledge about the mechanisms underlying IR and outstanding questions. Moreover, the application of IR in agronomy still requires more research to investigate for example how long IR can be ‘memorized’, how many treatments are needed, what are the potential side-effects, and whether these can be avoided. Finally, I will give some insights into the way plants ‘memorize’ past stress events through epigenetic changes, leading to long-lasting and in some cases even inter/trans-generational resistance.

94. Nematode Ecology: insights and applications

Thomais Kakouli-Duarte[1], Anna Karpinska[1], Anusha Pulavarty[1], Loriane Murphy[1], Tiernan Kavanagh[1]
1 South East Technological University, Kilkenny Road Campus, Carlow R93 V960, Ireland

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: nematodes, bioindicators, ecology, nematode communities, c-p scale

ABSTRACT:

Soil is a precious but non-renewable resource and a pillar for the stability of human societies and civilization, by primarily providing food security. A healthy soil provides additional desired ecosystem services. But, in the last decades, due to anthropogenic and other factors, humanity is challenged with soil degradation.

In terrestrial ecosystems a great deal of focus falls on physicochemical components of soil without consideration to its biotic elements, this one-sided approach additionally contributes to unhealthy soil. Establishing unfavourable conditions to soil biota causes loss of valuable soil ecosystem services. Biodiversity is a critical soil factor which increases the magnitude of each service and therefore soil health. Nematodes and their communities both directly contribute and indicate the support of other soil biota towards soil health. Nematodes constitute the highest proportion of and occupy the major trophic levels in soil food webs. They can thus be classified into functional guilds that can be utilised in nematode faunal analyses, which in turn aid towards concluding on the condition of the soil food webs.

This presentation will showcase work carried out in Ireland. During the ecological impact assessment of recycling derived fertilisers, in the part funded Interreg-NWE project ReNu2Farm (www.nweurope.eu/renu2farm), nematode assemblages were studied, revealing that organic waste struvites were sustainable replacements to mineral, non-renewable forms of imported phosphorus. In a currently ongoing study, aiming to evaluate the efficiency of the rewetting of Irish peatlands to restore the respective ecosystems, a similar approach is being followed, nematode assemblages are analysed to evaluate the success of this peatland restoration project. First results showed that healthy bog sites were dominated with algal and moss-feeding nematodes (families Achromadoridae and Teratocephalidae). Degraded bog habitats were dominated by plant parasitic nematodes, with more than 50% belonging to the family Pratylenchidae. Wasted peat grassland habitats had more omnivores belonging to Dorylaimidae. In a study characterising the effects of microplastics in the River Barrow, results revealed a decreasing pattern of species abundance and negatively affected nematode communities in sediments, in polluted sites. Motorway drainage lagoons act as sinks for motorway drainage runoff and can accumulate high levels of pollution. Studying M9 motorway lagoons in SE Ireland, nematode assemblage analyses demonstrated the stressed state of these habitats and pointed towards significant improvements required to remediate the waters before their release back to the environment. It is envisaged that this presentation will provide a view on the breadth of applications that nematode ecology can facilitate.

Acknowledgements: EU Interreg-NWE, Irish Research Council, Green Restoration Ireland Coop.

143. Phylogenomics of Nematoda - Thinking beyond the trees

<u>Oleksandr Holovachov</u>[1], Erna King[2], Qing Xue[3]
1 Swedish Museum of Natural History, Sweden
2 The Wellcome Sanger Institute, UK
3 Nanjing Agricultural University, China

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords:

ABSTRACT:

Continuous improvement in sequencing technologies increases access to more and better-quality genomic data that can be used to infer nematode phylogeny. However, since the first 18S rDNA based phylogenetic study, the overall topology of the nematode tree has not changed and is not expected to. Adding more genomes for more species as well as adding better quality genomes considerably improves tree topology in some lineages that have so far been underrepresented. However, the strategy of sequencing random selection of species should give way to more lineage focused and hypothesis driven projects, aimed at answering specific questions. Several of such questions will be discussed during the presentation.

54. Advanced technologies and potential impact on nematode management: State-of-the-Art and future potential.

Richard A. Sikora[1], **Sebastian Eves-van den Akker[2]**, **Leendert Molendijk[3]**, **Johannes Hallmann[4]**

1 Institute of Crop Science and Resource Conservation INRES, University of Bonn, Bonn, Germany
2 Department of Plant Sciences, University of Cambridge, Cambridge, UK
3 Wageningen University and Research, Field Crops, Lelystad, The Netherlands
4 Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Workshop - Oral
Keywords: advanced technologies

ABSTRACT:

Integrated nematode management relies on new technologies to improve crop health. This workshop is designed to discuss the impact of technologies, presently in the research pipeline, on nematode management programs. The advanced tools being covered will include: 1) remote sensing and precision agriculture, 2) gene editing, 3) digital support tools, 4) the microbiome and 5) biological control. The speakers will give short presentations designed to stimulate audience participation.

In these presentations, attempts will be made to 1) underscore positive and negative aspects of these technologies as it relates to practical use in nematode management and 2) estimate a timeline for broad market entry.

Discussion points to stimulate discussion will include: 1) why is remote sensing and precision agriculture not used in INM 2) genome editing based solutions to plant-parasitic nematodes will not be in fields any time soon 3) why are digital support tools not widely used and 4) is the microbiome for ecologists or for improved INM 5) where are the biological control products?

The positive impact of these technologies on nematode management on a global scale could be enormous. However, agricultural practice varies greatly by country, therefore, the saying ‘one size does not fit all’ will be reflected upon. We anticipate that the panel’s statements will generate an atmosphere that leads to productive discussion with the colleagues in the audience.

Oral COMMUNICATIONS

4. Distinct roles of E2fa and E2fb transcription factors in nematode feeding sites: regulators of DNA replication and in response to replication stress. *Janice de Almeida Engler, Danila Cabral, José Lozano, Geert Smant, Gilbert Engler and Lieven De Veylder*

5. Tricky parasites: How nematodes take their vitamins from plants. *Clarissa Hiltl, Sherouk Khalifa, Michelle Stankiewicz, Shiela P. Quiobe, Nora Druschel, Laura Oé and Florian M. W. Grundler*

6. NILR1 perceives a nematode ascaroside triggering immunity and is targeted by the nematode effector RHA1B. *Li Huang, Yulin Yuan, Chloe Lewis, Joanna Kud, Joseph Kuhl, Allan Caplan, Louise-Marie Dandurand and Inga Zasada*

7. Copper microRNAs modulate the formation of giant feeding cells induced by the root knot nematode *Meloidogyne incognita* in *Arabidopsis thaliana* and *Solanum lycopersicum*. *Stéphanie Jaubert*

8. Mining new interactions between PPN effectors and plant transcription factors via CrY2H. *Huy Xiang, Boris Stojilkovic and Godelieve Gheysen*

9. Unraveling Cytosolic Ca2 signaling in the early *Heterodera schachtii* infection. *M. Shamim Hasan, Chhana Ullah, Clement Pellegrin, Sakil Mahmud, Sharon Letia, Miroslaw Sobczak, Ute C. Vothknecht and Sebastian Eves-van den Akker*

10. *Pratylenchus* spp. dominate nematode food webs in a legume-based organic farming system. *Jan Henrik Schmidt, Morten Moeller, Miriam Athmann and Christian Bruns*

11. Unraveling the ecological dynamics of cave-dwelling nematodes in Dripkelder cave, South Africa. *Gerhard Du Preez, Marconi Souza Silva, Driekie Fourie, Chantelle Girgan, Edward Netherlands, Antoinette Swart and Rodrigo Ferreira*

12. Effects of agricultural intensification on soil nematode communities in freshwater streams. *Rosa Ana Salazar-García, Antonio Archidona-Yuste, Pablo Castillo and Gracia Liébanas*

13. Distribution of *Meloidogyne enterolobii* in vegetable crops in the southeastern United States. *Johan Desaeger*

14. Protected forests benefit soil biodiversity and function in the North of Portugal. *Carolina Duarte, Carlos Guerra and Sofia Costa*

15. Insights into the diversity and frequency of nematode communities in paddy fields. *Leidy Rusinque, Carla Maleita, Ana Rita Varela and Maria Lurdes Inacio*

16. Are EPNS attracted or repelled by the black truffle and its volatile organic compounds? A novel perspective for truffle beetle biocontrol. *Ivan Julià Brunat, Ivan Hiltpold, Ana Morton and Fernando Garcia del Pino*

17. Use of *Heterorhabditis bacteriophora* for the biological control of *Popillia japonica* larvae and evaluation of its impact on non-target soil biota. *Giulia Torrini, Immacolata Iovinella, Chiara Sciandra, Agostino Strangi, Stefania Simoncini, Giuseppe Mazza, Francesco Barbieri, Gian Paolo Barzanti and Francesco Paoli*

18. All strains are not the same: 42 international *Heterorhabditis bacteriophora* strains exhibit differential virulence rates to *Popillia japonica* larvae. *Anna Garriga, Hugo R. Monteiro, Rubén Beltrí, Duarte Toubarro and Nelson Simões*

19. Non-Effects of *Xenorhabdus* and *Photorhabdus* (Morganellaceae) unfiltered ferments on *Saccharomyces cerevisiae* during alcoholic fermentation of reconstituted grape must and on volatile composition of Tempranillo Must. *Ignacio Vicente-Díez*

20. Insecticide-free production of oilseed rape: Role of EPN to contribute to agro-ecosystem management. *Ralf-Udo Ehlers*

21. Rapid desiccation of entomopathogenic nematodes: Evaluating the physiological demands. *Itamar Glazer, Jayashree Ramakrishnan, Liora Salame, Mani Karthik Ananth , Reut Fledbaum, Mohammed Samara, Ahmed Nasser and Guy Mechrez*

22. Nematodes in sport turf: Strategies against the lack of nematicidal solutions. *Kristell Santander*

23. Potential management of *Meloidogyne enterolobii*, a regulated quarantine nematode species in the European Union. *Sebastian Kiewnick, Hemanth Gopal, Marine Poullet and Etienne Danchin*

24. Important agricultural nematodes and Integrated Nematode Managements (INM) in China. *Deliang Peng*

25. Nematicide efficacy at managing root-knot nematode in sweet potato in the United States. *Chang Liu, Zane Grabau and Rebeca Sandoval Ruiz*

26. Zoophytophagous mirid predators (Heteroptera: Miridae) induce systemic resistance in tomato against *Meloidogyne* spp. *Alejandro Expósito, Aida M Fullana, Alberto Urbaneja, Meritxell Pérez-Hedo, Pablo Urbaneja-Bernat, Jordi Riudavets, Ariadna Giné and Francisco Javier Sorribas*

27. Effect of interactions of plant-parasitic nematodes and *Verticillium dahliae* on peppermint. *Ilya Noskov, Katja Sommerfeld-Arnold and Johannes Hallmann*

28. The nematode-antagonistic fungi *Niesslia gamsii* and *Polydomus karssenii* are potential candidates for biocontrol of cyst and root-knot nematodes. *Milad Rashidifard, Wolfgang Maier and Samad Ashrafi*

29. *Allodiplogaster sudhausi* (Nematoda: Diplogasteridae), a potential predator for PPN control: Biology, production and safety. *Loel Bacolod Dalan, Silas Bett, Temesgen Addis Desta, Yitzhak Spiegel, Sigal Brown Miyara, Ohad Zuckerman, Wim Bert and Ralf-Udo Ehlers*

31. Parasite transmission in Svalbard reindeer in a rapidly changing climate: experimental studies on dispersal behaviour of a reindeer parasitic nematode. *Tirza Moerman*

32. Bioinformatic approach to identify gut related targets with potential to mitigate parasitic infection. *Liliana Jeziorska, James Dillon, Vincent O'Connor and Marcus Guest*

33. Targeted Proteomics as precise tool for biomarkers characterization of *Anisakis simplex* complex species: epidemiological implication. *Susana C. Arcos, Rosa González López, Sergio Ciordia, Marina Usieto, Fátima M. Santos, Yolanda Jiménez Ruíz, Lee Robertson and Mercedes Careche*

34. The MI2G02 effector of *Meloidogyne incognita* hijacks host plant trihelix transcription factor for parasitism. *Jianlong Zhao and Zhenchuan Mao*

35. *Meloidogyne incognita* and the host range paradox: RNAseq analysis of different infested hosts highlight the complexity of root-knot parasitism. *Victor Hugo Moura de Souza, Clement Pellegrin, Unnati Sonawala, Dio Sangwon Shin, Olaf Kranse, Junior Lusu Kika, Beatrice Senatori and Sebastian Eves-van den Akker*

36. Cross-Kingdom RNAi in plant root-knot nematode interactions. Ange Dussutour, Yara Noureddine, Martine da Rocha, Karine Mulet, Ann-Po Cheng, Arne Weiberg, Bruno Favery and Stéphanie Jaubert

37. SUGR: the Subventral Gland master Regulator of plant-parasitic cyst nematodes. *Anika Damm, Clement Pellegrin and Sebastian Eves-van den Akker*

38. Heat Tolerance of distinct geographic populations of *Meloidogyne incognita* is possessed by intrinsic thermal acclimation reaction: Nematode response to Global Warming. *Alkesh Hada, Patricia Bucki, Nathalia Sichov , Eyal Glanz, Noa Sela and Sigal Braun Miyara*

39. Transcription Factors in the plant-endoparasitic nematode interaction: Insights of gene regulatory Networks. *Jose Domínguez-Figueroa, Patricia Abril-Urias, Fernando Díaz-Manzano, Ángela Martínez-Gómez, Almudena Gómez-Rojas, Alba Carrión-Escudero and Carolina Escobar*

40. Do biodegradation products of dehydroascorbic acid contribute to its bioactivity as nematicidal compound and inducers of plant resistance? *Farzana Haque Tumpa, Eva Degroote, Satish Namdeo Chavan and Tina Kyndt*

41. Foliar nematode control on ornamental fern with new reduced risk nematicides and evaluating potential phytotoxicity of several new nematicides. *Zhiqiang Cheng*

42. New nematicidal agents from fungal endophytes. *Carmen Elisa Diaz, Maria Fe Andrés and Azucena Gonzalez-Coloma*

43. Melatonin S Protective Role: Enhancing Plant Growth and Mitigating Parasitic Nematode Infestation. *Yuliia Zhukova, Aryani Sianipar, Letia Sharon, Florian M.W. Grundler and M. Shamim Hasan*

44. Nematicidal evaluation of a coded compound against potato cyst nematodes (*Globodera pallida*). *Niru Tripathi, Simon Woods, Katarzyna Dybal, Grace Milburn and Matthew Back*

45. Pesticide selectivity: Finding novel targets in *Caenorhabditis elegans*. Henry Nvenankeng, *Fernando Calahorro, Jim Goodchild, Philippa Harlow, Lindy Holden-Dye and Vincent OConnor*

46. Characterisation and management of *Globodera rostochiensis* populations adapted to Kenyan sub-tropical conditions. *James Price, Kelsey Hunt, Solveig Haukeland, Calvince Orage, Onesmus Mwaura, Newton Nelsi, Danny Coyne and John Jones*

47. Nematode diversity and soil functions under different land uses at a regional scale. *Rui Carvalho, Carlos Guerra and Sofia Costa*

48. Plant-parasitic nematodes associated with chickpea from Ethiopia: damage potential of *Meloidogyne* spp. And identifying resistant varieties. *Habtamu Kefelegn, Beira H. Meressa, Marjolein Couvreur, Wim M. L. Wesemael, Misghina G. Teklu and Wim Bert*

49. Exploring the diversity and occurrence of major Plant-Parasitic Nematodes In vineyards across Northern and Central Spain. *Maria Del Mar González-Trujilo, Rubén Blanco-Pérez, Raquel Campos-Herrera and Sergio Álvarez-Ortega*

50. Nematode communities from strawberry fields: a comparison between morphology and mitometagenomics. *Dorota L. Porazinska, Eli Gendron, Clemen Oliveira and Johan Desaeger*

55. Identification and characterization of potato cyst nematode resistance genes. *Moray Smith, Ingo Hein and John Jones*

56. AI-powered holistic and dynamic plant-pathology to deliver new sources of resistance. *Siyuan Wei and Sebastian Eves-van Den Akker*

57. Identification of putative resistance-breaking effectors of *Globodera pallida* by systematic screening of a genomic region associated with virulence on *Gpa5*-resistant potatoes. *Arno Schaveling, Dennie te Molder, Stefan van de Ruitenbeek, Casper van Schaik, Geert Smant and Mark Sterken*

58. Unraveling the function of the cyst nematode resistance gene *Hs4* in different genomic backgrounds. *Annika Schildberg and Christian Jung*

59. Cyst nematodes counteract immunity by inhibiting activation of central nodes of a Solanaceae immune receptor network. *Lida Derevnina*

60. Use of virulent *Globodera pallida* lines obtained from experimental evolution to reveal candidate regions for the adaptation to potato resistances. *Océane Lechevalier, Magali Esquibet, Stéphanie Daval, Kévin Gazengel, Eric Grenier, Sylvain Fournet and Josselin Montarry*

61. European reference laboratories: a key tool for diagnosis and networking in the EU plant health field. *Maíra Grossi de Sá, Nicole Viaene, Sylvie Gamel, Nicole Damme, David Hatcher, Niels Vermassen, Françoise Munaut and Laurent Folcher*

62. Beech Leaf Disease, caused by the nematode *Litylenchus crenatae mccannii*, poses a threat to beech trees in Europe. *Nicole Viaene, Negin Ebrahimi, Annelies Haegeman, Anne Sophie van Bruggen, Nikica Ogris, Saša Širca, Thomas Prior, Ana Pérez Sierra and Mihaela Coman*

63. Detection of *Aphelenchoides besseyi* on rice seeds, a new official French method. *Justine Foucher, Damien Gonzalez, Céline Andro, Delphine Leroux-Bouly, Juliette Lechat, Valérie Grimault, Nicolas Denancé, Isabelle Sérandat, Thomas Baldwin, Amandine Le Van, and Jaiana Malabarba*

64. Possible spread of *Meloidogyne enterolobii* in potted ornamental plants in flooding water systems. *Anne Sophie van Bruggen, Rik Peters, Johnny Visser and Evelyn van Heese*

65. Two decades of epidemiological surveillance of the pine wood nematode in France. *Nicolas Mariette, Hoël Hotte, Anne-Marie Chappé, Marie Grosdidier, Géraldine Anthoine, Corinne Sarniguet, Odile Colnard, Emmanuel Kersaudy and Emmanuel Koen*

66. Chemical and physical methods to control the propagation of *Bursaphelenchus xylophilus* in wood materials. *Lee Robertson, Sara Santos, Susana C Arcos, Navas Alfonso and Teresa de Troya*

67. Wound-induced tissue-specific lignin accumulation as a root's immune response to cyst nematode infection. *Muhammad Shahzad Anjam, Luciano. M. Di Fino, Nageena Zahid, Shamim Hassan, Miroslaw Sobczak, Tonni G. Andersen, Florian. M.W. Grundler and Peter Marhavy*

68. The Perivitelline egg protein vitellogenin in *Heterodera Schachtii*. *Hannah Wileman, Roland Perry and Keith Davies*

69. Manipulation of plant nucleolar functions by MIEFF186, a novel root-knot nematode effector. *Ranty-Roby Sarah, Soule Salomé, Caravel Claire, Abad Pierre, Favery Bruno and Quentin Michael*

70. Changing perspectives on parasitism: on how plants accommodate, tolerate, and resist nematodes. *Geert Smant, Jaap-Jan Willig, Nina Guarneri, Mark Sterken and Jose Lozano Torres*

71. The effectorome of a plant parasitic cyst nematode. *Beth Molloy and Sebastian Eves-van den Akker*

72. E*luci*dating the role of MigPSY Peptides in plant root-knot nematode interaction. *Ching-Jung Lin, Henok Zemene Yimer, Dee Dee Luu, Alison Coomer Blundell, Maria Florencia Ercoli, Paulo Vieira, Valerie M. Williamson and Pamela C. Ronald*

73. Efficiency of organic amendments and crop mulching on soil biodiversity in horticultural crops with low carbon stocks and reduced water availability at the Central area of the Iberian Peninsula. *Jose Antonio López Pérez, Carlos García-Villarrubia and Sara Sánchez-Moreno*

74. Evaluation of 5 green manure species for the control of *Meloidogyne incognita* in a horticultural crop rotation under greenhouse at the Central area of the Iberian Peninsula. *Carlos Garcia-Villarrubia Bernabé, Marta María Moreno Valencia and José Antonio López-Pérez*

75. Heating tare soil for disinfestation from potato cyst nematodes: from laboratory to industrial implementation. *Negin Ebrahimi, Kenny Couvreur, Nancy de Sutter and Nicole Viaene*

76. Organic management of *Xiphinema Index* in Franciacorta Vineyards, North Italy. *Alessio Vovlas, Trifone D Addabbo, Alberto Troccoli, Elena Fanelli, Valentina Papeschi, Stefano Toninelli and Francesca De Luca*

77. Management of potato cyst nematodes using different organic amendments and cover crops. *Javier Lopez Robles and Jose Antonio López Pérez*

78. *Tagetes* species for root-knot nematode management in tomato agrosystems. *Cliven Njekete, Anne-Violette Lavoir and Caroline Djian-Caporalino*

79. Diversity and composition of soil and rhizosphere microbial communities associated with a susceptible *Prunus* hybrid rootstock under a *Meloidogyne* root gradient infection. *Ilenia Clavero-Camacho, Alba N. Ruiz-Cuenca, Carolina Cantalapiedra-Navarrete, Pablo Castillo and Juan Emilio Palomares-Rius*

80. Europe as a secondary distribution hub in the worldwide invasion of the potato cyst nematode *Globodera rostochiensis*. *Magali Esqibet, Eric Grenier and Josselin Montarry*

81. Soil nematode and microbiota evolution under different soil managements. *Alba N. Ruiz-Cuenca, Ilenia Clavero-Camacho, Antonio Archidona-Yuste, Carolina Cantalapiedra-Navarrete, Pablo Castillo and Juan E. Palomares-Rius*

82. Investigating nematode biodiversity in the Irish boglands. *Anusha Pulavarty, Tilman Klappauf, Douglas MacMillan and Thomais Kakouli-Duarte*

83. A broad overview of the biodiversity in the ring nematode genus *Xenocriconemella* De Grisse and Loof, 1965 (Nematoda: Criconematidae) in the Iberian Peninsula. *Antonio Archidona-Yuste, Carolina Cantalapiedra-Navarrete, Ilenia Clavero-Camacho, Alba Nazaret Ruiz-Cuenca, Guillermo León-Ropero, Jorge Martín-Barbarroja, Gracia Liébanas and Juan E. Palomares-Rius*

84. An update of regulated and recent discoveries of invasive alien plant-parasitic nematodes species in Canada. *Qing Yu*

95. A full-length SSU rRNA-based workflow for high-resolution monitoring of nematode communities reveals direct and indirect responses to plant-based manipulations. *Robbert van Himbeeck, Sara Cazzaniga, Sven van den Elsen , Johnny Visser and Johannes Helder*

96. Biomonitoring of microbial and chemical indoor air toxins with *C. elegans* nematodes. *Sari Paavanen-Huhtala, Karunambigai Kalichamy, Anna-Mari Pessi, Sirkku Hakkila, Annika Saarto, Maria Andersson and Paivi Koskinen*

97. Unveiling soil nematode traits to predict ecosystem functions in changing environments. *Manqiang Liu*

98. Nematode functional trait response to climate change in semi-arid systems. *Miguel Talavera, Jordi Moya-Laraño, Pilar M. Gavín-Centol, Paula Lillo, Antonio Archidona-Yuste, Diana H. Wall and Sara Sánchez-Moreno*

99. Soil nematode communities under a simulated climate change scenario in a semi-arid cropland. *Paula Lillo, Dorota L. Porazinska, Lee Robertson and Sara Sánchez-Moreno*

100. Biodiversity Soil Resilience (BSR): a new index to assess resilience in environmentally stressed ecosystems by including entomopathogenic nematodes. *Giambattista Maria Altieri, Josefina Garrido, Alcides Moino Junior, Cezary Tkaczuk, Vito Santarcangelo, Giuseppe Bari, Salustiano Mato and Eustachio Tarasco*

101. Development of calcium alginate beads containing the nematode *Steinernema feltiae* (SB 12[1]) and plant growth promoting rhizobacteria *Pseudomonas ogarae f113* GFP *Islam Ahmed Abdelalim Darwish, Daniel Martins, David Ryan and Thomais Kakouli-Duarte*

102. Efficacy of entomopathogenic nematodes against *Chrysomela populi* and *Gypsonoma aceriana* in Iran. *Reihaneh Gholami Ghavamabad, Seyedeh Masoomeh Zamani and Majid Pedram*

104. Update on Biodiversity of Entomopathogenic nematodes and their symbiotic bacteria in Italy. *Eustachio Tarasco, Elena Fanelli, Alberto Troccoli, Giulia Torrini, Alessio Vovlas, Giovanna Curto, Leonardo Marianelli, Mirella Clausi and Giancarlo Rappazzo*

105. Domestication of a nematode for biological management of insect pests. *Ralf-Udo Ehlers*

106. Elevational gradients and plant ontogeny as predictors of entomopathogenic nematode activity in natural oak (*Quercus*) populations. *Rubén Blanco-Pérez, Andrea Galmán, Alicia Pou and Xoaquín Moreira*

107. From soil to plant: strengthening carrot defenses against *Meloidogyne incognita* with vermicompost and arbuscular mycorrhizal fungi biofertilizers. *Aasha Rana, Lukman Ahamad, Aashaq Hussain Bhat, Harendra Kumar, Md. Nurul Hasan, Ishtiaq Ahmed, Shakoor Ahmed and Ricardo AR Machado*

108. *In vitro* parasitism of isolates of the nematode-trapping fungus *Arthrobotrys conoides* against *Meloidogyne incognita*. *Cafer Eken, Gulsum Uysal, Dudu Demir, Selda Caliskan, Emre Sevindik and Kardelen Caglayan*

109. Interactions of plant-parasitic nematodes with plant pathogenic soil fungi. *Rik Peters.*

110. *Clonostachys rosea* as a biological control agent for *Meloidogyne incognita*. *Paul Dahlin and Andrea Caroline Ruthes*

111. Bacterial cell cultures: Effects on the infectivity of *Meloidogyne hapla*. *Carlos Cruz, Adriana S. Mata, Paula V. Morais, Diogo N. Proença, Isabel Abrantes e Isabel Luci Conceição*

112. *Pochonia chlamydosporia* a biocontrol agent, biofertilizer, plant vaccine or abiotic plant stress protectant? *Luis Vicente López-Llorca*

113. Investigation of fluazaindolizine as a potential novel tool to manage *Xiphinema index*. *Emmanuel Tzortzakakis, Tim Thoden and Anastasia Chatzaki*

114. Host suitability of *Cucumis metuliferus* and *Citrullus amarus to Meloidogyne chitwoodi*, *M. enterolobii* and *M. luci*. *Aïda M Fullana, Carla Maleita, Duarte Santos, Isabel Abrantes, Francisco Javier Sorribas and Ariadna Giné*

115. Integrated *Globodera pallida* management as part of integrated Crop Management (ICM). The only route to sustainable potato production and maintaining the seed potato export market. *Leendert Molendijk and Johnny Visser*

116. Integrated *Meloidogyne* management, possibilities within the crop rotation to manage *Meloidogyne chitwoodi* and *M. fallax*. *Johnny Visser, Misghina G Teklu and Leendert Molendijk*

117. The IRAC International Nematode Working Group and nematicide mode-of action classification and labelling. *John A Wiles, Bruno Lovato, Julia Huggins, Tim C Thoden, Ursel Collienne, Ralf Nauen, Andrew Crossthwaite, Ekaterini Riga and Russell Eldridge*

118. Reklemel active: a novel tool for integrated nematode management? Key learnings from a global nematicide development project. *Thoden Tim, Yannis Stamatas, Raquel Abad and John Wiles*

119. Understanding excreted/secreted products in *Heterorhabditis bacteriophora*: activation material insights. *Sara Sreibr, Jana Ilgova, Pavel Dobes, Jacek Marciniak, Jiri Vorel, Jana Hurychova, Martin Kasny and Pavel Dobes*

120. Genomics of *in vitro* dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*. *Zhen Wang, Christopher Ogaya, Manoj Dhakal, Francisco Garcia, Verena Dörfler, Mike Barg, Bart Vandenbossche, Olaf Strauch and Carlos Molina*

121. SlugBot: Developing an autonomous monitoring and biocontrol system for slugs. *Jenna Ross OBE, Archita Barua, Faye McDiarmid and Tom Ashford*

122. Exploring the influence of entomopathogenic nematodes on aerial pest control in grape volatile composition. *Mar Vilanova, Miguel Puelles, Bianca S. Costa, Elisabet Vaquero, Javier Manzanares and Raquel Campos-Herrera*

123. Effects of chromium VI on the attraction, percentage penetration and reproduction of the entomopathogenic nematode *Steinernema feltiae in Galleria mellonella*. *Anique Godjo*

124. Defining climatic parameters for optimal timing of EPN application against apple codling moth. *Bart Vandenbossche, Mike Barg, Verena Dörfler, Hartmut Kaiser, Nikolina Grabovac, Thorsten Rocksch, Ralf-Udo Ehlers*

125. Advances in the isolation and characterization of endophytic fungi for the biological control of root-knot nematodes in vegetables in Mexico. *María Gabriela Medina-Canales, Manuel Silva-Valenzuela, Alejandro Tovar-Soto, Reyna Isabel Rojas-Martínez and Rosa Helena Manzanilla-López*

126. The biochemical diversity of the second-stage juvenile surface coat: its origin, role and interaction with *Pasteuria* endospores. *Keith Davies*

127. Dual nematode infection in *Brassica nigra* affects shoot metabolome and aphid performance in distinct contrast to single-species infection. *Jessil Ann Pajar, Stefanie Döll and Nicole M. van Dam*

128. Cyst nematodes are a rich source for the discovery of new nematode antagonistic fungi producing a multitude of new bioactive secondary metabolites. *Samad Ashrafi, Jan-Peer Wennrich, Marc Stadler and Wolfgang Maier*

129. The use of *Bacillus thuringiensis* Cry5 protein to control root-knot nematodes. *Yolanda Bel, Magdalena Galeano, Mireya Baños-Salmerón and Baltasar Escriche*

130. *Pochonia chlamydosporia* and chitosan: A new biocontrol strategy to manage plant parasitic nematodes. *Federico López-Moya*

131. Agronomy matters! Improving the performance of solanaceous trap crops for the management of potato cyst nematodes (*Globodera* spp.). *Matthew Back, Katarzyna Dybal, James Lee, Richard Griffiths, Tom Eyles, Ivan Grave, Alexander McCormack and Graham Tomlin*

132. The rice root-knot *Meloidogyne graminicola*: an evaluation of the aggressiveness level regarding rice and wheat cultivars mainly grown in France. *Killian Laurent, Maíra Grossi De Sá, Stéphane Bellafiore, Jamel Aribi, Luc Villain And Laurent Folcher*

133. Organic Production – management of root-knot nematodes, *Meloidogyne* spp. *Rosa Guilherme, Isabel Abrantes, Hermínio C. de Sousa, Mara Braga and Carla Maleita*

134. Population dynamics of stubby root nematodes, associated with “Docking disorder” of sugar beet, in field rotations with cover crops. *Nyambura Mwangi, Mark Stevens, Alistair Wright, Simon Edwards, Martin Hare and Matthew Back*

135. A transcriptomic perspective on the effects of temperature on tomato-*Meloidogyne enterolobii* interaction. *Duarte Santos, Victor Souza, Isabel Abrantes, Carla Maleita and Sebastian Eves-van den Akker*

136. Transcription factor WOX11 modulates tolerance to cyst nematodes via adventitious lateral root formation. *Jaap-Jan Willig, Nina Guarneri, Thomas van Loon, Sri Wahyuni, Ivan E. Astudillo-Estévez, Lin Xu, Viola Willemsen, Aska Goverse and José L. Lozano-Torres*

137. Cellular dynamics underlying *Globodera pallida* effector rbp-1 recognition and function. *Vera Putker, Qi Zheng, Ana Catarina Silva, Erik J. Slootweg, Maikel Zerdoner, Andelko Zivkovic, Octavina C.A. Sukarta and Aska Goverse*

138. G-protein Signalling Promotes the Susceptibility of Plants to a Biotrophic Plant Pathogenic Nematode. *Sharon Letia, Shamim M.D. Hasan and Florian M.W. Grundler*

139. Exploring genomic insights into draft genomes of *Heterodera* species. *Akshita Jain, John Wainer, Jacqueline Edwards, Brendan Rodoni and Timothy Sawbridge*

140. Detection of *Meloidogyne incognita* infection by measuring the root electrical capacitance. *Renáta Petrikovszki, Fanni Bárányos, Gergely Boros, Amelita Gerda Molnár and Imre Cseresnyés*

141. Nematodes of coffee in Kenya: Challenges and prospects in the identification process. *Joseph Maosa, Denis Gitonga, Zipporah Githinji, Huu Tien Nguyen, Marjolein Couvreur, Laura Cortada, Celestine Oduor and Danny Coyne*

142. Comparison of two real-time PCRs to identify *Meloidogyne enterolobii*. *Daniel Apolonio Silva de Oliveira, Paul Mooijman, Tim Warbroek, Aron van Duijnhoven, Robin du Pré, Anne Sophie van Bruggen, Evelyn van Heese and Gerrit Karssen*

144. Tools to prevent the spread of root-knot nematode *Meloidogyne luci* in potato. Barbara Geric Stare, Nik Susič, Uroš Žibrat, Melita Theuerschuh, Andrej Vončina, Janez Lapajne and Saša Širca

145. Management of plant-parasitic nematodes with cover crops in rotations with open-field vegetable crops. *Lirette M. Taning, Louis Lippens, Ellen Formesyn, Sander Fleerakkers and Wim M.L. Wesemael*

146. Efficacy of TYMIRIUM® as different soil application methods against plant parasitic nematodes on tobacco, citrus, vegetables, and potato, banana in Africa and the middle east. *Hany Monir, Tia Ferreira and Driekie Fourie*

147. Plant pathogenic nematodes behave badly: Uncovering the first case of field-relevant nematicide resistance. *Christoph Andreas Braun, Ralf Nauen and Marc Rist*

148. Efficacy of TYMIRIUM® technology in protecting row crops against key nematode pests. *Benedikt Kurtz, Hany Monir, Driekie Fourie and Tia Ferreira*

149. Development of integrated *Meloidogyne* spp. management with digital support: a case study. *Josep Izquierdo Casas*

150. Complex plant-microbe interactions determine the performance of root-knot nematodes on plants. *Olivera Topalovic, Mette Vestergaard, Stefan Geisen and Flemming Ekelund*

151. Soil bacterial, fungal, and nematode community structures and co-occurrence network structures in coniferous plantations and broadleaves secondary forests. *Yudai Kitagami and Yosuke Matsuda*

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176. Using novel bioinformatic approaches to identify candidates for the potato Cyst Nematode resistance genes G*pa5* and H1. *Thomas M. Adams, Yuhan Wang, Lynn H. Brown, Xinwei Chen, Yuk Woon Cheung, Vanessa Young, Drummond T. Todd and Micha M. Bayer*

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4. Distinct roles of E2Fa and E2Fb transcription factors in nematode feeding sites: regulators of DNA replication and in response to replication stress

Janice de Almeida Engler[1], Danila Cabral[1], José Lozano[2], Geert Smant[2], Gilbert Engler[1], Lieven De Veylder[3]

1 INRAE, Université Cote d'Azur, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France

2 Wageningen University Laboratory of Nematology, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

3 Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, B-9052, Belgium, Center for Plant Systems Biology, VIB, Ghent, B-9052, Belgium

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: cell cycle, E2F, *Arabidopsis*, nematodes, galls, giant cells, DNA replication.

ABSTRACT:

Plant host cell-cycle hyperactivation is essential for nematode feeding-site ontogenesis, but the balanced mitotic and endoreduplication cycles must occur for an accurate homeostasis. Alterations on core cell-cycle gene expression is well-known to disturb root-knot as well as cyst-nematode feeding-site development. We investigated in root-knot nematode induced galls the activity of E2FA and E2FB transcription factors that control both the mitotic and endocycle through the activation of S phase cell-cycle genes. The role of the two plant E2F activators, during cell-cycle progression in galls, was compared to syncytia induced by cyst-nematodes. E2FA and E2FB transcript localization accompanied by functional investigations revealed that up or downregulation of E2FA and E2FB hampered feeding-site development leading to largely reduced gall development and nematode reproduction. Transcript analysis of galls upon E2FA and E2FB loss-of-function compared to wild-type revealed differential selected target gene expression. Although our results imply the functional interplay of E2FA and E2FB for gall development, we recognize that E2FA alone commands and sustains cell division as well as the endocycle in galls, whereas E2FB is likely partaking in nematode-induced feeding-site initiation. Recent studies strongly indicate the involvement of both E2FA and E2FB in response to replication stress caused by nematode parasitism. I close this abstract highlighting the importance of technology development to assist on the understanding of root-knot nematodes-induced galls ontogeny.

5. Tricky parasites: How nematodes take their vitamins from plants

Clarissa Hiltl[1], Sherouk Khalifa[1], Michelle Stankiewicz[1], Shiela P. Quiobe[1], Nora Druschel[1], Laura Oé[1], Florian M. W. Grundler[1]
1 INRES, Molecular Phytomedicine, University of Bonn, Germany

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: vitamin B metabolism, cyst nematodes, nutrient availability, parasitism

ABSTRACT:

Heterodera schachtii second-stage juveniles invade the root of their host and move intracellularly towards the vascular cylinder, where they induce the formation of a plant-derived syncytium and become sedentary. The hypertrophic and hypermetabolic syncytium serves as the sole nutritional source for the developing juveniles. Due to this dependency, it is crucial for *H. schachtii* to successfully initiate and maintain the syncytium in order to complete its lifecycle and produce progeny. Transcriptome data of *Arabidopsis thaliana*-derived syncytia compared to uninfected root tissues revealed an increase in the expression of genes involved in metabolic processes, including the biosynthetic pathways of several vitamins. These metabolites are essential nutrients, as they cannot be stored or synthesized by humans and, presumably, all other animals. *A. thaliana* lines over-expressing single or multiple steps of the biosynthesis of vitamin B1 (VB1) and vitamin B7 (VB7) were differently susceptible to infection by *H. schachtii*, confirming their important function during cyst nematode parasitism. Notably, our work has identified homologs of VB1 and VB7 biosynthetic genes, and their high expression during feeding stages, in *H. schachtii*. Using RNAi to down-regulate these genes, we observed defects in the nematode's development. We assume that *H. schachtii* can perform the full or partial biosynthesis of VB1 and VB7, which serves as an evolutionary benefit for its parasitism by ensuring a continuous supply of these vitamins. Nevertheless, the simultaneous up-regulation of the host homologs during infection implies a highly sophisticated adaptation of host and parasite metabolism to ensure successful biotrophic parasitism without starving the host.

6. NILR1 perceives a nematode ascaroside triggering immunity and is targeted by the nematode effector RHA1B

Li Huang[1], Yulin Yuan[1], Chloe Lewis[1], Joanna Kud[2], Joseph Kuhl[1], Allan Caplan[1], Louise-Marie Dandurand[1], Inga Zasada[3], Fangming Xiao[1]
1 University of Idaho, USA
2 University of Arkansas, USA
3 USDA-ARS, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: nematode-associated molecular pattern (NAMP), Ascr18, pattern recognition receptor, NAMP-triggered immunity, nematode effector RHA1B

ABSTRACT:

Animals and plants use pattern recognition receptors (PRRs) to perceive conserved molecular patterns derived from pathogens thereby activating the immune responses. The most abundant nematode ascaroside Ascr18 is a nematode-associated molecular pattern (NAMP) that induces immune signaling and enhances resistance to pathogens in various plant species. We found the *Arabidopsis* NEMATODE-INDUCED LRR-RLK1 (NILR1) protein physically interacts with the Ascr18 elicitor, as indicated by a specific direct interaction between NILR1 and Ascr18, and NILR1 is genetically required for Ascr18-triggered immune signaling and resistance to both *Pseudomonas* bacterium and root knot nematode, as manifested by the abolishment of these immune responses in nilr1 mutants. These results suggest NILR1 is the immune receptor of the nematode NAMP Ascr18, mediating Ascr18-triggered immune signaling and resistance to pathogens. The Ascr18-NILR1 pair is the first example of ligand-receptor identified in the innate immunity in plants or animals against nematode infection. Significantly, the potato NILR1 (termed StNILR1) also recognizes Ascr18 and is required for resistance to cyst nematode *Globodera pallida*. On the other hand, successful plant-parasitic nematodes use stylet to deliver effectors, which are produced in the pharyngeal glands, into the plant cell cytoplasm to suppress host defenses, including NAMP-triggered and PRR-mediated immunity. We found the *G. pallida* effector RHA1B, which is a functional ubiquitin ligase, directly interacts with StNILR1 and promotes its degradation via the 26S proteasome. Thus, these results suggest that *G. pallida* utilizes the effector RHA1B to interfere with the NAMP-triggered immunity by targeting the NAMP receptor PRR for proteolysis, thereby promoting parasitism.

7. Copper microRNAs modulate the formation of giant feeding cells induced by the root knot nematode *Meloidogyne incognita* in *Arabidopsis thaliana* and *Solanum lycopersicum*.

Stéphanie Jaubert[1]
1 Institut Sophia Agrobiotech

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: plant, root-knot nematode, copper, epigenetic, microRNA

ABSTRACT:

The redifferentiation of root parenchyma cells into giant feeding cells induced by Root knot nematodes (RKN) is the result of a massive transcriptional reprogramming of host cells. Since RKN induce similar feeding cells in roots of thousands of plant species, RKN are thought to manipulate conserved plant molecular pathways. MicroRNAs are small non-coding RNAs (20-22 nucleotides), with many microRNA families highly conserved, that regulate gene expression at the post-transcriptional level. Our work aims to investigate the role of plant microRNAs in the control of the massive transcriptional reprogramming observed during formation of RKN induced feeding cells.

We identified two microRNA families, miR408 and miR398, as upregulated in *Arabidopsis thaliana* and *Solanum lycopersicum* roots infected by root-knot nematodes. In plants, the expression of these two conserved microRNA families is known to be activated by the SPL7 transcription factor in response to copper starvation. By combining functional approaches, we deciphered the network involving these microRNAs, their regulator and their targets. MIR408 expression was located within nematode-induced feeding cells in which it co-localised with SPL7 expression and was regulated by copper. Moreover, infection assays with mir408 and spl7 KO mutants or lines expressing targets rendered resistant to cleavage by miR398 demonstrated the essential role of the SPL7/MIR408/MIR398 module in the formation of giant feeding cells. Our findings reveal how perturbation of plant copper homeostasis, via the SPL7/MIR408/MIR398 module, regulate the development of nematode-induced feeding cells.

8. Mining new interactions between PPN effectors and plant transcription factors via CrY2H

Huy Xiang[1], Boris Stojilkovic[1], Godelieve Gheysen[1]
1 Ghent University, Department of Biotechnology, Proeftuinstraat 86 N1 building, Gent, Belgium
2 Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: root-knot nematode, effector hub, CrY2H-seq, plant transcription factors.

ABSTRACT:

During the infection process of host plants, plant pathogens secrete effectors to enhance their parasitic abilities, and plant parasitic nematodes (PPN) are no exception. By interacting with specific plant proteins, these effectors manipulate and regulate host functions to facilitate successful parasitism. To comprehend their mechanisms, the discovery of their interacting proteins represents a critical step. To date, numerous PPN effectors and their target proteins have been identified and confirmed, encompassing functions such as suppressing host immunity, hijacking signal pathways, and influencing cell division and differentiation.

Traditional methods often suffer from low efficiency and sensitivity, necessitating the adoption of novel technologies. Over the past decade, advancements in omics research have ushered in new directions for studying plant pathogen-host interactions. Here, we use an emerging technology known as CrY2H-seq, enabling group-to-group interaction screening (Trigg, Shelly A., et al. 2017). In this system, protein interactions activate Cre recombinase, resulting in the fusion of coding sequences of the interacting partners. Subsequent next-generation sequencing facilitates the identification of these interactions on a large scale. In comparison to traditional Y2H methods, CrY2H-seq allows for comprehensive screening of bait and prey libraries in a streamlined fashion. This innovative approach offers a fresh avenue for large-scale and targeted screening.

Our study employed 82 candidate effectors from *Meloidogyne incognita* and over 1400 transcription factors from *Arabidopsis thaliana* as bait and prey libraries for CrY2H screening. The screening was executed successfully, and PCR amplification results for interaction pairs were subjected to ONT-sequencing. Bioinformatic analysis revealed complex interaction networks between effectors and transcription factors. Moreover, by integrating known validated transcription factor-effector interaction data, certain established hubs were corroborated and supplemented with additional data. Interestingly, new transcription factors were also identified as potential effector hubs. The successful application of this technique in studying effector and plant host protein interactions introduces fresh perspectives for large-scale screening of interaction networks and the exploration of new central hubs.

9. Unraveling cytosolic Ca2 signaling in the early *Heterodera schachtii* infection

M. Shamim Hasan[1], Chhana Ullah[2], Clement Pellegrin[3], Sakil Mahmud[4], Sharon Letia[1], Miroslaw Sobczak[5], Ute C. Vothknecht[4], Sebastian Eves-van den Akker[3], Florian M.W. Grundler[1]

1 Molecular Phytomedicine, INRES, University of Bonn, Bonn, Germany
2 Department of Biochemistry, Max Planck Institute for Chemical Ecology, Hans-Knoell- strasse 8, 07745 Jena, Germany
3 The Crop Science Centre, Department of Plant Sciences, University of Cambridge, CB2 3EA, UK
4 Plant Cell Biology, IZMB, University of Bonn, Kirschallee 1, 53115 Bonn, Germany
5 Department of Botany, Institute of Biology, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159, 02-787 Warsaw, Poland

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: *Arabidopsis*, sedentary endoparasitic nematodes, Ca2 signatures, calcium sensory protein, phytohormone, immune response

ABSTRACT:

Pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) on the cell surface and then trigger appropriate immune responses in the affected plant cells. Calcium (Ca2+), as a central secondary messenger, is able to encode the signals generated by the PRRs into Ca2+ signatures, which are recognized by various intracellular Ca2+-binding proteins and subsequently decoded by mostly transcriptional reprogramming into different downstream responses. Very little is known about Ca2+-mediated signaling responses in the early phase of plant-nematode interactions. Here, we show that infection of the host root by the parasitic cyst nematode *Heterodera schachtii* induces cytosolic Ca2+ and activates several calcium-sensitive proteins involved in the modulation of defense responses. Our previous work indicated that the expression of several genes responsive to Ca2+ signaling is significantly upregulated in *Arabidopsis* roots at the early stage of nematode infection. Using genetically encoded biosensors, we show for the first time that a pronounced cytosolic Ca2+ burst occurs during the early nematode infection process. A comprehensive microscopic, transcriptomic, biochemical, and genetic analysis of the processes triggered by the nematode-induced Ca2+ signatures reveals a link between the Ca2+ signaling network and the modulation of plant defense hormones during the observation period. Our results provide new insights into the mechanisms of Ca2+ signaling-mediated molecular responses in orchestrating plant-nematode interactions.

10. *Pratylenchus* spp. dominate nematode food webs in a legume-based organic farming system

Jan Henrik Schmidt[1], Morten Moeller[2], Miriam Athmann[2], Christian Bruns[2]
1 Julius Kuhn Institute, Braunschweig, Germany
2 University of Kassel, Witzenhausen, Germany

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: long-term experiment, crop rotation, legume diversity, organic fertilizer, nematode abundance and diversity

ABSTRACT:

The diversity and abundance of nematodes in arable soils predominantly depend on farm management. Organic farms frequently grow legumes for soil fertility and fodder production. We ask if and how 1) frequent cropping of legumes affects nematode abundance and diversity, and 2) different types of organic fertilizer affect nematode trophic groups. Nematodes were examined in an organic long-term field experiment (LTFE) near Kassel in Germany. Three different crop rotations (farm type) with two non-orthogonal fertilization systems each (four replicates) were studied in 2022 and 2023: Two stockless farms with a focus on economy (farm 1, 1-year-clover grass, potatoes, carrots, faba bean, wheat, spelt) and soil fertility (farm 2, 1-year-alfalfa or clover grass, potatoes, wheat, faba bean, oat, spelt) and one mixed farm focusing on forage production (farm 3, 2-year-clover grass, potatoes, wheat, faba bean, silage rye). We determined nematodes in the unfertilized controls (mulched clover grass) and in treatments fertilized with biogas digestate (farm 1), green waste compost (farm 2) and slurry and manure compost equivalent to 2.0 livestock units (LU) ha-1 (farm 3). In April 2022, soil samples were taken in spelt on farm 1 and in the first year of the clover grass on farm 3, while in April 2023 samples were taken in clover grass (farm 1), alfalfa grass (farm 2), and in the second year of clover grass on farm 3. Nematodes in 100 ml soil aliquots were extracted with an Oostenbrink elutriator followed by 48h incubation in Oostenbrink dishes to obtain clean suspensions. Nematodes were counted in aliquots reflecting 10% of the total suspension. About 100-150 individuals were identified to the genus level with the diagnostic keys of Andrassy, Bongers, and Brzesky.

Across treatments and years, we found 1982, 2219, and 3048 nematodes 100 ml-1 soil in farm 1, 2, and 3, respectively. A vast density of herbivores (2360 individuals 100 ml-1 soil) occurred in farm 3 that was twice as high as in farm 1 and 2. High densities of *Pratylenchus* (1125 individuals 100 ml-1 soil) were found in farm 3 compared to 546 and 209 individuals 100 ml-1 soil in farm 1 and 2, respectively. Recently applied composts in farm 3 and 2 reduced bacterivores (-14%) and increased omnivores (+100%) in farm 2 compared to the control. Biogas slurry application in farm 1 increased bacterivores (+30%) and predators (+100%) compared to the control. In conclusion, farm 3 contained many good hosts (legumes) for *Pratylenchus* spp. emphasizing further monitoring to prevent damage to upcoming crops in the rotation. The increased number of omnivores after compost application in farm 2 highlights the possibility to improve the soil food web structure by agronomic decisions. Furthermore, the low number of *Pratylenchus* spp. in this farming system highlights crop rotation as an effective nematode control strategy.

11.Unravelling the ecological dynamics of cave-dwelling nematodes in Dripkelder cave, South Africa

Gerhard Du Preez[1], Marconi Souza Silva[2], Driekie Fourie[1], Chantelle Girgan[3], Edward Netherlands[1], Antoinette Swart[3], Rodrigo Ferreira[2]

1 Unit for Environmental Sciences and Management, North-West University, South Africa
2 Centro de Estudos em Biologia Subterrânea, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Campus Universitário, CEP 37200-90, Brasil
3 Nematology Unit, Biosystematics, Agricultural Research Council-Plant Health and Protection, Private Bag X134, Queenswood 0121, South Africa
4 Department of Zoology and Entomology, University of the Free State, PO Box 339, Bloemfontein, 9300, South Africa

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: cave Ecology, ecological filtering, nematode diversity, seasonal variability

ABSTRACT:

Caves, with their unique habitats, have long intrigued scientists, especially in the realm of biodiversity and ecological interactions. Despite this interest, the ecological significance of cave-dwelling nematodes had remained largely unexplored. Our study focused on investigating the role of selected environmental factors over temporal (seasonal) and spatial (site location) scales on the community dynamics and functional diversity of cave-dwelling nematodes in Dripkelder Cave, South Africa. Multiple terrestrial sites within this cave were investigated over the four seasons of a one-year period, considering various microhabitat characteristics including carbon availability, moisture content, temperature, pH, and microbial activity. Our study reported 50 nematode taxa from Dripkelder Cave of which multiple taxa including *Amphidelus* sp., *Fictor* sp., *Pseudacrobeles* sp., and *Wilsonema* sp., among others, were first reports from caves in southern Africa. The nematode communities were subject to significant ecological influences. Notably, the periodic influx of surface materials in the cave entrance chamber introduced food sources (as indicated through the measurement of active carbon), which resulted in bacterial blooms and the correlated increase in bacterivore nematodes. This influx of materials, together with a physical restriction, differentiated the entrance chamber nematode community from those associated with habitats deeper in the cave. Intriguingly, the entrance chamber presented the greatest temporal similarity in nematode community structure with deeper areas presenting less temporal stability. One theory is that the nematode community of the entrance chamber was ‘maintained’ through the periodic influx of nematode taxa from a relatively stable surface pool of taxa. Additionally, observed temporal trends further evidenced the effect of seasonality. An example of this was the rapid response of nematode colonizers (*Rhabditis* sp.) to the introduction of food and their later transitioning to dauer larvae. Finally, the presence of diverse trophic groups in more isolated sections of the cave highlighted the potential complexity of cave food web interactions involving nematodes. This study sheds light on the intricate dynamics of cave-dwelling nematodes, suggesting a more complex ecosystem role than previously suggested. Our findings underscore the need for further exploration of these understudied habitats to better understand their ecological intricacies.

12. Effects of agricultural intensification on soil nematode communities in freshwater streams

Rosa Ana Salazar-García[1], Antonio Archidona-Yuste[1], Pablo Castillo[1], Gracia Liébanas[2]
1 IAS-CSIC, Instituto de Agricultura Sostenible-CSIC, Córdoba
2 Departamento Biología Animal, B. Vegetal y Ecología. Universidad de Jaén, Jaén

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: land-use change, agriculture intensification, biotic homogenization, freshwater ecosystems, beta diversity

ABSTRACT:

Land use change and agricultural intensification are major threats to biodiversity. Specifically, the conversion of complex natural ecosystems to simplified managed ecosystems leads to marked alterations and subsequent biotic homogenization in the assembly of communities. However, agriculture has not only effects on soil where it is cropped, its effect is extended to the surrounding aquatic networks due to the flushing of sediment and agrochemical pollutants. This is a major disturbance to aquatic environments and their biodiversity. Surprisingly, this issue has received limited attention in soil invertebrate communities living in freshwater ecosystems such as nematodes. The overall aim of our study was to evaluate how land-use change alters community assembly in soil nematodes in freshwater ecosystems and to test if this leads to biotic homogenization. To this end, our study was conducted within the basin of the Guadalquivir River (southern Spain), particularly in the upper course of the main river, which is surrounded by an extensive olive growing area mixed with unmanaged natural environments. We selected five tributaries of the Guadalquivir River located in natural and agricultural ecosystems, each divided into three parts according to the course of the stream (i.e. upper, middle and lower course), and resulting in a total of 50 sampling sites collected from the streambed. Changes in alpha diversity between ecosystems were detected resulting in a loss of species along the river courses surrounded by the agricultural ecosystems. This species loss led to an increase of taxonomic similarities and a strong biotic homogenization of nematode communities along the river courses due to the conversion from natural to agricultural systems. Definitively, the ability and valuable role of soil nematodes as bio-indicators is demonstrated in freshwater ecosystems.

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13. Distribution of *Meloidogyne enterolobii* in vegetable crops in the southeastern United States

Johan Desaeger[1]
1 University of Florida, Entomology and Nematology, Wimauma, FL 33598, USA
2 Clemson University, Plant and Environmental Sciences, Clemson, SC 29634, USA
3 University of Georgia, Dept. of Plant Pathology, Tifton, GA, 31793, USA
4 North Carolina State University, Entomology and Plant Pathology, Raleigh, NC 27695, USA
5 University of Florida, Entomology and Nematology, Gainesville, FL 32611, USA
6 United States Department of Agriculture USDA-ARS, Charleston, SC 24914, USA
7 North Carolina Department of Agriculture and Consumer Services, Raleigh, NC 27601, USA

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: *Meloidogyne enterolobii*, southeastern USA, vegetables

ABSTRACT:

A focused effort sponsored by the USDA National Institute of Food and Agriculture’s Specialty Crop Research Initiative was done to investigate the presence and distribution of *Meloidogyne enterolobii* (M.e.) in Florida, Georgia, South Carolina and North Carolina. The spread of M.e., a highly virulent root-knot nematode (RKN) species, is potentially devastating to specialty crop production in the southeastern United States. This species can cause significant losses in yield and quality, and its quarantined status jeopardizes both interstate and international trade. A major concern is the ability of M.e. to infect and damage crop genotypes that are resistant to the other major species of RKN, including sweet potato, cucumber, watermelon and tomato. More than one thousand samples across the four states were collected from mostly vegetable growing areas from 2018-2023. Nematode species identification was conducted using nonspecific primer sets followed by species-specific primers and DNA sequencing of selected populations. M.e. was found in all four surveyed states and was especially prevalent on Asian vegetables, pepper, tomato, and sweet potato. In Florida, M.e. was found on 20 different crops, and together with M. *incognita* it was the most found RKN species. In North Carolina M.e. was found on sweet potato and its rotational crops in 16 counties, primarily in the central Coastal Plains region of the state. In Georgia, M.e. was found on sweet potato in two fields. In South Carolina no M.e. was discovered in commercial fields, but one positive sample was found in a home garden. There was limited variability among M.e. populations from our study. Four M.e. populations from North Carolina had similar virulence on sweet potato. Phylogenetic trees based on COX2 and tRNA-His DNA fragments grouped 20 M.e. populations from our study and nine M.e. populations from other countries together. Additional studies are ongoing to develop and evaluate vegetable germplasm with resistance to M.e., and test the efficacy of nematicides, cover crops and rotations as integrated management strategies to help manage this emerging nematode threat.

14. Protected forests benefit soil biodiversity and function in the North of Portugal

Carolina Duarte[1], Carlos Guerra[2], Sofia Costa[1]
1 CBMA - Centre of Molecular and Environmental Biology, Braga, Portugal
2 i-Div - German Centre for Integrative Biodiversity Research, Leipzig, Germany

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: conservation, edaphoclimatic conditions, exotic vs. native forests, structure footprint, trophic groups

ABSTRACT:

This study addresses a significant gap in nature conservation policy, which is mainly concerned with aboveground terrestrial species, while soil biodiversity remains largely overlooked. In the context of the diverse ecosystems found in Northern Portugal s forests, this research investigates the impact of protected areas and forest typology (native vs. exotic) on soil nematode biodiversity and functions. In the scope of the Soil Recon Project (10.54499/PTDC/BIA-CBI/2340/2020) we used soil nematode communities as bioindicators to: (i) assess differences in soil biodiversity and functions between protected and non-protected areas, as well as between native and exotic forest types, and (ii) infer how protected areas deal with pressures and drivers influencing soil biodiversity and functions. The findings reveal that ongoing conservation efforts in Northern Portugal are producing some significant effects on the soil system. Protected areas exhibit a larger Structure and Omnivore footprints, along with an increased abundance of fungivorous and omnivorous nematodes. Notably, native forest areas are better suited for safeguarding soil biodiversity and ecological functioning, highlighting their importance in conservation strategies. Key influencers on soil functional biodiversity include soil organic carbon, apparent density, soil moisture, and deciduous forest cover. Positive associations were observed between higher nematode trophic groups and soil organic carbon and moisture, while negative associations were identified with apparent density. In conclusion, conservation efforts focusing on native forests must extend their considerations to encompass the effects on soil biodiversity and function. A holistic approach is imperative for integrated protection that addresses both aboveground and belowground components, ensuring a comprehensive and sustainable conservation outcome.

15. Insights into the diversity and frequency of nematode communities in paddy fields

Leidy Rusinque[2], Carla Maleita[3], Ana Rita Varela[5], Maria Lurdes Inacio[1]
1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, Portugal
2 Centre for Functional Ecology Science for People & the Planet (CFE), Department of Life Sciences, University of Coimbra, Coimbra, Portugal
3 Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, University of Coimbra, Coimbra, Portugal
4 GREEN-IT Bioresources for Sustainability, ITQB NOVA, Oeiras, Portugal
5 Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Évora, Portugal

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: survey, prevalence, frequency, rice

ABSTRACT:

Rice (*Oryza sativa* L.), one of the main cultivated crops worldwide, is the staple food for more than half of the global population. It is grown in more than 100 countries and provides more than 20% of calorie intake worldwide. According to the European Commission, the total area growing rice in Europe is 425.000 ha, being Portugal the fourth largest rice producer with an area equivalent of 28.000 ha, and a production yield of 5.6 tons/ha. Currently, rice is part of the Portuguese strategy for the promotion of cereal production, however, there are still some weaknesses that need to be addressed, such as the reduced options to control pests/diseases. Plant parasitic nematodes (PPN) are considered one of the most important soil-borne pests, causing yield losses worldwide of up to USD 175 billions/year, therefore, representing an important constrain to agricultural production. Among the PPN species known to be associated with rice, only 29 species are reported to be responsible for significant yield losses. Many rice PPN are considered pests of tropical production, but climate changes and the trade activity are promoting their spread northwards meaning that temperate rice agro-systems will be affected. During 2021-2023, a survey on the main Portuguese rice production areas (basins of rivers Tejo-Sorraia, Mondego, and Sado) was undertaken. A total of 98 samples were collected and analysed. Nematodes were extracted from soil using the tray method and then fixed and identified to genus level using identification keys. For molecular analysis, total genomic DNA was extracted from single nematodes and used for sequencing of the partial ITS (TW81/AB28), 28S rDNA (D2A/D3B), 18S (CL-f3/1912R) and cytochrome oxidase I (JB3/JB5) regions. These regions yield single fragments of the expected fragment size, 550 bp, 750 bp, 985 bp and 447 bp, respectively, and the obtained sequences showed 99.8% to 100% similarity with accessions in the GenBank database. A total of 13 genera were associated to rice, among which the most prevalent species were *Helicotylenchus dihystera*, present in 38% of the fields followed by Aphelenchus avenae (19%), *Aphelenchoides bicaudatus* (19%) and *Pratylenchus crenatus* (11%). Mg, the most serious pest of rice, was not detected on our survey. This is the first survey to assess the PPN species found associated with Portuguese paddy fields.

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16. Are EPNs attracted or repelled by the black truffle and its volatile organic compounds? A novel perspective for truffle beetle biocontrol

Ivan Julià Brunat[1], Ivan Hiltpold[2], Ana Morton[1], Fernando Garcia del Pino[1]
1 Universitat Autonoma de Barcelona, Bellaterra 08193, Spain
2 Agroscope, Nyon 1260, Switzerland

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: attraction behavior, *Steinernema*, *Tuber melanosporum*, VOCs, *Leiodes cinnamomeus*, chemical ecology.

ABSTRACT:

The European truffle beetle, *Leiodes cinnamomeus*, is the most important pest in black truffle (*Tuber melanosporum*) plantations. Entomopathogenic nematodes (EPNs) are a promising biological control agent against *L. cinnamomeus*. EPNs may employ multiple sensory cues for their host-seeking behaviors, such as volatile organic compounds (VOCs) and CO2 gradients. We report for the first time the ability of truffle fruitbodies to attract EPNs, identifying some VOCs that appear to play a key role in this attraction. We conducted olfactometer assays to investigate the attraction behavior of *Steinernema feltiae* and *Steinernema carpocapsae* towards both *T. melanosporum* fruitbodies and larvae of *L. cinnamomeus*. Subsequently, a chemotaxis assay using agar plates was performed to determine which of the 14 main VOCs emitted by the fruitbodies elicited an attraction behavior to *S. feltiae* at two different concentrations. Both EPN species were attracted to mature fruitbodies of *T. melanosporum*, which may enhance the likelihood of encountering *L. cinnamomeus* during field applications. *L. cinnamomeus* larvae in the presence of truffles did not significantly affect the behavior of EPNs 24 hours after application, underscoring the importance of the chemical compounds emitted by truffles themselves. Chemotaxis assays showed that four long-chain alcohol compounds emitted by *T. melanosporum* fruitbodies attracted *S. feltiae*, especially at low concentration, providing a first hint in the chemical ecology of a still-eluded ecological system of great economical value. Further studies should be conducted to gain a better understanding of the tritrophic interactions between *T. melanosporum*, EPNs, and *L. cinnamomeu*s, as this knowledge may have practical implications for the efficacy of EPNs in the biological control of this pest.

17. Use of *Heterorhabditis bacteriophora* for the biological control of *Popillia japonica* larvae and evaluation of its impact on non-target soil biota

Giulia Torrini[1], Immacolata Iovinella[1], Chiara Sciandra[2], Agostino Strangi[1], Stefania Simoncini[1], Giuseppe Mazza[1], Francesco Barbieri[1], Gian Paolo Barzanti[1], Claudia Benvenuti[1], Francesco Paoli[1], Giuseppino Sabbatini Peverieri[1], Leonardo Marianelli[1]
1 CREA Research Centre for Plant Protection and Certification, Florence, Italy
2 Department of Life Sciences, University of Siena, Siena, Italy

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: Entomopathogenic nematodes, Integrated Pest Management, Japanese Beetle, Persistence, Priority Pest

ABSTRACT:

Popillia japonica (Coleoptera: Scarabaeidae) is a scarab beetle native to Japan and accidentally introduced to Italy from North America in 2014. The high reproductive rate, the pathway of introduction, and the dispersal capability, as well as the polyphagous diet, led the EU to include this pest in the priority list. *Popillia japonica* spends most of its life cycle as a grub in the soil. At this stage, the grubs damage turf grasses, hay meadows, soccer pitches, and golf courses and are susceptible to biological control agents present in the soil, such as entomopathogenic nematodes (EPNs).

As part of the Horizon 2020 Project 'IPM-POPILLIA', a large number of natural EPN strains have been collected from the soil in the outbreak area in Northern Italy along the Ticino River. The most virulent indigenous strain of *Heterorhabditis bacteriophora* (POP 16) was used in field trials to assess its effectiveness in containing the *P. japonica* larval population and its persistence in soil. Since September 2021, these trials have been carried out in the Piedmont region in three meadows with different irrigation management (non-irrigated, semi-irrigated, and irrigated fields).

Two months after the treatments, a significant reduction of *P. japonica* larvae was observed in plots treated with EPNs (>90%) compared to untreated ones. Regarding persistence, *H. bacteriophora* was isolated again after the winter season and at lower concentrations one and two years after treatments, depending on irrigation management.

Furthermore, in the irrigated meadow, the effects of EPNs on non-target soil biota were also evaluated, comparing soil arthropod assemblages with a Before-After Control-Impact experimental design. Compared to the control, no evident negative effect on non-target edaphic arthropods was disclosed on soil treated with EPNs. However, some taxa showed an increase in abundance, suggesting that some changes may occur in the long term. These results evidenced that the use of the selected EPN indigenous strain may be an excellent candidate for the integrated management of *P. japonica*, with no impact on non-target species, and at the same time highlighting the importance of evaluating the effects on soil biota to preserve the key- functioning of the ecosystem.

18. All strains are not the same: 42 international *Heterorhabditis bacteriophora* strains exhibit differential virulence rates to *Popillia japonica* larvae

Anna Garriga[2], Hugo R. Monteiro[1], Rubén Beltrí[1], Duarte Toubarro[1], Nelson Simões[1]
1 Centro de Biotecnologia dos Açores. Faculdade de Ciências e Tecnologia, Universidade dos Açores, Portugal
2 Dept. Biologia Animal, Vegetal i Ecologia. Universitat Autonoma Barcelona, Spain

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: biological control, phenotype, entomopathogenic nematodes

ABSTRACT:

Entomopathogenic nematodes (EPNs) are biological control agents of great potential against insect pests. However, the phenotypic and genetic diversity of these nematodes requires careful selection, as not all strains infect and behave equally. Usually, this selection against a specific pest is made using laboratory biological tests, which makes the process time-consuming. With sufficient knowledge of phenotype and genotype correlation, EPNs virulence could be predicted through genetic markers. In order to obtain this information from the EPN *Heterorhabditis bacteriophora*, a wide screen of strains needs to be tested in the same insect and conditions to standardize the phenotypical trait of virulence. For our work, we assessed the virulence as lethal time for 42 *H. bacteriophora* strains in laboratory conditions against the third instar of *Popillia japonica* as our target pest. To achieve the maximum genetic variability, we experimented with two commercial strains and native strains from 12 countries worldwide. The initial analysis shows a lethal time 50 (LT50) that ranged from 60 hours (LU1, HU2, FA16) to 120 hours (AU1, Kuba, CT52) with an even distribution. Moreover, there is no correlation between virulence and the geographical origin of the strains. These results will permit the clustering of strains by phenotype and correlate the virulence rate to a genetic profile obtained from SNPs in the genome.

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19. Non-Effects of *Xenorhabdus* and *Photorhabdus* (Morganellaceae) unfiltered ferments on *Saccharomyces cerevisiae* during alcoholic fermentation of reconstituted grape must and on volatile composition of Tempranillo must

Ignacio Vicente-Díez[1], Pilar Morales[1], Ramon González[1], Mar Vilanova[1], Alícia Pou[1] and Raquel Campos-Herrera[1]
1 Instituto de Ciencias de la Vid y del Vino (ICVV), Gobierno de La Rioja, CSIC, Universidad de La Rioja, Finca La Grajera Ctra. Burgos Km. 6 Salida 13 Lo-20, Logroño, 26007, Spain

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: biopesticides, non-target effects, sustainable agricultural practices

ABSTRACT:

Viticulture, a crucial global crop, annually yields over seven million tons of grapes, half used in winemaking. However, traditional viticultural practices and synthetic chemical pesticides pose significant challenges. The toxins produced by *Xenorhabdus* and *Photorhabdus*, symbiotic bacteria of entomopathogenic nematodes, hold promise for pest and disease control in vineyards. Yet, their potential adverse effects on grapes and their microbiota remain unexplored. This study hypothesizes that unfiltered ferments (UFs) from these bacteria do not impact the yeast-driven fermentation process in winemaking or the volatile profile of must grapes. We conducted two bioassays to test this, each performed twice at different times/places. The first involved microfermentation assays in 50 mL Falcon tubes containing 15 mL of synthetic must media and a 1:10 ratio of bacterial ferments. We assigned three tubes per treatment group, using deionized water and TSB as controls and *X. nematophila* and *P. laumondii* UFs as treatments. We examined two *S. cerevisiae* strains (EC1118 and IFI 473). We maintained the cultures for ten days at 25 ºC in a non-agitated and temperature-regulated, the tubes were closed with fermentation locks. We monitored CO_2 production daily. The second bioassay involved applying bacterial UFs to Tempranillo grapes and then analyzing the total volatile composition in the grapes (free and glycosidically-bound fractions) using solid-phase extraction/gas chromatography-mass spectrometry (SPE/GC-MS), 24 hours post-application. Our results indicate that applying *Xenorhabdus* and *Photorhabdus* UFs to the synthetic must did not significantly affect the fermentation efficiency of either S. cerevisiae strain as assessed by CO_2 release. Furthermore, the analysis showed no significant effects on the volatile composition of Tempranillo grapes. These findings suggest that using *Xenorhabdus* and *Photorhabdus* in vineyard pest management does not adversely affect the yeast-driven fermentation process in winemaking or the volatiles responsible of the aromatic quality of grapes. This compatibility underscores their potential as eco-friendly alternatives in integrated pest management strategies in viticulture, aligning with the growing demand for sustainable agricultural practices.

20. Insecticide-free production of oilseed rape: Role of EPN to contribute to agro-ecosystem management

Ralf-Udo Ehlers[1]

1 Biocontrol Biotech Consult, Duesternbrook 1, 24211 Rastorf, Germany

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: oil seed rape, control of stem flea beetle, establishment, support of natural control potential

ABSTRACT:

The major source of vegetable oil in Northern Europe is oilseed rape (OSR, *Brassica napus*). Worldwide, OSR is the second-largest source of protein meal and the third-largest source of vegetable oil. The crop is attacked by a wide range of insect pests. The most common are *Delia radicum* (L.), *Dasineura brassicae* (Winn.), *Psylliodes chrysocephala* (L.), *Brassicogethes aeneus* (Fab.), *Ceutorhynchus assimilis* (Payk.), *C. pallidactylus* (Marsh.) and *C. napi* (Gyll). Almost all these pests have developed resistance against pyrethroide insecticides. Together with limited innovation in insecticide development, time is not far that chemical control strategies will not be available. In this case farmers will depend on the natural antagonistic potential. Parasitoids and predators can provide up to 80% control. Laboratory and field tests evaluated the potential of EPN to contribute to the natural control potential. The pests are all susceptible to EPN and pupate in the soil providing a biomass of > 15 kg per ha and year, a huge food source for *Steinernema* and *Heterorhabditis* spp. However, checking 11,000 soil sample of arable crop rotation winter wheat, winter barley and OSR, only 0,2% were positive for EPN. Results on application of EPN against the major problem, the stem flea beetle, will be presented and the potential for future agro-ecosystem IPM with re-establishment of EPN and without insecticides will be discussed.

Since insecticides are lacking for oilseed rape pest control, producers need to make use natural enemies. All major pests pupate in the soil and are potential host for EPN, however EPN are seldomly found. Field and lab results on control of the cabbage stem flea beetle are presented and re-establishment of EPN discussed.

21. Rapid desiccation of Entomopathogenic nematodes: evaluating the physiological demands

Itamar Glazer[1], Jayashree Ramakrishnan[1], Liora Salame[1], Mani Karthik Ananth [1], Reut Fledbaum[1], Mohammed Samara[1], Ahmed Nasser [1], Guy Mechrez [1], Dana Ment[1]
1 ARO Volcani Center, Israel
2 Faculty of Agriculture, Food & Environment the Hebrew University of Jerusalem, Israel

Area: Entomopathogenic nematodes
Type: Oral
Keywords: rapid desiccation, stress tolerance, foliar application, survival mechanism

ABSTRACT:

Rapid desiccation (RD) of EPNs is a major limiting factor for their effectiveness against foliar pests. Current formulation technology for improving EPNs shortcomings of low viability and erratic infectivity is met with limited success. Our research focused to identify and evaluate the unique demands arising from EPNs physiology under RD. EPNs desiccated at low humidities were characterized for their survival, rate of water loss (gravimetric), and the bioprotectant (Trehalose) accumulation patterns. Measures were determined in bare EPNs and ones protected with silica Pickering emulsion gel (SPEG) and Titanium Pickering emulsion (TPE) formulations. In-vitro trials indicated, that at 52 and 64% RH, formulation enhanced EPNs survival by 168 hrs (SPEG, TPE) in comparison to 16 hrs in the unprotected EPNs in control (water). While gravimetric water loss indicated retention of water by SPEG and TPE formulation in comparison to control desiccated for 24 hrs. Trehalose accumulation patterns was also measured in EPNs formulated in SPEG and TPE in comparison to control (water) desiccated under low humidity. In this study, we identified the mechanism of protection rendered by formulation to EPNs IJs by Confocal Laser Scanning Microscopy (CLSM). These studies enable to understand requirements from EPNs at RD to deliver consistent and reliable results for above-ground applications.

23. Potential management of *Meloidogyne enterolobii*, a regulated quarantine nematode species in the European Union

Sebastian Kiewnick[1], **Hemanth Gopal**[1], **Marine Poullet**[2], **Etienne Danchin**[2]
1 Julius Kuehn-Institut, Federal Research Centre for Cultivated Plants, Germany
2 Genomics & Adaptive Molecular Evolution, Institut Sophia Agrobiotech INRAE, France

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Meloidogyne enterolobii*

ABSTRACT:

The root-knot nematode *Meloidogyne enterolobii* Yang & Eisenback (syn. *M. mayaguensis*) has been receiving a lot of attention following a first report in two Swiss greenhouses in the year 2008. Due to its potential wide host range and the ability to reproduce on host plant carrying resistance against other tropical *Meloidogyne* species, *M. enterolobii*] has been identified as a mayor threat to crops worldwide. In 2009, the European Plant Protection Organization (EPPO) performed a risk analysis, which concluded to recommend this species for regulation and consequently placement on the EPPO A2 list. Based on numerous interceptions over the past years, it was also concluded that *M. enterolobii* fulfilled the conditions provided in Article 3 and Section 1 of Annex I to Regulation (EU) 2016/2031 in respect of the Union territory and therefore should be listed in Part A of Annex II to Implementing Regulation (EU) 2019/2072 as Union quarantine pest. The measures for all plants for planting in relation to *M. enterolobii* apply therefore from 11 January 2023 onward. It is still unknown how widespread *M. enterolobii* might be present in the EU, as there was only one new finding reported from Portugal in recent years. As there are highly virulent tropical *Meloidogyne* species, such as *M. incognita* present, *M. enterolobii* might have been overlooked in previous surveys. In contrast to initial studies, virulent *M. incognita* populations have demonstrated similar levels of virulence and damage potential compared to *M. enterolobii*. Recent interceptions of *M. enterolobii* in *Ficus microcarpa* plants demonstrated possible routes of introduction into the EU. Plants, infected with M. *enterolobii* were found in Belgium, Germany, The Netherlands and Italy. Once introduced, few options for chemical or biological control are available. As plant resistance is not yet established, knowledge on host range, potential to adaptation to non- and poor-hosts as well as the genetic diversity of *M. enterolobii* is needed. Greenhouse experiments with seven *M. enterolobii* populations from different geographical regions revealed differences in their host range in comparison to published reports, as well adaption to poor hosts within two generations. This will leave few options for farmers to control this nematode by using a non- or poor host in rotation with high values crops. In conclusion, these findings underline the importance of regulating *M. enterolobii* as a quarantine species in the EU as the risk for high value crops, especially in southern Europe, is very high.

24. Important agricultural nematodes and Integrated Nematode Management (INM) in China

Deliang Peng[1]
1 Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: root-knot nematodes, cyst nematodes, Integrated Nematode Management

ABSTRACT:

Plant parasitic nematodes (PPN) are one of the most devastation pest in agricultural production in China. With global climate change, cropping systems innovation, and globalization of trade, crop nematode diseases caused by PPN have become the second largest diseases in agricultural crops and seriously threatening the sustainability of modern agriculture in China. Plant-parasitic nematodes are responsible for Chinese agricultural losses amounting to an estimated 100 billion RMB yuan annually.

The most important nematode from the survey are the root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* and *Globodera* spp.), the pine wilt nematode *Bursaphelenchus xylophilus*, stem rot nematode *Ditylenchus destructor*, *Aphelenchoides besseyi*, root lesion nematodes (*Pratylenchus* spp). and the reniform nematode *Rotylenchulus reniformis*. *Meloidogyne enterolobii* is capable of infecting the roots of almost any crop, causing alone 65% losses, more than any other root-knot nematode species. Cereal cyst nematodes (CCN, *Heterodera avenae* and *H. filipjevi*) are the most economically important plant-parasitic nematode on cereal crops in wheat production area in China. The CCN has been confirmed to distribute 16 provinces in China. Yield losses estimated were 18%-35% in Henan, 11-18% in Hebei, and 15%-28.24% in Qinghai, The population dynamics and life cycle of *H. avenae* was one generation. There are two cyst nematodes (*H. avenae* and *H. filipjevi*) occurrence in the wheat production area of China.

Integrated Nematode Management (INM) have been developed including rotation, agriculture methods, biological agents, nematicides. Fluopyram (flu) it is widely used as a nematicide product due to its low toxicity, low environmental impact, high efficiency, and multifunctionality. A cationic star polymer (SPc) was constructed to load fluopyram (flu) and prepare a flu nanoagent. A series of industry standards and Technical code of practice for resistance evaluation, identification, detection, monitoring, prevention and control root-knot nematode disease, cyst nematode disease and stem rot nematode disease have been released by the Ministry of Agriculture and Rural Affairs. The advance of Integrated Nematode Management is reviewed in this paper.

25. Nematicide efficacy at managing root-knot nematode in sweet potato in the United States.

Chang Liu[1], **Zane Grabau**[2], **Rebeca Sandoval Ruiz**[2]
1 Mississippi State University, 46 Old Bully Blvd, Mississippi State, Mississippi. USA
2 University of Florida, 1881 Natural Area Dr, Gainesville, Florida. USA

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: sweet potato, *Meloidogyne enterolobii*, *Meloidogyne incognita*, management, nematicide.

ABSTRACT:

Root-knot nematode (*Meloidogyne* spp., RKN) is the most important nematode species affecting sweet potatoes in the United States. In 2018-2023, two field trials were conducted at Live Oak, Florida to test the efficacy of fluazaindolizine (Salibro), fluopyram (Velum Prime), fluensulfone (NIMITZ), oxamyl (Vydate), and 1,3-Dichloropropene (Telone II) for managing root-knot nematode on sweet potato. For trial A in 2018-2021, RKN population were identified as *M. incognita* (SRKN). Six treatments were applied with following rate (a.i.): 1) Fluazaindolizine 1.12 kg/ha, 2) Fluazaindolizine 2.24 kg/ha, 3) Fluazaindolizine 2.24 L/ha + oxamyl 9.35 L/ha, 4) 1, 3-D 84 kg/ha, 5) Fluopyram 238 g/ha, and 6) Oxamyl 2.14 kg.ha. For trial B in 2022-2023, RKN population were identified as *M. enterolobii* (Guava root-knot nematode). Five treatments were applied with following rate (a.i.): 1) Fluensulfone 1.96 kg/ha, 2) Fluensulfone 3.93 kg/ha, 3) Fluazaindolizine 2.25 kg/ha, 4) 1,3-D 78.8 kg/ha, 5) Fluopyram 0.25 kg/ha. 1,3-D was applied as a broadcast shank application 3-4 weeks before planting. The other treatments were soil-directed broadcast applications approximately 1 week before planting. Tubers were dug, sorted into marketable and unmarketable categories and weighed to assess yield. Soil samples were taken at midseason (approximately 2 months after planting) and at harvest, and nematodes were extracted and assessed under microscope. For Trial A, in 2018, no treatment effects were observed on root-knot nematode abundance at mid-season. Treatments with fluazaindolizine significantly reduced *M. enterolobii*. There were minimal effects on free-living nematodes. In summary, 1,3-D was the most effective nematicide tested for RKN management, but other fluorinated nematicides may be useful depending on RKN pressure at site.

26. Zoophytophagous mirid predators (Heteroptera: Miridae) induce systemic resistance in tomato against *Meloidogyne* spp.

Alejandro Expósito[1], Aida M Fullana[1], Alberto Urbaneja[2], Meritxell Pérez-Hedo[2], Pablo Urbaneja-Bernat[3], Jordi Riudavets[3], Ariadna Giné[1], Francisco Javier Sorribas[1]
1 Departament of Agri-Food Engineering and Biotechnology, UPC, BarcelonaTech, Esteve Terradas 8, 08860 Castelldefels, Barcelona, Spain
2 Entomology Unit, IVIA, Carretera Moncada-Náquera km 4,5., 46113 Moncada, Valencia, Spain.
3 Sustainable Plant Protection Programme, IRTA, Ctra. Cabrils km 2, 08348 Cabrils, Barcelona, Spain.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: zoophytophagous predators, root-knot nematodes, induced resistance, *Solanum lycopersicum*

ABSTRACT:

We evaluated the phytophagy of *Macrolophus pygmaeus* and *Nesiodiocoris tenuis* in triggering plant defences against *Meloidogyne*. In the first two pot experiments the tomato cv. Bodar were exposed to 15 nymphs of *M. pygmaeus* or *N. tenuis* per plant for 24 or 48h. Then, plants were inoculated with 200 second-stage juveniles (J2) of *M. incognita* or *M. javanica* per plant and kept in a growth chamber for 40 days. Nematode infectivity and reproduction were assessed, along with the expression of the genes PIN2 and PR1, related to the jasmonic (JA) and salicylic (SA) acid, at 0, 7 and 40 days after nematode inoculation (DANI). In the first experiment, *N. tenuis* reduced 60% the infectivity and 70% the reproduction regardless of the exposure time. In the second experiment, *N. tenuis* and *M. pygmaeus* reduced 33% and 28% the infectivity and 37% and 24% the reproduction. In the leaves exposed to *N. tenuis* and *M. pygmaeus*, the PIN2 was upregulated 9 and 14-fold at 0 DANI and 9 and 20-fold at 40 DANI, but in roots, only was upregulated 2.5-fold at 0 DANI in plants exposed to *M. pygmaeus*. In the following two pot experiments, the susceptible cv. Roma and the resistant cv. Caramba were exposed for 48h to 15 nymphs of *M. pygmaeus* per plant and inoculated with 200 J2 of *M. incognita* or 600J2 of a community composed of *M. hapla*, *M. arenaria*, and *M. javanica*. *M. pygmaeus* reduced the infectivity and reproduction by 37% and 53%, when plants were inoculated with *M. incognita* and by 52% and 37%, when plants were inoculated with the nematode community in the susceptible tomato but not in the resistant. Also, the preference of *M. pygmaeus* females for inoculated (after 14 DANI) vs. uninoculated resistant or susceptible plants was assessed in a Y-tube olfactometer or in an insect cage. After 1, 2, 4, 24, 48 and 72h of releasing the insects, the number of *M. pygmaeus* per plant were counted and the offspring after 14 days was evaluated. The insect s preference and offspring were not affected by nematode infection, irrespective of the plant or nematode population. Finally, a greenhouse experiment was conducted, exposing the susceptible tomato cv. Bodar to 15 nymphs of *N. tenuis* or *M. pygmaeus* 24 h before transplanting in a soil infested with *M. arenaria*, *M. incognita* and *M. javanica*. The initial and final soil populations, disease severity, and the nematode reproduction at the end of the crop were assessed. Plants exposed to *M. pygmaeus* reduced the disease severity by 32% and the reproduction by 38%, but not those exposed to *N. tenuis*.

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27. Effect of interactions of plant-parasitic nematodes and *Verticillium dahliae* on peppermint

Ilya Noskov[1], Katja Sommerfeld-Arnold[2], Johannes Hallmann[1]
1 Julius Kühn Institute (JKI) - Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11/12, Braunschweig, 38104, Germany
2 Julius Kühn Institute (JKI) - Institute for Plant Protection in Horticulture and Urban Green, Messeweg 11/12, Braunschweig, 38104, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: *Verticillium dahliae*, *Paratylenchus projectus*, *Meloidogyne hapla*, *Mentha x piperita*

ABSTRACT:

Peppermint (*Mentha x piperita*) is a medicinal and aromatic plant used for its essential oil and leaves. Cultivation of peppermint often experiences yield losses and quality reductions. *Verticillium dahliae*, a soil-borne fungus, and plant parasitic nematodes are among the most important and destructive pathogens in mint production. Plant parasitic nematodes cause direct plant damage but might also promote secondary pathogens leading to synergistic yield losses. Our study investigated whether this is also the case for peppermint.

The pathogenicity of *V. dahliae* on peppermint was examined alone as well as in the presence of the ectoparasitic nematode *Paratylenchus projectus* or the endoparasitic species *Meloidogyne hapla*. We measured plant biomass, reproduction of the nematodes, and incidence of *V. dahliae* in stems, leaves and roots of peppermint. Additionally, we studied whether the time of pathogen inoculation has an effect on the severity of peppermint damage.

Our results confirmed that the presence of two pathogens, *V. dahliae* and nematodes, reduced plant growth more than application of a single pathogen. The reduction in plant growth caused by *V. dahliae* and nematodes was greater than that caused by *V. dahliae* alone. In presence of *V. dahliae* the majority of plants showed typical symptoms such as wilting, browning of the vascular tissue in stems and discoloration of leaves. Symptoms caused by the nematodes were not detected. It could be shown that the presence of *V. dahliae* negatively affected the reproduction of both nematode species, indicating competition for plant resources between both pathogens. Furthermore, plant growth turned out to be lower when *V. dahliae* was inoculated 7 days before the nematodes than vice versa. Overall, our results confirmed a strong interaction between *V. dahliae* and nematodes. The presence of fungus plus nematode resulted in synergistic yield losses.

28. The nematode-antagonistic fungi *Niesslia gamsii* and *Polydomus karssenii* are potential candidates for biocontrol of cyst and root-knot nematodes

Milad Rashidifard[1], Wolfgang Maier[1], Samad Ashrafi[1]
1 Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany
2 Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: Cyst nematodes, antagonistic fungi, biological control, root-knot nematodes, defence genes, gene expression, qRT-PCR

ABSTRACT:

Cyst nematodes (*Heterodera* spp. and *Globodera* spp.) are among the most devastating plant-parasitic nematodes causing substantial yield loss. A series of screenings targeting symptomatic eggs of the cereal cyst nematode *Heterodera filipjevi* collected from fields in Turkey where nematode damage was substantially reduced, led to the discovery of several recently described nematode antagonistic fungi. The main aim was to investigate whether the isolated fungi have a wider host range and can also parasitise root-knot nematodes (*Meloidogyne* spp.). Among the isolated fungi, two distantly related species, namely *Niesslia gamsii* and *Polydomus karssenii*, were selected to investigate the potential of these fungi in (1) reducing the reproduction and penetration rate of *M. hapla* in tomato plants and (2) inducing the plant defence against nematode. Tomato plants were first inoculated with each fungus individually and five days later treated with *M. hapla*. Plants inoculated only with *M. hapla* or only with either of the fungi, and completely uninoculated plants served as controls. The data was collected 3- and 7-days, and eight weeks after nematode inoculation (dani). A significant reduction ($P < 0.05$) was recorded in the number of eggs per root system and consequently in Rf value when the plants were inoculated with *N. gamsii* (80377 and 26.8) and *P. karssenii* (79833 and 26.6), respectively, compared to those of the untreated control (117012 and 39). Treating the tomato plants with either of the fungi significantly reduced the nematode penetration at 3 and 7 dani. An induced defence reaction was observed in plants pre-inoculated with either of the fungi alone. Plants inoculated with *N. gamsii* and challenged later with nematodes showed a significant reduction in the transcript level of PR1, PR3 and PR5 genes, and an increase in the transcript level of ACO gene. However, significant upregulations in PR1, ACO and CAT genes were recorded in plants pre-inoculated with *P. karssenii* and treated with nematodes. We provide the first evidence for substantial antagonistic potential of the recently described fungal species *N. gamsii* and *P. karssenii*, originally isolated from *H. filipjevi*, against *M. hapla* via different mechanisms under greenhouse conditions. In addition to direct egg parasitism, our study suggests that both fungi can also act against root-knot nematodes indirectly by inducing salicylic acid (SA), Jasmonate (JA) and ethylene (ET) biosynthesis pathways. *Niesslia gamsii* plays a role in modulating SA and JA responses as well

as ET biosynthesis, while *P. karssenii* triggers genes involved in SA response, ET biosynthesis and the production of antioxidants.

29. Allodiplogaster sudhausi (Nematoda: Diplogasteridae), a potential predator for PPN control: Biology, production and safety

Loel Bacolod Dalan[1], Silas Bett[1], Temesgen Addis Desta[2], Yitzhak Spiegel[3], Sigal Brown Miyara[3], Ohad Zuckerman[4], Wim Bert[1], Ralf-Udo Ehlers[5]

1 Department of Biology, Nematology Research Unit, Ghent University, Ledeganckstraat, 9000 Ghent, Belgium
2 e-nema GmbH, Klausdorfer Str. 28-36, 24223 Schwentinental, Germany
3 Nematology Unit, ARO, The Volcani Center, Derech Hamacabim 68, Bet Dagan 50250, Israel
4 Copia Agro & Food, 1 Shenkar St., Herzliya 4672501, Israel
5 Biocontrol Biotech Consult, Düsternbrook 1, 24211 Rastorf, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: biocontrol, *Meloidogyne*, *Allodiplogaster sudhausi*, predatory nematode, production, safety

ABSTRACT:

Allodiplogaster sudhausi is a predatory diplogasterid nematode with promising biocontrol potential against plant-parasitic nematodes. Since high quality nematode material from liquid culture was tested in glasshouse experiments on cucumber, tomatoes and sweet pepper significant reductions of galling indices and number of infested plants were recorded. An Environmental Risk Assessment using multi-dimensional scaling, maturity index and diversity indices to analyze the nematode community structure could not report any significant effects on non-target nematode populations. Reproduction is by self-fertilizing hermaphrodites. Dauer juveniles (DJs) hatch from eggs. Production in monoxenic liquid culture with different bacterial food sources reached > 150,000 nematodes ml^{-1}. Shelf life is limited to one months. Life History Trait analysis of *A. sudhausi* was carried out using hanging drops with 5×109 cells ml-1 of *E. coli* strain OP50 at 25°C. *A. sudhausi* completes its life cycle within 8 days (egg to egg). Hatching of DJs takes around 31 h. In hanging drops, low frequency of males (6%) developed from recovered DJs 5 days after hatching. A single *A. sudhausi* has a total fertility rate of 117. *Allodiplogaster sudhausi* has a mean generation time (T) = 0.17, a population doubling time (PDT) of 1.72 and a lifespan of 27 days. The intrinsic rate of natural increase rm= 0.42 d-1. In monoxenic liquid culture, *A. sudhausi* grows best at 25°C, both in Erlenmeyer flasks and lab scale bioreactors. *Panagrolaimus* sp. was most susceptible to predation (89%), followed by DJs of *Steinernema feltiae* (68%), *S. carpocapsae* (40%) and *Heterorhabditis bacteriophora* (14%). Cannibalism of *A. sudhausi* on its DJ stages was quite low (11%). This study supplements information on existing data regarding life cycle parameters of *A. sudhausi*, its population dynamics in monoxenic liquid culture, its survival in different formulations as well as its predation efficacy.

Predator with high control potential for PPN, mass production and formulation possible, safe for non-target nematode populations

31. Parasite transmission in Svalbard reindeer in a rapidly changing climate: experimental studies on dispersal behaviour of a reindeer parasitic nematode.

Tirza Moerman[1]
1 NMBU, Norwegian University of Life Sciences, Norway

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: Ecology, animal parasitic nematode, experiment

ABSTRACT:

The High Arctic, in particular, is rapidly changing and this may have strong effects on parasitic nematodes, their negative impacts on host performance and hence parasite-host dynamics. Animal parasitic nematode dispersal under changing temperatures and moisture regimes may be a vital factor in understanding contact rates with the host and parasite transmission, but this has been rarely studied. We conducted one field and one laboratory study on the dispersal behaviour of a parasitic nematode (*Ostertagia gruehneri*) of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) on Svalbard. 1) In a field experiment we exposed introduced *O. gruehneri* to a combination of temperature and moisture treatments and studied the effect of these treatments on vertical movement and distribution of *O. gruehneri*. We hypothesized that experimentally manipulated warm and dry conditions would result in vertical movement of *O. gruehneri* to deeper soil layers and cold and moist conditions in movement to the vegetation layer where the parasitic nematodes are more likely accessible to Svalbard reindeer. 2) In a laboratory experiment we tested if and how far *O. gruehneri* actively can disperse from reindeer faeces. We placed reindeer faeces with *O. gruehneri* larvae in horizontal and angled trays with soil and studied the presence of *O. gruehneri* at different distances from the faeces. We expected *O. gruehneri* to actively disperse out and away from reindeer faeces but to have a limited horizontal dispersal range. 1) Results indicate that drier but not warmer conditions negatively affected the number of *O. gruehneri* retrieved in the vegetation layers but not in the soil layers. Differences in the total number of *O. gruehneri* retrieval suggest a mortality rather than the expected movement effect. 2) Preliminary results suggest that *O. gruehneri* could actively disperse from faeces, but horizontal dispersal distance is short. These experimental studies provide a fundamental insight into environmental effects on parasitic nematode dispersal. Moreover, these results could have implication for indicating parasite infection risks and hotspots under changing climates, drier conditions might indirectly benefit Svalbard reindeer and infection risk might be highest close to deposited faeces.

32. Bioinformatic approach to identify gut related targets with potential to mitigate parasitic infection.

Liliana Jeziorska[1], James Dillon[1], Vincent O'Connor[1] Marcus Guest[2]
1 University of Southampton, UK
2 Syngenta UK Limited, Jealott's Hill, Warfield, Bracknell RG42 6EY, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords:

ABSTRACT:

Plant- and animal-parasitic nematodes are a significant problem in agriculture, causing disruptions in food security and large losses in the global economy (1-3). In both contexts, chemical/drug treatments are important routes to mitigation. Since investigating novel mitigation strategies is challenging given the complex life cycle of parasites, *Caenorhabditis elegans* can provide a platform to facilitate target identification and novel treatment strategies.

C. elegans is a free-living nematode and established model organism that shares many anatomical and genetic features with parasitic nematodes. The cuticle is highly impermeable which prevents free access of chemicals into the worm. Thus, compounds can be delivered more efficaciously through the intestine, which is an essential tissue for organism viability. The nematode intestine, exemplified by C. elegans, has a simple anatomy made up of 20 epithelial cells, called enterocytes, forming a tube. Enterocyte structure is polarised and contains basolateral and apical domains, with the apical region consisting of proteins expressed on the microvilli surface, in the terminal web and exposed to the gut lumen. These luminally exposed molecules can readily interact with chemicals, and if these interactions disrupt key functions, they will produce phenotypes which impact organism viability, becoming attractive targets for developing novel treatments.

To identify such targets, a sequential bioinformatic pipeline was designed. This approach involved highlighting genes with selective expression in the intestine, predicted plasma membrane localization and predicted apical localization. This was further filtered by assessing if mutated alleles of the candidate genes resulted in perturbed phenotypes. Candidates were also assessed for predicted protein structure and membrane topology, and this approach was reinforced by literature verification. The pipeline identified 17 membrane-localised and two extracellular proteins which are exposed to the lumen and functions include nutrient uptake, response to drugs, and/or control of gut motility. These candidates are potentially susceptible to mitigation.

I will use *C. elegans* to investigate if these candidates are tractable targets. Utilising innate or engineered susceptibility to small peptides or polypeptides (toxins or nanobodies), I will determine if target function can be disrupted. Effects will be assayed using established lethality, feeding, or developmental assays. This approach can be also extended to other target tissues such as pharynx and sensory neurons that have privileged exposure to environmentally applied chemicals. This work highlights how *C. elegans* biology can be used to efficiently identify and validate candidate targets for the mitigation of parasitic infestations.

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33. Targeted proteomics as precise tool for biomarkers characterization of *Anisakis simplex* complex species: epidemiological implication

Susana C. Arcos[1], Rosa González López[1], Sergio Ciordia[2], Marina Usieto[3], Fátima M. Santos[2], Yolanda Jiménez Ruíz[1], Lee Robertson[4], Mercedes Careche[3], Alfonso Navas[1]
1 Museo Nacional de Ciencias Naturales, CSIC, Spain
2 Centro Nacional de Biotecnología, CSIC, Spain
3 Instituto de Ciencia y Tecnología de Alimentos y Nutrición, CSIC, Spain
4 Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, CSIC, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: proteomics, *Anisakis*, biomarkers

ABSTRACT:

The transcriptome and proteome of *Anisakis simplex* s.s., *A. pegreffi* and their hybrid genotypes were previously compared. Comparing the levels of expressed proteins and using the criteria of Character Compatibility, a set of twenty-eight expressed proteins from near of 20,000 were unambiguously inferred as significant taxonomic biomarkers within the complex. Some of these proteins are specific allergens of *Anisakis* (Ani s7, Ani s8, Ani s12, and Ani s14), while others are common nematode venom allergens (*Ancylostoma* secreted). The remaining markers consist of four unknown or non-characterized proteins, five different proteins related to innate immunity, four proteolytic proteins (metalloendopeptidases), a lipase, a mitochondrial translocase protein, a neurotransmitter, a thyroxine transporter, and a structural collagen protein. From the data obtained in the differential proteomics study and the in-silico digestion of the sequences deposited in the Uniprot repository, a list of representative proteotypic peptides for each protein was chosen and monitored by Parallel Reaction Monitoring (PRM) in independent replicates of the taxonomic entities tested.

The data obtained from the PRM acquisition were analyzed in Skyline. Chromatograms were aligned based on peptide RTs, and peptide abundance was determined relative to a pooled sample. Protein total abundance was calculated by median intensity of assigned peptides, and statistical significance was assessed using Fisher s one-way ANOVA with FDR adjustment. Univariate and multivariate ROC analyses evaluated discriminatory protein diagnostic potential. Multiple marker panels achieved AUC > 0.86 and >80% predictive accuracy for distinguishing between the 3 taxonomic entities.

34. The Mi2G02 effector of *Meloidogyne incognita* hijacks host plant trihelix transcription factor for parasitism

Jianlong Zhao[1], Zhenchuan Mao[1]
1 Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, China

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: root-nematode, effector, giant cell, interaction

ABSTRACT:

Root-knot nematodes (RKNs) cause huge agricultural losses every year. They secrete a repertoire of effectors to facilitate parasitism through the induction of plant-derived giant feeding cells, which serve as their sole source of nutrients. However, the mode of action of these effectors and host targeted proteins remain largely unknown. In this study, we investigated the role of the effector Mi2G02 in *Meloidogyne incognita* parasitism. Host-derived Mi2G02 RNA interference in *Arabidopsis thaliana* affects giant cells development, whereas the ectopic expression of Mi2G02 promotes root growth and increases plant susceptibility to *M. incognita*. We used various combinations of approaches to study the specific interactions between Mi2G02 and *A. thaliana* GT-3a, a trihelix transcription factor. GT-3a knockout in *A. thaliana* affected feeding site development, resulting in the production of fewer egg masses, whereas GT-3a overexpression in *A. thaliana* increased susceptibility to *M. incognita* and also root growth. Moreover, we highlight the role of Mi2G02 in maintaining GT-3a protein stabilization by inhibiting the 26S proteasome-dependent pathway, leading to a suppression of TOZ and RAD23C expression, promoting nematodes parasitism. Thus, this work enhances our understanding of the manipulation of the role and regulation of a transcription factor by a pathogen effector through interfering proteolysis pathway to reprogram genes expression for nematode feeding cells development.

35. *Meloidogyne incognita* and the host range paradox: RNAseq analysis of different infested hosts highlight the complexity of root-knot parasitism

Victor Hugo Moura de Souza[1], Clement Pellegrin[1], Unnati Sonawala[1], Dio Sangwon Shin[1], Olaf Kranse[1], Junior Lusu Kika[1], Beatrice Senatori[1], Sebastian Eves-van den Akker[1]
1 University of Cambridge, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords:

ABSTRACT:

The root-knot nematodes (*Meloidogyne* spp.) have been identified as one of the greatest threats to crop production in tropical and sub-tropical areas, with *M. incognita* likely the single most important plant pathogen globally. It damages important crops, is widespread in agricultural areas, and is complex to manage. Additionally, *M. incognita* presents a paradox: although it has an extremely intimate biotrophic interaction with its hosts, it also has the broadest host range of any obligate parasite (>3,000 species), encompassing representatives from almost every order of flowering plants.

To understand this 'host-range paradox', we sought to determine whether and how *M. incognita* tunes its gene expression in different hosts. We carried out transcriptomic profiling of ten different infected plant species, carefully selected to have representatives across the phylogenetic tree of flowering plants. Galls and symptomless adjacent root fragments were collected from each host 25 days after inoculation. Total RNA was extracted and sequenced. The principal component analysis grouped the hosts into three distinct groups based on global gene expression. While some *M. incognita* genes were always expressed regardless of the host, sets of host or group-specific genes were identified. Unexpectedly, we found that nematodes infecting distantly related plants presented extremely similar expression profiles, while those infecting closely related plants had distinct expression profiles. When analysing the predicted secretome, several genes overlap between groups of hosts with few specific to a particular plant. In conclusion, *M. incognita* tunes gene expression regardless of the phylogenetic distance between hosts, physiology of infection or global gene expression.

36. Cross-kingdom RNAi in plant-nematode interactions.

Ange Dussutour[1], Yara Noureddine[3], Martine da Rocha[1], Karine Mulet[1], Ann-Po Cheng[2], Arne Weiberg[2], Bruno Favery[1], Stéphanie Jaubert[1]
1 Institut Sophia Agrobiotech, France
2 University of Munich (LMU), Germany
3 INRAE Bourgogne-Franche-Comté, France

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: cross-kingdom, RNAi, *Meloidogyne*, miRNA, ncRNA, silencing

ABSTRACT:

Transkingdom and inter-organism silencing refers to a new pathway of molecular dialogue in parasitic interactions. This process consists of the exchange of non-coding RNAs (ncRNAs) between two organisms that may belong to different kingdoms. This process has been identified in the interaction between the vertebrate parasitic nematode *Heligmosomoides polygyrus* and the mouse (Buck et al. 2014) or between the fungus *Botrytis cinerea* and the model plant *Arabidopsis thaliana* (Weiberg et al. 2013). In both cases, pathogens were shown to secrete ncRNAs into host tissues to silence host genes involved in the immune response. Since then, only a few articles have highlighted the key role of ncRNA exchange in host-parasite interactions. Nematodes of the genus *Meloidogyne* are among the most important agricultural pests. To date, no study has been conducted to characterise the exchange of ncRNAs during plant-nematode interactions. The aim of this PhD project is to determine whether RKN secrete ncRNAs into root cells and to characterise the role of inter-organism silencing in plant-nematode interaction. Initial sequencing analyses have identified several candidates: nematode small ncRNAs and their target plant messenger transcript. Direct identification and functional analyses are needed to validate and e*luci*date the role of the identified nematode small ncRNA/plant mRNA pairs in plant-nematode interactions. This project will open new perspectives in the study of host-parasite dialogue and the development of new pest management strategies.

37. SUGR: the SUbventral Gland master Regulator of plant-parasitic cyst nematodes

Anika Damm[1], Clement Pellegrin[1], Sebastian Eves-van den Akker[1]
1 The Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: plant-parasitic nematodes, effectors, effector production

ABSTRACT:

Plant-parasitic cyst nematodes are devastating pests that have evolved unique and intimate relationships with their host plants. At the core of these interactions are effectors, nematode proteins enabling the colonisation of plant tissues. Effector production is restricted to two specialised sets of gland cells in the nematode. Investigation of this spatial confinement allowed us to discover the first gene in any plant-parasitic nematode that regulates effector production: the SUbventral Gland Regulator SUGR.

We used RNA interference to show that SUGR is required for plant invasion and controls the expression of 297 genes, including half of all known cell wall degrading enzymes and 42 effectors. Furthermore, SUGR, in conjunction with the transcription factors that it also activates, directly binds the promoters of effector genes in a partially overlapping manner. Together, this reveals a regulatory network underpinning effector production. Interestingly, at the top of this network, transcription of *SUGR* itself is activated by effectostimulins: plant-derived small molecule signals.

These discoveries allow us to build a working model for effector regulation that ultimately describes a careful balancing of resource-sparing when you must, and promoting parasitism when it counts. Excitingly, these findings could form the first step towards much-needed, novel approaches to nematode control targeting effector production.

38. Heat tolerance of distinct geographic populations of *Meloidogyne incognita* is possessed by intrinsic thermal acclimation reaction: nematode response to global warming

Alkesh Hada[1], Patricia Bucki[1], Nathalia Sichov [1], Eyal Glanz[1], Noa Sela[2], Sigal Braun Miyara[1]

1 Department of Entomology, Nematology and Chemistry Units, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan 7505101, Israel

2 Department of Plant Pathology and Weed Research, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan 7505101, Israel

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: climate change, global warming, heat tolerance, embryogenesis, plasticity, root-knot nematode, thermal time, transcriptomic regulation

ABSTRACT:

Research interest in the mechanisms enabling plant-parasitic nematodes to adjust their physiological performance and cope with changing temperatures has intensified in light of global warming. Here, we show that geographically distinct populations of the root-knot nematode *Meloidogyne incognita*, prevalent in the three main pepper-growing regions in Israel Carmel Valley (Carmel), Jordan Valley (JV), and the Arava Rift (Arava) possess persistent differences in their thermal acclimation capacity, which affect embryonic development. The optimal temperature for embryonic growth completion for the Carmel population was 25°C, 25 and 30°C for the JV population, and 30°C for the Arava population. Juvenile survival indicates that at the lowest temperature tested at 20°C, compared with the Arava population, Carmel population gained the highest survival rates throughout the experimental duration while at 33°C, Arava population gained the highest survival rate throughout time duration. This tendency was further reflected by roots penetration assay as well whole plant infection studies, while Carmel population demonstrated increased J2s penetration at 25°C compared to the JV and Arava populations, the opposite trend was found at 33°C, where increased penetration of tomato roots was observed for the Arava population compared to the Carmel and JV populations. Next, for studying the molecular mechanism underlying population s thermal adaption, we performed transcriptomic analysis. RNA of infected roots at 25, 30, and 33°C was extracted and sequenced using Illumina sequencing PE150, which identified an extensive set of genes in different populations cultured on plants roots under different temperatures. Gene ontology and KEGG-pathway-based analysis were used to further investigate the functions of the differentially expressed genes (DEGs). Results so far indicate that Carmel population possess higher plasticity compared with Arava, as reflected by significant increase in differential expressed genes along the temperatures. The transcriptome profiles presented in this study provide insight into the transcriptome complexity and will contribute to further understanding the thermal adaptation and help to predict eco-evolutionary trends under temperature change scenarios.

39. Transcription factors in the plant-endoparasitic nematode interaction: insights of gene regulatory networks

Jose Domínguez-Figueroa[1], Patricia Abril-Urias[1], Fernando Díaz-Manzano[1], Ángela Martínez-Gómez[1], Almudena Gómez-Rojas[1], Alba Carrión-Escudero[1], Carolina Escobar[1]
1 Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha, Toledo, Spain.

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: transcription factors, auxin regulated genes, galls, giant cells, syncytia

ABSTRACT:

Plant parasitic nematodes (PPNs) are known to infect and damage a wide variety of crops causing serious agronomic losses worldwide [1]. Among these obligate parasites, cyst (CNs) and root-knot nematodes (RKNs) show a similar sophisticated parasitism behavior by inducing specialized feeding sites that become the sole source of nutrients for the nematode´s development. RKNs enter the root through the elongation zone and subtly migrate to the root meristem, where they enter the vascular cylinder and induce a group of transfer cells called giant cells (GCs) inside a newly formed feeding site organ called gall [2]. In contrast, CNs form syncytia which involves the fusion of adjacent cells already differentiated. Although the formation of feeding sites requires a crucial maximum of auxin in both types of parasitism, the ontogeny of the syncytia and galls is strikingly different and there are important divergences in the molecular mechanisms involved in their formation [3]. These processes of *de novo organogenesis* involve manipulating of different gene regulatory networks within the cells orchestrated by the nematode's effectors, which hijack some molecular transducers of established plant developmental processes, such as lateral root formation or root regeneration [4,5]. The dramatic transcriptomic changes occurring in these feeding sites suggests a key regulatory role of plant Transcription Factors (TFs) in coordinating both, the new organogenesis and the plant´s responses against the nematode.

We have investigated the auxin transduction pathways through the analysis of promoters of several genes that are key during feeding site establishment. We found that those promoters active in galls/GCs (e.g. *AHP6*, *LBD16*) contain only canonical AuxRe elements in the proximal promoter regions. In contrast, promoters active in syncytia such as *pmiR390a* and *pGATA23* carry AuxRe overlapping core cis-elements for other TF families (i.e., bHLH, bZIP). Furthermore, *in silico* transcriptomic analysis in *Arabidopsis thaliana* showed only a few common upregulated IAA responsive genes in both types of feeding sites despite the high number of IAA induced genes present in both interactions as compared to uninfected roots [3]. This is consistent with the robust evidence that combinatorial TF pairing is often sufficient to determine regulatory specificity and control of gene expression [6]. We also conducted a comprehensive analysis of TFs transcriptomic data available focusing on CNs and RKNs interactions in *Arabidopsis*. We believe that knowing the role of plant-transcription factors participating in these processes becomes essential for a deeper understanding of the Plant-Nematode interaction and provides an opportunity for the future development and design of targeted control strategies.

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Both first and second authors have contributed equally to this work

40. Do biodegradation products of dehydroascorbic acid contribute to its bioactivity as nematicidal compound and inducers of plant resistance?

Farzana Haque Tumpa[1], Eva Degroote[1], Satish Namdeo Chavan[2], Tina Kyndt[1]
1 Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Proeftuinstraat 86 N1, Ghent, 9000 Belgium
2 ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, 500030, India

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: dehydroascorbic acid, biodegradation product, hydrolysis, phytotoxic, nematicide, IR-stimulus

ABSTRACT:

Exogenous treatment with the oxidized form of Ascorbic Acid (AsA), that is dehydroascorbic acid (DHA), activates induced resistance (IR) against the root-knot nematode *Meloidogyne graminicola*. Additionally, our previous studies showed that DHA was highly nematicidal to second stage juveniles of *M. graminicola* in in-vitro condition and can control nematode infection in both pot and field studies. Foliar application of 10 mM DHA was found to protect rice plants from *M. graminicola* for at least 14 days after its application. After hydrolysis, in aqueous solution DHA spontaneously decomposes to DKG (2,3-dioxo-L-gulonate) and then to oxalic acid, formic acid, threonic acid and hydrogen peroxide. In the present study, the IR and nematicidal effect of these biodegradation products produced from DHA was evaluated, to investigate if they could contribute to DHA-bioactivity. Nematicidal assays revealed that 10 mM DHA, 10 mM formic acid, 10 mM oxalic acid, or 10 mM threonic acid are strongly nematicidal (100% mortality), while 10 mM hydrogen peroxide is mildly nematicidal (54%) to second stage juveniles. On the other hand, 10 mM DKG is not nematicidal (0% mortality). In case of plant bio-assays to evaluate induced resistance, foliar application of 10 mM formic acid was found to be strongly phytotoxic. 10 mM DKG and 10 mM oxalic acid also showed minor phytotoxicity. On the other hand, 10 mM DHA, 10 mM hydrogen peroxide or 10 mM threonic acid are not phytotoxic. Only DHA and H202 can induce resistance against nematodes through foliar application, while foliar treatment with the other degradation products did not have significant effects on rice susceptibility to nematodes. In conclusion, some of the DHA biodegradation products are nematicidal, while only hydrogen peroxide can contribute to IR-stimulation.

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- In conclusion, some of the DHA biodegradation products are nematicidal, while only hydrogen peroxide can contribute to IR-stimulation.

41. Foliar nematode control on ornamental fern with new reduced risk nematicides, and evaluating potential phytotoxicity of several new nematicides

Zhiqiang Cheng[1]
1 University of Hawaii at Manoa, USA

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: plant-parasitic nematodes, chemical control, biological control, crop safety

ABSTRACT:

Aphelenchoides fragariae is a species of foliar nematode that is an increasingly widespread pathogen of ornamental plants with a wide host range, attacking more than 250 plants species in 47 plant families. The most recognizable symptom of foliar nematode infection is the interveinal lesions on leaves. Previously, chemical treatments using active ingredients (a.i.) such as oxamyl and parathion were effective against foliar nematodes. However, due to environmental concerns and their potentially high toxicity, these chemicals are no longer available for foliar nematode control in USA. The overall goal of this project was to determine the effectiveness of several new, reduced-risk nematicides against foliar nematodes on several popular ornamental plants in Hawaii. Specific objectives were to determine: 1) the efficacy of several new nematicides for managing foliar nematodes on palapalai fern, *Microlepia strigosa*, and 2) if these new nematicides have phytotoxicity on commonly grown ornamental plants in Hawaii: *Microlepia strigosa*, *Frangipani*, *Rhaphiolepis indica*, *Hibiscus*, *Phalaenopsis*, and *Anthurium andraeanum*. Foliar nematodes were extracted from infected fern tissues using the Baermann funnel method. These nematodes were cultured in the lab using carrot discs and the cultures were refreshed every 5-7 weeks prior to inoculation onto *M. strigosa*. New nematicides ESP 715 (a.i. fluopyram), MBI 304 (a.i. *Chromobacterium* spp. strain extract), and Majestene (a.i. *Burkholderia* spp. strain extract) were tested against Aphelenchoides fragariae on M. strigosa. Foliar nematode abundance was measured prior to treatments and at the end of the experiment. Foliar nematode damage severity (0-5 scale) was measured weekly post treatment for 6 weeks. In addition, ESP 715, MBI 304, and Majestene were examined for phytotoxicity on *M. strigosa*, *Frangipani*, *R. indica*, *Hibiscus*, *Phalaenopsis*, and *A. andraeanum* at various rates. All experiments included a no chemical control. Tested plants received three applications of the designated nematicides at 14-day intervals in the phytotoxicity experiment. Our results indicated that fluopyram did not significantly suppress foliar nematode abundance but did reduce foliar nematode damage severity. *Burkholderia* and *Chromobacterium* did not statistically suppress foliar nematode abundance but did reduce the numbers by 65.7% and 75.8%, respectively. *Burkholderia* and *Chromobacterium* also reduced foliar nematode damage severity. No visual foliar phytotoxicity symptoms were observed under any treatments throughout the study, except for fluopyram on M. strigosa (visible phytotoxicity, but low at 1 on a 1-5 phytotoxicity scale). These results suggested that *Burkholderia* and *Chromobacterium* could potentially be used to manage *A. fragariae* on *M. strigosa*.

42. New nematicidal agents from fungal endophytes

Carmen Elisa Diaz[2], Maria Fe Andrés [1], Azucena Gonzalez-Coloma[1]
1 Instituto de Ciencias Agrarias. CCSIC. Madrid, Spain
2 Instituto de Productos Naturales. CSIC. Santa Cruz de Tenerife, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: endophytic fungi, secondary metabolites, nematicidal compounds, root-knot nematodes

ABSTRACT:

The indiscriminate use of synthetic pesticides has generated environmental and public health problems. In recent years, the use of these products and the number of active ingredients has been limited by very restrictive legislation in the EU and other countries. Therefore, there is a growing need for alternative control agents, including natural products and biocontrol. In this context, botanical and fungal natural products are a low-risk and sustainable source of new biopesticide ingredients.

From selected host plants (medicinal and/or endemic species) producing bioactive natural products, we have isolated a series of fungal endophytes. These endophytic fungi have been cultured in a liquid medium and the resulting extracts tested for biological activity against root-knot nematode (*Meloidogyne javanica*) to assess their potential as bionematicides. From a large collection of plans species biosourced, we have found that the Lauraceae tree Persea indica (endemic to the Canary Islands Laurel Forest) yielded a large number of endophytes with the ability to produce nematicidal compounds (9 nematicidal isolates from a total of 16). Among them, the isolate YCC4 has been submitted to a bio-guided chemical study to identify the active metabolite/s. The chemical structures, biological effects of the active compounds, and future development as a nematicidal will be discussed.

43. Melatonin’s protective role: Enhancing plant growth and mitigating parasitic nematode infestation

Yuliia Zhukova[1], Aryani Sianipar[1], Letia Sharon[1], Florian M.W. Grundler[1], M. Shamim Hasan[1]
1 Molecular Phytomedicine, INRES, University Bonn, Bonn, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: Sedentary endoparasites, melatonin, plant growth, resistance, biostimulant

ABSTRACT:

Melatonin (N-acetyl-5-methoxytryptamine) is a non-toxic biological molecule synthesized in bacteria, plants and animals. At least 20 plant families produce melatonin in various tissues, where it functions as an important second messenger and antioxidant that is critical for coping with a range of abiotic and biotic stressors. Our study investigates the effects of exogenous melatonin application on enhancing plant resistance to cyst nematodes (CNs) and root-knot nematodes (RKNs) in *Arabidopsis thaliana*, *Beta vulgaris* and *Solanum lycopersicum*. Various infection and plant growth parameters were systematically evaluated to determine the effects on plants and nematodes. Exogenous melatonin treatment with milimolar concentrations resulted in a significant reduction in nematode infection, as evidenced by a decrease in the number of females and associated nematode feeding sites for CNs by 45% in *A. thaliana*, as well as number of cysts up to 70% in *B. vulgaris*. Against RKNs, melatonin reduced number of galls by 30% in *A. thaliana*. It also prevented severe infection in *S. lycopersicum* indicated by a decline in galling index and reproduction rate by 47% and 59%, respectively. In addition, the application of melatonin has positive effects on plant growth and yield, such as increased number of leaves, root and fruit weight. Our ongoing research aims to further explore the mechanisms underlying melatonin-mediated plant resistance and to gain a more comprehensive understanding of the signaling pathways involved. All our results underline the potential of melatonin as a promising biostimulant to help plants fight nematode infestations.

44. Nematicidal evaluation of a coded compound against potato cyst nematodes (*Globodera pallida*)

Niru Tripathi[1], Simon Woods[1], Katarzyna Dybal[1], Grace Milburn[1], Matthew Back[1]
1 Harper Adams University, Newport, Shropshire, UK

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: chemical nematicides, exposure, *Globodera pallida*, juveniles, movement

ABSTRACT:

Potato cyst nematodes (PCN) are devastating pests of potatoes, threatening global food security due to the yield loss they cause in potatoes and their resilience and persistence. The eggs inside PCN cysts can remain viable for over two decades, and thus once introduced, PCN is very difficult to eradicate. Various control measures including the use of chemical nematicides have been proposed for PCN. However, due to their negative impact on the environment and human health, many widely used nematicides are banned or have restricted use. Therefore, the discovery and development of novel nematicides with high nematode-specific activity with low acute mammalian toxicity and overall better environmental compatibility are needed for sustainable crop production. A novel coded compound was tested under in-vitro, glasshouse, and field conditions to understand its effectiveness against PCN (*Globodera pallida*). Movement of the *G. pallida* juveniles (J2s) was significantly reduced when pre-exposed to the compound at 6.25-50 mg l-1 for 24, 48, or 96h. Similarly, an inhibition of hatching was observed following a 24-hour pre-exposure to the same concentrations. However, the chemical did not suppress the nematodes under glasshouse and field conditions.

45. Pesticide selectivity: finding novel targets in *Caenorhabditis elegans*

Henry Nvenankeng[1], Fernando Calahorro[1], Jim Goodchild[2], Philippa Harlow[2], Lindy Holden-Dye[1], Vincent OConnor[1]
1 University of Southampton, UK
2 Syngenta, Jealotts Hill International Research Centre Bracknell, UK

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: nicotinic acetylcholine receptors, ion channels, target selectivity, nematode control

ABSTRACT:

Parasitic nematodes infect and cause yield declines and health issues in plants and animals when not appropriately treated. The control of these parasites largely relies on synthetic chemicals which are effective yet are subject to increasing regulatory control because they are highly condemned for their off-target impact on crops, host animals, and their wider environmental impacts. This study investigates a nicotinic acetylcholine receptor EAT-2, as a novel selective pharmacological target to control parasitic worms.

There is a strong rationale for targeting EAT-2 as a potentially novel pharmacophore to deliver selective nematicidal chemicals. It has a ligand binding site that is distinct from other receptors in the same class. Furthermore, an auxiliary protein EAT-18 with no known homologs in other animals is required for EAT-2 functional expression. Importantly, in the free-living nematode, *Caenorhabditis elegans*, where it was first discovered, it is a major regulator of a vital behaviour i.e., feeding (McKay et al., 2004) and EAT-2 expression is also found in parasitic species where it may perform a similar functional role.

Here, *C. elegans* is used to screen for EAT-2 selective chemicals. In a bioassay that mimics the presence of food by triggering feeding behaviors in *C. elegans* and the PPN *Globodera rostochiensis*, mecamylamine, a known inhibitor of EAT-2 has been used to block this feeding response. This provides evidence that EAT-2 may be pharmacologically targetable in the context of the whole organism. We have developed a screening platform in *C. elegans* to identify candidate EAT-2 selective compounds. This approach uses a genetic background deficient in other major cholinergic receptors in a whole organism screening platform to bias for EAT-2 modulators by phenotypically scoring for feeding disruption and other key behaviours like egg laying, motility and aversive behaviour. Potential hit compounds are investigated in other paradigms to confirm their selectivity for EAT-2. This is followed by exposure of PPNs to the chemical and scoring for impact.

McKay, J. P., Raizen, D. M., Gottschalk, A., Schafer, W. R., & Avery, L. (2004). eat-2 and eat-18 Are Required for Nicotinic Neurotransmission in the *Caenorhabditis elegans* Pharynx. Genetics, 166(1), 161-169. https://doi.org/10.1534/GENETICS.166.1.161

46. Characterisation and management of *Globodera rostochiensis* populations adapted to Kenyan sub-tropical conditions

James Price[1], Kelsey Hunt[2], Solveig Haukeland[3], Calvince Orage[4], Onesmus Mwaura[4], Newton Nelsi[3], Danny Coyne[4], John Jones[1]
1 Cell & Molecular Sciences Department, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK
2 School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK
3 International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
4 International Institute of Tropical Agriculture (IITA), Nairobi, Kenya

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: PCN, resistance, giant cysts

ABSTRACT:

Potato production in East Africa is being seriously impacted by the potato cyst nematode (PCN) *Globodera rostochiensis*, where it has been recorded in at least three countries. In Kenya, it is widespread in all major potato growing regions, often at very high densities (Mburu et al., 2020, doi.org/10.3389/fpls.2020.00670). Consecutive cropping of potato on the same land and a sub-tropical climate have influenced PCN biology. For example, unusually large cysts have been regularly recovered. We have analysed the biological properties of these ‘giant cysts’. Giant cysts contained more eggs than those from UK fields. Egg size did not differ from UK populations and there was no difference in overall lipid content or lipid profile in J2 from giant cysts, compared to control samples. The nematodes in giant cysts were also genetically indistinguishable from any other *G. rostochiensis* sampled. When grown under UK glasshouse conditions, the offspring of nematodes from giant cysts were no different in size from those grown from control cysts, indicating that gigantism is not a heritable trait and may simply reflect favourable conditions for PCN under Kenyan farming systems.

To date, all the PCN tested from Kenya, including those from giant cysts, are avirulent on potato cultivars containing the H1 resistance gene. New potato lines that combine H1 resistance with other preferred traits required by growers in the region have been developed and tested. Field tests show that the lines performed well in the field including high levels of tolerance to PCN, were favoured by growers and provided excellent control of PCN. Progress towards the registration and scale up of seed tuber production of these new cultivars will be presented.

47. Nematode diversity and soil functions under different land uses at a regional scale

Rui Carvalho[1], Carlos Guerra[2], Sofia Costa[1]
1 Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal
2 German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: ecosystem functions, soil biodiversity, conservation, regional scale, land-use

ABSTRACT:

Soil biodiversity is vital in providing essential ecosystem functions that ultimately affect human well-being, but it is threatened by land-use intensification and global change. However, a scarcity of belowground ecology studies at an appropriate scale limits our understanding of land-use impacts and hinders management decisions and policies to alleviate their consequences. Due to their wide distribution in all soil types, large abundance, and functional diversity, nematodes are particularly useful indicators of soil biodiversity and function. We conducted a regional analysis of soil nematode communities across the North of Portugal, sampling six land-use types (annual and perennial agriculture, pasture, urban areas, and exotic and native forests), and encompassing protected areas for nature conservation.

Results suggest that pastures and native forests harbour higher total nematode abundance and diversity, whereas exotic forests display reduced nematode abundance and less diverse communities. The latter also had lower nutrient availability and food-web complexity, being markedly different from native forests, pastures and agriculture soils. We further found increased structure and omnivore metabolic footprints in protected areas, indicating a more regulated system and suggesting (aboveground) conservation practices are mirrored belowground.

Understanding soil biodiversity-function relationships under different land-use types can lead to more effective nature conservation policies, enhancing soil-based ecosystem services whilst promoting resilience of ecosystems.

48. Plant-parasitic nematodes associated with chickpea from Ethiopia: damage potential of *Meloidogyne* spp. and identifying resistant varieties

Habtamu Kefelegn[1], Beira H. Meressa[2], Marjolein Couvreur[1], Wim M. L. Wesemael[3], Misghina G. Teklu[5], Wim Bert[1]
1 Nematology Research Unit, Department of Biology, Ghent University, Campus Ledeganck, Ledeganckstraat 35, B-9000 Ghent, Belgium
2 College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box, 307, Jimma, Ethiopia
3 Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Burg Van Gansberghelaan 96, B-9820 Merelbeke, Belgium
4 Laboratory for Agrozoology, Department of Plants and Crops, Ghent University, Coupure links 653, B-9000 Ghent, Belgium
5 Plant Research, Plant Sciences Group, Wageningen University and Research Centre, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords:

ABSTRACT:

Chickpea is one of the most important legume crops in Ethiopia, however, its production is far below the average international production due to biotic and abiotic stressors. Plant-parasitic nematode infestation is widespread in chickpea-growing areas worldwide. The distribution and population density of plant-parasitic nematodes of chickpea was determined from September to December 2021. Ten plant-parasitic nematode taxa were identified in 304 fields sampled in the main chickpea-growing areas of Ethiopia. According to prominence values, *Pratylenchus* had the highest value, followed by *Rotylenchulus* and *Meloidogyne* spp., *Helicotylenchus*, *Hoplolaimus*, *Scutellonema* and *Quinisulcius*, *Criconemoides* and *Ditylenchus* were less prominent. Morphological and molecular data, including D2-D3 of 28S rDNA, ITS of rDNA and *COI*, *COII* and Nad5 of mtDNA sequences, revealed the presence of *Meloidogyne javanica*, *Meloidogyne* sp., *Pratylenchus delattrei*, *Rotylenchulus parvus*, *Scutellonema clathricaudatum*, *Helicotylenchus caudatus* and *Quinisulcius capitatus*. *Pratylenchus delattrei*, *Rotylenchulus parvus*, *Scutellonema clathricaudatum*, and *Helicotylenchus caudatus* are the first reports of chickpea. Nineteen chickpea varieties were evaluated under glasshouse conditions at the Institute of Agricultural and Fisheries Research (ILVO), Belgium, to investigate their resistance/tolerance to two tropical *Meloidogyne* species in a temperature-controlled growth chamber at ILVO. Based on the qualitative assessment, mainly counting egg mass per plant, these varieties showed different responses to *Meloidogyne javanica* and *Meloidogyne* sp. Further research on resistance and damage threshold was conducted on six selected chickpea varieties at Wageningen University and Research, Netherlands. Based on Seinhorst yield loss models for several biomass variables (shoot height, fresh shoot and root weight, number of pods, pod weight, number of seeds, and seed mass weight per plant), *Meloidogyne javanica* and *Meloidogyne* sp. showed different values for these chickpea varieties, and significantly reduced their yield. The Seinhorst population dynamics model demonstrated that chickpea varieties differ significantly in their maximum

multiplication rate and maximum population density. The current results provide baseline information on nematode pest occurrences in chickpeas in Ethiopia, which will enable growers, agricultural officers, policymakers, and extension advisors to pay attention to and develop management strategies in Ethiopia.

49. Exploring the diversity and occurrence of major Plant-Parasitic nematodes in vineyards across Northern and Central Spain

Maria Del Mar González-Trujilo[1], Rubén Blanco-Pérez[2], Raquel Campos-Herrera[1], Sergio Álvarez-Ortega[3]
1 Instituto de Ciencias de la Vid y del Vino (ICVV), Gobierno de La Rioja, CSIC and Universidad de La Rioja) Logroño, La Rioja, Spain
2 Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Spain
3 Universidad Rey Juan Carlos, Móstoles, Madrid, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: viticulture, abundance, distribution patterns, plant damage, environmental factors, management strategies

ABSTRACT:

Vineyards are pivotal to Spain’s agronomy and social fabric, representing one of the most relevant crops due to extensive cultivation -the largest globally- remarkable productivity, deep-rooted historical tradition, and substantial contribution to agricultural and wine sector employment. Plant-parasitic nematodes have often been associated with a reduction in the growth and productivity of grapevines in regions worldwide. These nematodes act as significant pathogens and cause increased crop losses by serving as virus vectors and interacting with other root pathogens. Given Spain’s diverse viticulture management and landscapes, which extend a wide range of soil and climatic conditions, an ideal scenario exists to explore the distribution patterns and ecological preferences of important plant-parasitic nematodes affecting vineyards. Moreover, information on the incidence and distribution of the main plant-parasitic nematodes in Spanish vineyards remains limited. This study hypothesized that plant-parasitic nematode diversity, distribution, and incidence might differ across central and northern Spain, influenced by diverse environmental factors (soil properties, climate). Through extensive sampling, this research investigated the diversity and distribution of predominant plant-parasitic nematode genera in vineyards across La Rioja, Madrid, and Toledo. Preliminary results revealed that dagger (*Xiphinema* spp.), ring (*Criconemoides* spp. and *Mesocriconema* spp.), lesion (*Pratylenchus* spp.), spiral (*Helicotylenchus* spp.), and pin nematodes (*Paratylenchus* spp.) were prevalent. Among them, dagger, spiral, and ring, nematodes were the most widespread, occurring in 76%, 73%, and 61% of the sampled vineyards, respectively. These findings underscore the need for a deeper understanding of the diversity, abundance, and distribution patterns of plant-parasitic nematodes to design effective management and control strategies for Spain’s diverse agroecosystems.

50. Nematode communities from strawberry fields: a comparison between morphology and mitometagenomics

Dorota L. Porazinska[1], Eli Gendron[1], Clemen Oliveira[2], Johan Desaeger[2]
1 Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA
2 Department of Entomology and Nematology, University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598, USA

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: identification, metagenomics, mitochondrial sequencing, species, taxonomic resolution,

ABSTRACT:

Plant parasitic nematodes are among the most damaging pests in agricultural fields. Current nematode management heavily relies on the use of soil fumigants, but is known to negatively impact soil biodiversity, soil processes, and overall soil health. More sustainable agricultural activities must adopt more nematode species-specific practices such as cover crops or targeted biologicals. However, the adoption of nematode pest specific practices requires the ability to accurately identify species.

Here, we evaluated nematode biodiversity in four Florida strawberry fields, at four depths, under varying management histories (three conventional fields: R1, C1, C2 and one organic: O1), using both classic morphological identification and more recently developed mitochondrial metagenomics (mtMG). By bypassing the need for morphological inspection, taxon-specific primers, or limited taxonomic resolution of gene markers, while also allowing high throughput processing, mtMG could allow for more comprehensive and accurate analysis not only of pests but the entire nematode communities.

Due to the increased capacity to identify nematode species, we expected to recover higher richness and significantly different communities with the mtMG method than with the morphological method. However, due to the known impacts of management practices on the diversity of nematode communities, we expected that richness and community composition would also depend on the field and depth with overall patterns being independent of the method.

As expected, nematode communities assessed by the mtMG method deviated from those based on morphology by generally recovering higher nematode richness and significantly different compositions. Differences were largely driven by the distinct detection of different trophic groups, with mtMG recovering animal parasitic and entomopathogenic taxa while morphology recovering omnivorous and predatory taxa. Furthermore, despite significant differences in the recovered taxa, both methods identified management history (field) and depth as significant factors shaping nematode communities with both pointing to fields C2 and O1 driving the observed differences.

Our analyses highlight the limitations and advantages of both methods. On one hand, while the mtMG method captures a wide diversity of species and reduces the time and effort needed to do so, it struggles to recover taxa absent in the reference database. In contrast, while the morphology method effectively captures nematodes at the trophic level, it falls short in identifying species and

morphologically ambiguous life stages. Thus, to obtain the most comprehensive description of nematode communities, the use of both methods is encouraged.

55. Identification and characterization of potato cyst nematode resistance genes

Moray Smith[1], Ingo Hein[1], John Jones[1]
1 James Hutton Institute, UK
2 University of Dundee, UK
3 University of St Andrews, UK

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: resistance genes, NLRs, Potato, Potato Cyst Nematode

ABSTRACT:

The nucleotide-binding leucine-rich repeat (NLR) gene family is a highly conserved yet diverse source of plant disease resistance. They are abundant in many plant species and have been characterised as being effective against many species of pathogen. Despite this, many genetic loci associated with disease resistance have not been characterised to the individual gene level. This limits our understanding of how they function and their utilisation in protecting economically important crops.

The aim of this PhD is to identify and characterise novel resistance genes against the potato cyst nematode (PCN). A combination of single-molecule real-time resistance gene enrichment sequencing (SMRT-RenSeq) and marker genotyping was used to identify two candidates for the H2 PCN resistance gene. Throughout the process of identification and characterisation by transformation into a susceptible background, several drawbacks of this method were identified. The H2 gene is currently only available in a tetraploid background which severely impacts the effectiveness of SMRT-RenSeq and mapping based approaches. Recent advances in sequencing technologies offer an alternative approach for resolving candidates in complex tetraploid genomes which are being pursued. A combination of ultra-long and duplex nanopore sequencing will be carried out to create a haplotype-phased genome, allowing isolation of the full gene structure of the resistant haplotype of H2.

Another source of PCN resistance, the H3 resistance gene, has also remained elusive. Unlike H2, H3 is more widely deployed across potato cultivars. This diversity can be exploited to identify genetic elements shared by H3 positive cultivars. A series of computational methods have been developed that use sequence data from cultivars with and without the resistance to identify genes associated with the resistant phenotype.

56. AI-powered holistic and dynamic plant-pathology to deliver new sources of resistance

Siyuan Wei[1], Sebastian Eves-van Den Akker[1]
1 The Crop Sciences Centre, Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral

Keywords: plant-parasitic nematodes, *Arabidopsis thaliana*, *Heterodera schachtii*, genome-wide association study, multiparent advanced generation inter-cross population, high-throughput phenotyping

ABSTRACT:

Plant-parasitic nematodes are a major, and in some cases dominant, threat to food security. The major barrier to identifying new sources of resistance is phenotyping. To address this constraint, we designed and built a bespoke high-throughput, low-cost, and semi-automated phenotyping system that combines custom 3D-printed hardware and deep-learning-powered trait recognition.

Using this unprecedented capability, we screened the *Arabidopsis* Multiparent Advanced Generation Inter-Cross (MAGIC) population for susceptibility to the beet cyst nematode *Heterodera schachtii*. All 527 recombinant inbred lines, each with 20 biological replicates (approx. 10,000 plants), were phenotyped 45 times over the following 90-days (some ~400,000 phenotyping events, including tens of millions of nematodes).

This exemplifies a truly holistic (i.e. whole plants) and dynamic (i.e. the whole life cycle) phenotyping approach that sets a precedent in pathology in general. Using these extensive data, we explored novel aspects of strategic and academic merit. Firstly, we used genome-wide association studies to map the plant-loci that contribute to nematode phenotypes (i.e. novel susceptibility and resistance loci). Secondly, we define, with high confidence, new fundamental features of the nature of parasitism.

Taken together, we demonstrate the power of AI to deliver a step change in our understanding of, and ability to control, plant-parasitic nematodes.

In this project, I focused on the model plant-parasitic nematode pathosystem (*Arabidopsis thaliana*: *Heterodera schachtii*) because the diverse and well-characterised plant populations and genetic backgrounds provide rich resources for GWAS. To harness these resources, I have prototyped a new high-throughput phenotyping method empowered with deep-learning trait recognition to circumvent the phenotyping bottleneck. I have then used this new capability to screen the susceptibility and resistance of the *Arabidopsis thaliana* Multiparent Advanced Generation Inter-Cross (MAGIC) population: 500 lines, each with 20 biological replicates, every other day for 90 days. This enormous data set of literally millions of nematodes has allowed us to uncover new fundamental features of parasitism and new sources of resistance. Now, I am working on the characterization of the nature of the resistant/susceptible genotypes that I have discovered.

57. Identification of putative resistance-breaking effectors of *Globodera pallida* by systematic screening of a genomic region associated with virulence on *Gpa5*-resistant potatoes

Arno Schaveling[1], Dennie te Molder[1], Stefan van de Ruitenbeek[1], Casper van Schaik[1], Geert Smant[1], Mark Sterken[1]
1 Laboratory of Nematology, Wageningen University Research, Wageningen, The Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: effectors, resistance, potato, PCN

ABSTRACT:

Potato cultivation is under constant threat by a range of pests and diseases, including the potato cyst nematode (PCN) species *Globodera pallida*. In the 1990s, the *Gpa5* resistance locus from the related species *Solanum verneii* was introduced into potato to control *G. pallida* populations. However, over the last decade, it has become clear that *G. pallida* field populations have overcome *Gpa5*-mediated resistance. The limited durability of this resistance shows that *G. pallida* populations can easily adapt and overcome resistances employed in the field. The genetic and molecular basis driving the adaptations of *G. pallida* to *Gpa5* is unknown. Recently, a genomic region was identified that significantly associated with virulence on *Gpa5*-resistant potato cultivars. By mapping whole genome sequencing data to a newly constructed *G. pallida* genome, we confirmed that this region was under selection. We are exploring the hypothesis that virulence of *G. pallida* on *Gpa5*-resistant cultivars is determined by allelic variation in effector genes located within this region. To this end, we used five criteria to identify a subset of putative effector genes that are most likely to be responsible for breaking *Gpa5* resistance in potato. Currently, by expressing the putative effectors in a *Gpa5* background, we aim at pinpointing the effector responsible for overcoming *Gpa5* resistance. During the ESN2024, we hope to share the success story of a widely applicable screening method for the identification of resistance-breaking effectors in parasitic nematodes.

1. A region on the *Globodera pallida* genome consistently associates with virulence on Gpa5-resistant potatoes.
2. Using five selection criteria to screen all transcripts within this region, we identified a subset of genes that classify as putative resistance-breaking effectors .
3. Expressing these putative effectors in a Gpa5 background aims to pinpoint the effector responsible for breaking Gpa5 resistance.

58. Unraveling the function of the cyst nematode resistance gene *Hs4* in different genomic backgrounds

Annika Schildberg[1], Christian Jung[1]
1 Plant Breeding Institute, Kiel University, Kiel, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords:

ABSTRACT:

The beet cyst nematode (BCN) *Heterodera schachtii* is a main pest of sugar beet (*Beta vulgaris*) and many other species from the Amaranthaceae and Brassicaceae plant families. Sugar beet and its close relatives are highly susceptible to the BCN, while distant relatives from the genus *Patellifolia* are fully resistant. Sugar beet lines carrying translocations from the *P. procumbens* chromosome 1 are resistant to the BCN. Recently, the *Hs4* gene was cloned from a *P. procumbens* translocation attached to the beet chromosome 9. The gene encodes an ER-bound rhomboid-like protease. A homolog with 60 % polypeptide similarity is present in *B. vulgaris*. The *Hs4* gene was expressed in susceptible sugar beet hairy roots resulting in resistance to the BCN.

In an ongoing project, we searched the genomes of different *Beta* and *Patellifolia* species for the presence of *Hs4* or its homolog. We found that all *Patellifolia* contained the *Hs4* gene and the homolog could be detected in all Beta species. Then, we studied the expression of these two genes in several *Beta* and *Patellifolia* species. While *Hs4* is expressed more prominently in *Patellifolia* roots compared to leaves, the homolog is expressed conversely in beets.

We were also questioning whether *Hs4* functions in distantly related species. We therefore transformed the gene into the model plant *Arabidopsis thaliana* under the transcriptional control of two different promoters. *Hs4*-transgenic T3 lines showed significantly reduced cyst numbers. Moreover, the *Hs4* expression correlated with the cyst number indicating that the *Hs4* gene confers resistance even in distantly related species. Recently, we started transforming oilseed rape (*Brassica napus*) using the same *Hs4* constructs.

59. Cyst nematodes counteract immunity by inhibiting activation of central nodes of a Solanaceae immune receptor network

Mauricio P. Contreras[1], Hsuan Pai[1], Muniyandi Selvaraj[1], AmirAli Toghani[1], David M. Lawson[2], Yasin Tumtas[3], Cian Duggan[3], Enoch Lok Him Yuen[3], Clare E.M. Stevenson[2], Adeline Harant[1], Abbas Maqbool[2], Chih-Hang Wu[4], Tolga O. Bozkurt[3], Sophien Kamoun[1] and Lida Derevnina[1*]
1 The Sainsbury Laboratory, University of East Anglia, Norwich, UK
2 Department of Biochemistry and Metabolism, John Innes Centre, Norwich, UK
3 Department of Life Sciences, Imperial College, London, UK
4 Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan
** Present address: Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge, UK*

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: plant-nematode interactions, NLR activation, inhibition, helper NLR

ABSTRACT:

Plant pathogens deploy effectors that suppress plant immune responses and promote disease. Despite their important role in pathogenicity, our understanding of the mechanisms underlying effector function remain relatively limited. We previously identified SS15 a SPRYSEC-type effector derived from the potato cyst nematode *Globodera rostochiensis* as a potent suppressor of immunity in Solanaceous plants. SS15 targets the function of helper NLRs, namely NRC2 and NRC3, that are central nodes of a complex immune signalling network. Using biochemical and cellular approaches, we demonstrate that SS15 binds to and inhibits NRC2 oligomerization and plasma membrane association, which are critical for immune signalling. To overcome this suppression, we introduced mutations in the SS15-NRC2 binding interface and bioengineered an NRC2 variant that evades inhibition and restores NRC2 function in the presence of SS15. Our work exemplifies how a deeper mechanistic understanding of effector biology can provide valuable insights for developing novel strategies to generate disease resistant crops.

60. Use of virulent *Globodera pallida* lines obtained from experimental evolution to reveal candidate regions for the adaptation to potato resistances

Océane Lechevalier[1], Magali Esquibet[1], Stéphanie Daval[1], Kévin Gazengel[1], Eric Grenier[1], Sylvain Fournet[1], Josselin Montarry[1]
1 INRAE, Le Rheu, France

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: *Globodera pallida*, adaptation, phenotyping, RNAseq, GenomeScan

ABSTRACT:

The use of alternative methods to control populations of cyst nematodes has accelerated since the banning of the most efficient nematicides. The pest-resistant plant varieties have also become an indispensable solution to this problem. However, this solution is not without risk, since it has been shown that some nematode populations can overcome these resistances. In order to understand the mechanisms of adaptation of the nematode *Globodera pallida* to potato resistances, a combination of phenotypic, transcriptomic and genomic approaches has been initiated on lines obtained from experimental evolutions. The objectives are to identify the genetic basis of *G. pallida* adaptation to (1) different resistance QTLs and (2) to a combination of QTLs. This work involved phenotyping to characterize the level of virulence of selected lines on potato genotypes carrying different resistance factors. These initial results illustrate the overcome of resistances by virulent lines after 10 generations. A first RNAseq approach revealed candidate genes responsible for suppressing plant immunity and therefore potentially involved in adaptation. The nematode lines were then sequenced and analyzed using a GenomeScan approach. This analysis highlighted genomic regions under selection and potentially involved in the adaptation mechanism. First results showed for instance that genes involved in the adaptation to resistant QTLs from different wild Solanaceae are located in distinct genomic regions. These results will have an impact on the design of efficient and sustainable management strategies, independent of the use of controversial chemicals.

61. European reference laboratories: a key tool for diagnosis and networking in the EU plant health field

Maíra Grossi de Sá[1], Nicole Viaene[2], Sylvie Gamel[1], Nicole Damme[2], David Hatcher[1], Niels Vermassen[2], Françoise Munaut[3], Laurent Folcher[1]
1 ANSES - Plant Health Laboratory - Nematology Unit, France
2 ILVO - Plant Sciences Unit, Belgium
3 Françoise Munaut, European Commission - DG SANTE - Unit G1, Plant Health, Belgium

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: EURL, NRLs laboratories, plant-parasitic nematodes, diagnostic methods, EU networking, plant health, regulation

ABSTRACT:

The increasing world globalization and trade of agricultural commodities have become a challenge to food security and plant health services. European Reference Laboratories (EURLs) in the field of Plant Health have been designated by the European Commission (EC) to provide high-level expertise in plant pest diagnosis and to support the EU Member States (MSs) for the detection and identification of regulated and emerging pests. The EURLs contribute to the improvement and harmonisation of the diagnostic methods used for official controls and other official activities performed to ensure the application of the EU plant health law (Regulation (EU) 2016/2031). By assisting the National Reference Laboratories (NLRs) of all 27 Member States (MSs), the EURLs contribute to early and focused interventions at EU borders and in its territory. The EURL for Plant Parasitic Nematodes (PPNs) was created to improve accurate detection of PPNs. At the same time, it was asked to establish a network between the EC, the EURL and the NRLs, which supports the EU surveillance framework of quarantine and emerging PPNs. In the EU, 39 nematode species, which cause substantial economic losses in agricultural crops or forestry, are regulated as quarantine pests, a few examples are *Meloidogyne enterolobii*, *M. chitwoodi* and *M. fallax*, *Bursaphelenchus xylophilus*, *Globodera rostochiensis* and *G. pallida*. For new threats, such as the rice root-knot nematode *M. graminicola*, temporary measures have been put in place by the Implementing Regulation (EU) 2022/1372. The EURL for PPNs provides coordinated assistance to NRLs by disseminating high standard diagnostic practices and information on analytical methods, distributing reference materials, as well as giving training courses. Inter-laboratory tests are organized to assess the diagnostic competence and robustness of the EU NRL network and are open to other countries of interest for the EU. Validation of analytical methods and research on reliable diagnostic approaches resulted in the publication of EURL protocols for the detection and identification of six regulated nematode species so far. In addition, the EURL for PPNs provides recommendations to the EC regarding the most reliable and robust diagnostic methods to be included in legal acts aiming at preventing, controlling and eradicating major PPNs. Therefore, the EURL for PPNs is a crucial part of the EU s efforts to ensure agriculture and food safety by acting as a key tool enabling early detection of potential threats and supporting PPN surveillance.

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62. Beech Leaf Disease, caused by the nematode *Litylenchus crenatae mccannii*, poses a threat to beech trees in Europe

Nicole Viaene[1], Negin Ebrahimi[1], Annelies Haegeman[1], Anne Sophie van Bruggen[2], Nikica Ogris[3], Saša Širca[4], Thomas Prior[5], Ana Pérez Sierra[6], Mariana Groza[7], Mihaela Coman[7], Marie-Jo Hurley[8]

1 Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium
2 Netherlands Food and Consumer Products Safety Authority (NVWA), Wageningen, the Netherlands
3 Slovenian Forestry Institute (SFI), Ljubljana, Slovenia
4 Agricultural Institute of Slovenia (KIS), Ljubljana, Slovenia
5 Fera Science, York, United Kingdom
6 Forest Research (FR), Surrey, United Kingdom
7 National Phytosanitary Authority (NPA), Ilfov, Romania
8 Department of Agriculture, Food and the Marine (DAFM), Dublin, Ireland

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: culture, *Fagus*, leaf microbiome, *Litylenchus*, risk, survey

ABSTRACT:

Beech Leaf Disease (BLD) has been expanding in several states in north-east America since 2012. Infected trees exhibit dark interveinal bands on the leaves, leaf deformation and bud abortion in spring, leading to canopy thinning and tree mortality. Both American beech (*Fagus grandifolia*) and European beech (*F. sylvatica*) are affected. The etiology and spread of the disease is not fully understood, however it is certain that *Litylenchus crenatae* subsp. *mccannii* is involved [1], a nematode species isolated from symptomatic beech leaves and buds. In Europe, no BLD symptoms on beech trees nor *Litylenchus* spp. have been reported to date. Within the Euphresco project 2020-A-334, FAGUSTAT, we increased awareness of BLD in Europe and made the first assessment of its status in the region. In 2021 and 2022, surveys were performed on *Fagus* spp. in the six participating countries (Belgium, the Netherlands, Romania, Slovenia, United Kingdom and Ireland). We collected over 500 samples of beech leaves, buds and nuts in forests, parks, botanical gardens and nurseries and extracted the nematodes. Although look-alike BLD symptoms were observed, very few nematodes (e.g., *Aphelenchoides* sp., *Plectus* sp., *Panagrolaimus* sp.) were found, none belonging to *Litylenchus*. Information sheets on BLD were drafted to increase public awareness and communicated via several channels with stakeholders in nurseries, public parks, plant health inspections services, botanic gardens, and ornamental businesses. Although the pathways of BLD transmission are still not completely understood, we explored the possibilities of entry and establishment of the disease in Europe. The role of a vector or microorganisms contributing to disease development has been suggested but is still unknown. We studied the microbiome of symptomatic as well as asymptomatic leaf samples from 20 trees, although *L. crenatae* was not found. Metabarcoding showed that the microbiome varied greatly between trees, hence differences between symptomatic and asymptomatic leaves were strongly tree-dependent. In general, leaves from forest trees had a higher bacterial and fungal richness than leaves from nurseries. *Pseudomonas* and *Erwinia* were among the top five taxa in our study, genera that were previously reported to be more prevalent in leaves affected by BLD [2]. Lastly,

culturing *L. crenatae* via different methods was trialed (carrot discs, callus tissue, fungal cultures and beech sapling inoculation) to obtain large numbers for resistance testing of F. sylvatica cultivars but did not succeed in multiplying the nematode. The information gathered to date will be used in support of a pest risk analysis for Europe.

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63. Detection of *Aphelenchoides besseyi* on rice seeds, a new official French method.

Justine Foucher[1], Damien Gonzalez, Céline Andro, Delphine Leroux-Bouly, Juliette Lechat, Valérie Grimault, Nicolas Denancé, Isabelle Sérandat, Thomas Baldwin, Amandine Le Van, Jaiana Malabarba
1 Groupe d'Étude et de contrôle des Variétés Et des Semences (GEVES), 25 rue Georges Morel, 49071 Beaucouzé, France.

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords:

ABSTRACT:

Rice is one of the most important crops in the world and is a staple food for a large part of the global population. However, the rice white tip nematode, *Aphelenchoides besseyi*, is one of the main pests on this culture and has been ranked among the ten most important nematodes in terms of economic losses. This free-living nematode is an ectoparasite, living on the foliar part of the rice plant. When the seeds are developing, *A. besseyi* can move close to the seeds and can stay in a quiescent state between the glumes of the seeds for several years. This ability to survive on seeds contributes to the significant risk of this nematode spreading through the exchange of rice seeds around the world. Today, *A. besseyi* has a wide geographical distribution and is reported in several European Union countries. Because of these risks of economic loss and its presence in Europe, this nematode is listed by the European Commission as a non-quarantine regulated pathogen on rice seeds. GEVES, designated by French Ministry of Agriculture as a national reference laboratory (NRL) for plant health, is specialised in the detection of non-quarantine regulated pests on seeds, such as *A. besseyi* on rice seeds.

In order to limit the spread of infected seeds, it is essential to have an official method to identify infected seed lots. Based on the different detection methods already available in the literature, the GEVES NRL tested several nematode extraction techniques and tested a PCR confirmation step in order to avoid false positive or negative results, which could have serious consequences for the management of this disease. This is why the different performance criteria of each technique have been evaluated to ensure that the results are as reliable as possible.

This work led to the development of a new method adapted for the detection of *A. besseyi* on rice seeds, which will be submitted to the French Ministry for official recognition. This new method improves the detection of *A. besseyi* on rice seeds and therefore helps to limiting the spread of this important pathogenic nematode.

64. Possible spread of *Meloidogyne enterolobii* in potted ornamental plants in flooding water systems

Anne Sophie van Bruggen[1], Rik Peters[2], Johnny Visser[2], Evelyn van Heese[1]
1 Netherlands Institute for Vectors, Invasive Plants and Plant Health (NIVIP), National Plant Protection Organization (NPPO), NVWA, Wageningen, The Netherlands
2 Wageningen University & Research (WUR), Business Unit Field Crops, Lelystad, the Netherlands

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: quarantine, root-knot nematode, measures

ABSTRACT:

M. enterolobii is a tropical root-knot nematode causing severe symptoms in a wide range of host plants. This *Meloidogyne* species can overcome an important resistance in tomato and pepper. Therefore, *M. enterolobii* is listed as a European Union quarantine organism since April 2022. In the Netherlands this nematode has been intercepted frequently in imported potted ornamental plants, especially *Ficus microcarpa*, all these consignments were destroyed. An action plan was drafted describing measures to be applied in case of findings in greenhouses of ornamental potted plants. In 2023 a survey has been performed at 33 growers of *F. microcarpa*, *M. enterolobii* was found at three companies. All plants at these companies in the same watering system as the infested plants were declared infested, because of the risk of spread of *M. enterolobii* in flooding water systems. This measure had a serious impact, because large greenhouse area's (up to 5 ha) were connected to one shared watering system.

An experiment was conducted by WUR in cooperation with NIVIP to study the dissemination of *M. enterolobii* from infested plants into the water during flooding. Fourteen tomato plants in 3 L pots with potting soil were used as model plants. Six-week-old tomato plants were inoculated with *M. enterolobii* and 12 weeks after inoculation each pot was placed in a separate plastic container and flooding was applied weekly, for a period of 8 weeks. Three infested *Zelkova* plants from an intercepted consignment were treated in the same way. After flooding, the leftover water in each container was collected and *Meloidogyne* nematodes were extracted and counted. From the water of eight tomato plants and from all three *Zelkova* plants *M. enterolobii* was isolated. The numbers of *M. enterolobii* found were low, but risk of spread of this nematode in flooding water systems cannot be excluded.

65. Two decades of epidemiological surveillance of the pine wood nematode in France

Nicolas Mariette[1], Hoël Hotte[1], Anne-Marie Chappé[1], Marie Grosdidier[2], Géraldine Anthoine[3], Corinne Sarniguet[1], Odile Colnard[4], Emmanuel Kersaudy[1], Marie-Thérse Paris[1], Emmanuel Koen[6], Laurent Folcher[1]

1 ANSES, Plant Health Laboratory, Nematology Unit, Le Rheu, France
2 INRAE, Unité BioSP, Avignon, France
3 ANSES, Plant Health Laboratory, Coordination of Reference Activities Unit, Angers, France
4 Bureau de la transition pour une production agricole durable, Sous-direction de l'accompagnement des transitions agroécologiques et alimentaires, DGAL, Paris, France
5 Pole Sante des Forêts, Direction régionale de l'alimentation, de l'agriculture et de la forêt de Nouvelle-Aquitaine, Bordeaux, France
6 Sous-direction de la santé et de la protection des végétaux, Direction générale de l'alimentation, Ministère de l'agriculture et de l'alimentation, Paris, France

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: *Bursaphelenchus xylophilus*, priority quarantine pest, national monitoring program, *Monochamus* spp., first report, forest

ABSTRACT:

The installation of a plant pest into a new area can have economic, environmental and/or social consequences. This is the case for the pine wood nematode (PWN) *Bursaphelenchus xylophilus* which is one of the most important coniferous pests worldwide, causing massive tree mortality wherever it has been established. It thus receives a great scrutiny like in European Union where it is categorised as a priority quarantine pest, obliging each state member to monitor and to manage this pathogen on its territory. Our study takes stock of the first 20 years (2000-2019) of the monitoring implemented in metropolitan France to track PWN. Our work had three main objectives. The first one was to describe PWN monitoring, namely how it is organised and whether it has led to the nematode's detection. Secondly, we wished to investigate what the levels of PWD expression for host pines infected by *B. xylophilus* could be in France. Finally, we wanted to find out whether other *Bursaphelenchus* species had been found on French territory during these two decades of PWN monitoring. To meet these three objectives, we used and analysed data from samples collected in the framework of the French monitoring programme implemented from 2000 to 2019 to track both PWN in its host pines, its insect vector and in wood-based commodities imported or in circulation in metropolitan France. This monitoring, which was regularly reinforced across the time, consisted to sample and analyze more than 17,000 wood samples and 66,000 insects over this period. Although PWN has never not been detected in pine stands or within its insect vector, some wood-based commodities inspected during the monitoring were contaminated. This monitoring has also showed the presence of other *Bursaphelenchus* species whose it was the first description in metropolitan France for most of them. If metropolitan France is still free of PWN, this study emphasizes the need to stay vigilant, as climatic conditions of metropolitan France would be particularly suitable to this pest. Besides, we propose some

improvements that could be implemented to the monitoring to make it more efficient. Finally, such a monitoring is also the occasion to enhance our knowledge of the *Bursaphelenchus* genus.
Reference: N. Mariette, H. Hotte, A.M. Chappé, M. Grosdidier, G. Anthoine, C. Sarniguet, O. Colnard, E. Kersaudy, M.T. Paris, E. Koen, L. Folcher, Two decades of epidemiological surveillance of the pine wood nematode in France reveal its absence despite suitable conditions for its establishment. Annals of Forest Science 80, 21 (2023). https://doi.org/10.1186/s13595-023-01186-8

66. Chemical and physical methods to control the propagation of *Bursaphelenchus xylophilus* in wood materials

Lee Robertson[1], Sara Santos[1], Susana C Arcos[2], Navas Alfonso[2], Teresa de Troya[1]
1 Instituto de Ciencias Forestales, ICIFOR-CSIC, Ctra. de la Coruña KM. 7, 28040Madrid, Spain.
2 Laboratorio Nacional de Referencia de Nematodos Fitopatógenos, Museo Nacional de Ciencias Naturales, MNCN-CSIC, C/ Jóse Gutiérrez 5, Madrid, Spain.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Bursaphelenchus*, wood treatment, biocide, ISPM15

ABSTRACT:

The pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (PWN) is the causal agent of ‘Pine wilt disease’ affecting coniferous forests, and since its detection in 1999 in Portugal, has obligated the European Union to take action to prevent its propagation and dispersion (Decision 2006/133/CE). The current control measures have been unsuccessful in preventing the dispersal of this organism, as it continues to be detected in wood material through commercial trade. These measures to control PWN are covered by International Standards for Phytosanitary Measures No. 15 (ISPM-15) for packaging material and ISPM 39 for solid wood. Although these Standards are applied in the wood industry, those materials have been found infested with PWN. There may be several reasons, including, inadequate application of the Standards, post-treatment contamination, or resistance forms of the nematode. This paper presents the results obtained from two national research projects. One focuses on the application of chemicals recommended in ISPM 39 against wood decay organisms (fungi and insects) in wood and permitted by the Regulation of Biocidal Products, and the other on the development of physical treatments addressed in ISPM 15.

Experiments using commercial wood treatment products and their active ingredients show that almost all of them are effective against the PWN. In consequence, timber protected with commercialized wood products may be a good way to control PWN in the wood industry.

Concerning the physical treatments the use of traditional ovens, microwaves and ultrasounds (mono and multimode) are studied. The results in oven heat treatment showed that the most effective temperatures to kill the nematode are closer to 70°C. Those using microwaves showed that the most effective microwave device is the multimode and the effectiveness depends on the wood dimensions. Finally, the results obtained with ultrasonic equipment show that ultrasounds (Hz 50/60, 230V) affect nematode survival both in vitro and in infected wooden specimens.

All previous results show that there exist alternatives to ISPM 15 and ISPM 39 to control PWN, and that further research is required to assess the different possible combinations of chemical and physical treatments.

67. Wound-induced tissue-specific lignin accumulation as a root´s immune response to cyst nematode infection

Muhammad Shahzad Anjam[1], *Luci*ano. M. Di Fino[1], Nageena Zahid[1], Shamim Hassan[2], *Miroslaw Sobczak[3], Tonni G. Andersen[4], Florian. M.W. Grundler[2], Peter Marhavy[1]*
1 Umeå Plant Science Centre (UPSC), Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences (SLU), Umeå, Sweden
2 Rheinische Friedrich-Wilhelms-University of Bonn, Department of Molecular Phytomedicine, Bonn, Germany
3 Department of Botany, Warsaw University of Life Sciences, Warsaw, Poland
4 Max Planck Institute for Plant Breeding Research, Köln, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: cyst nematodes, plant wounding response, lignin accumulation, tissue-specific defense, MYB transcription factors, MYB15 transcription factor

ABSTRACT:

Plant-parasitic nematodes pose a significant threat to global crop productivity, especially cyst nematodes, a group of root-infecting sedentary endoparasites. These nematodes establish long-term feeding structures in the root vasculature to extract nutrients, causing substantial yield losses. To achieve this successful interaction, invasive juveniles (J2) pass through various cell files, leaving behind a trail of mechanically damaged root cells that trigger local plant responses. In this study, we reveal a localized defense mechanism in plant roots whereby lignin, a component of secondary cell walls, rapidly accumulates in cells adjacent to the wounded cells. Intriguingly, similar to the nematodes, single-cell laser ablation induces lignin biosynthesis, which depends on MYB transcription factors, notably MYB15. This lignin induction enhances the mechanical strength of cells, fortifying their resistance against nematode infection. Moreover, we discern that the plant s response to wounding is tailored to specific cells, manifesting heightened lignin accumulation when the endodermis cell layer is subjected to mechanical breach. Overall, our study unveils that the local signaling triggered by single-cell wounding, leading to lignin deposition, represents a tissue-specific defense mechanism, thus strengthening defense responses against infiltration by small invaders.

68. The perivitelline egg protein vitellogenin in *Heterodera schachtii*

Hannah Wileman[1], Roland Perry[1], Keith Davies[1]
1 University of Hertfordshire, Hatfield, Hertfordshire, UK.

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: Cyst nematodes, Vitellin, Development, Comparative Phylogenetics

ABSTRACT:

Plant-parasitic nematodes (PPN) are economically important crop pests. Cyst nematodes develop within the egg, from embryogenesis to the second-stage juvenile. Within the egg, the nematode is bathed in perivitelline fluid which contains the protein vitellin, formed of the precursor protein vitellogenin. In *Caenorhabditis elegans*, this protein has roles in nematode development by transporting lipids, providing amino acids, influencing post-embryonic phenotypes, and providing some environmental protection against bacterial infection. Vitellogenin is a complex of two 170 kDa polypeptides and two smaller subunits of 115 kDa and 88 kDa. Vitellogenin has not yet been fully characterised in PPN, some vitellogenin encoding genes (vit) have been identified within the genomes of some species, but the proteins have only been partially characterised in *Heterodera glycines*. The focus of this work has been to further understand the role of vitellogenin in other species of PPN, particularly *H. schachtii*.

Bioinformatic tools were used to determine the vit genes of economically important PPN. These genes were incorporated into a phylogenetic tree for analysis and protein domains were aligned and compared. Cyst homogenates of *H. schachtii* were used to determine the molecular weight of vitellogenin proteins by western blotting, using an anti-vitellogenin antibody. Furthermore, genomic DNA sequences containing the putative *H. schachtii* vit gene were cloned for sequencing.

The phylogenetic tree clustered the vit genes from different species into four groups: one cluster of root-knot nematodes, two of cyst nematodes, and one of *Caenorhabditis* spp. The comparison of protein domains showed that the *Caenorhabditis* spp. domains were relatively uniform whereas the PPN species were more diverse. Initial dot blot experiments have shown that the anti-vitellogenin antibody recognises proteins within cyst samples of *H. schachtii* and *Globodera pallida*. In western blot experiments, the molecular weight of the putative vitellogenin proteins in *H. schachtii* were 142 kDa, 110 kDa, 49 kDa, 35 kDa and 16 kDa. Vitellogenin nucleotide sequences will be determined by the successful cloning of the genomic DNA region.

This work has shown that PPN have diverging vitellogenin gene sequences but can mostly be clustered by their defined groups. By inferring the relationship between these genes, this forms the basis for further investigation of vitellogenin in PPN. In the use of an anti-vitellogenin antibody that can recognise proteins in *H. schachtii*, aspects of the proteins could be compared to other species. This work forms a basis to further understand the function of vitellogenin in *H. schachtii* and other cyst nematodes.

This work is the first to document the presence of vitellogenin genes in plant-parasitic cyst nematodes on a comparative basis. Furthermore, the molecular weight of vitellogenin proteins in *Heterodera schachtii* have now been determined and are comparable to other species.

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69. Manipulation of plant nucleolar functions by MiEFF186, a novel root-knot nematode effector.

Sarah Ranty-Roby[1], Salomé Soule[1], Claire Caravel[1], Pierre Abad[1], Bruno Favery[1], Michael Quentin[1]
1 INRAE-Université Cote d Azur-CNRS, UMR Institut Sophia Agrobiotech, FR-06600 Sophia Antipolis France

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: effector, plant-nematode interactions, nucleolus

ABSTRACT:

Root knot nematodes (RKNs) are endoparasitic worms that invade plant roots causing economically important damages worldwide as they have a wide host range. Furthermore, RKN populations are predicted to geographically spread and to increase with climate warming. Through their stylet (a syringe-like organ), these pests inject proteins, known as effectors, into plant cells to manipulate diverse functions to their advantage and to escape plant defenses. Indeed, RKNs induce in the root the formation of giant polynucleate cells, which constitute their feeding sites to drain plant nutrients, thus affecting plant yield. Understanding the molecular dialog between the plant and root knot nematodes is therefore of high interest to build new strategies of plant protection against these parasites.

A combination of comparative genomics and transcriptomics allowed the prediction of RKN secreted effectors. In situ hybridization confirmed effector expression in RKN salivary glands, indicating they could be injected in planta. Agro-infiltration of GFP-tagged effectors in tobacco helped select RKN effectors targeting the plant cell nucleus. Yeast two-hybrid screening identified potential targets of the effector MiEFF186 involved in ribosome biogenesis in the tomato plant. Co-localization of MiEFF186 and its target revealed the interaction in the plant nucleolus. This suggests RKNs manipulate plant nucleolar functions to establish feeding sites in planta. Functional analysis of these targets will be presented.

70. Changing perspectives on parasitism: on how plants accommodate, tolerate, and resist nematodes

Geert Smant[1], Jaap-Jan Willig[1], Nina Guarneri[2], Mark Sterken[1], Jose Lozano Torres[1]
1 Wageningen University, Wageningen, The Netherlands
2 University of Amsterdam, Amsterdam, The Netherlands

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: tolerance, resistance, susceptibility, phenotypic plasticity, root system architecture, *Arabidopsis*, parasitism

ABSTRACT:

The dominant perspective in research on molecular nematode-plant interactions views nematodes as master manipulators of host plant development and growth. Herein, an important role is assigned to nematode effectors changing host cell structure and function to provide the feeding parasites access to the flow of assimilates in plants. Moreover, nematode effectors are thought to act as essential modulators of host innate immunity enabling persistent infections. While acknowledging the importance of effectors, we approach nematode parasitism from a different perspective asking if and how plants adapt to mitigate the impact of stress by root-feeding nematodes, or even benefit from it. At the conference, we will report on our recent work focusing on root system architecture plasticity as a mechanism underlying tolerance to root-feeding nematodes. Also, we have conducted a genome-wide association study on plant responses to root-feeding nematodes and used the data to disentangle the genetic architectures underlying susceptibility, tolerance, and resistance of *Arabidopsis* to the beet cyst nematode *Heterodera schachtii*. Based on our findings, we conclude that specific plant adaptations to parasitism can also be explained as a response to nematode-induced abiotic stresses.

71. The effectorome of a plant parasitic cyst nematode

Beth Molloy[1], Dio S. Shin[1], Jonathan Long[1], Clement Pellegrin[1], Beatrice Senatori[1], Paulo Vieira[2], Peter Thorpe[3], Anika Damm[1], Mariam Ahmad[1], Kerry Vermeulen[1], Lida Derevnina[1], Siyuan Wei[1], Alexis Sperling[1], Estefany Reyes Estévez[1], Samuel Bruty[1], Victor Hugo Moura de Souza[1], Olaf Prosper Kranse[1], Tom Maier[4], Thomas Baum[4], Sebastian Eves-van den Akker[1]

1 The Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK
2 Mycology and Nematology Genetic Diversity and Biology Laboratory, United States Department of Agriculture - Agricultural Research Service, Beltsville, Maryland, 20705, USA
3 The Data Analysis Group, School of Life Sciences, University of Dundee, Dow St, Dundee DD1 5EH, UK
4 Department of Plant Pathology, Entomology and Microbiology, Iowa State University, 2213 Pammel Dr., Ames, IA, 50011, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: effectors, effector identification, evolution, plant-parasitic nematodes, transcriptional networks

ABSTRACT:

Plant-parasitic nematodes constrain global food security. During parasitism, they secrete effectors into the host plant from two types of pharyngeal gland cells. These effectors elicit profound changes in host biology to suppress immunity and establish a unique feeding organ from which the nematode draws nutrition. Despite the importance of effectors in nematode parasitism, there has been no comprehensive identification and characterisation of the effector repertoire of any plant-parasitic nematode. To address this, we advance techniques for gland cell isolation and transcriptional analysis to define a stringent annotation of putative effectors for the cyst nematode *Heterodera schachtii* at three key life-stages. We define 659 effector gene loci: 293 ‘known’ high-confidence homologs of plant-parasitic nematode effectors, and 366 ‘novel’ effectors with high gland cell expression. In doing so we define a comprehensive ‘effectorome’ of a plant-parasitic nematode. Using this effector definition, we provide the first systems-level understanding of the origin, deployment and evolution of a plant-parasitic nematode effector repertoire.

Within the effectorome we identify 345 ‘effector families’ with extremely skewed membership: the 5 largest families contain a fifth of effectors, in contrast 41 % of all effectors are the only member of their family in *H. schachtii*. Cross-referencing effectors with an orthologous gene clustering of 61 species reveals that the effectorome is assembled from a diversity of genetic resources, which arose across evolutionary time: 20 % of effector sequences predate nematodes altogether, 53 % of effector sequences arose since the last common biotrophic ancestor with *Rotylenchulus reniformis* and 7 % are H. schachtii specific ‘orphan’ effectors. By cross-referencing the effectorome with life-stage specific transcriptional data for both parasite and host, we generate transcriptional networks to interrogate co-expression throughout infection between effectors, between nematode transcription factors and effectors, and between effectors and host plant genes. These data provide a platform for hypothesis generation, ultimately to accelerate the interrogation of complex plant-nematode interactions. The robust identification of the

comprehensive effector repertoire of a plant-parasitic nematode will underpin our understanding of nematode pathology, and hence, inform strategies for crop protection.

72. Elucidating the role of MigPSY peptides in plant root-knot nematode interaction

Ching-Jung Lin[1], Henok Zemene Yimer[2], Dee Dee Luu[1], Alison Coomer Blundell[1], Maria Florencia Ercoli[1], Paulo Vieira[3], Valerie M. Williamson[1], Pamela C. Ronald[1], Shahid Siddique[2]
1 Department of Plant Pathology and Genome Center, University of California, Davis, California, USA
2 Department of Entomology and Nematology, University of California, Davis, California, USA
3 USDA-ARS Mycology & Nematology Genetic Diversity & Biology Laboratory, Beltsville, MD 20705, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: PSY, root-knot nematode, root growth, plant-parasitic nematode, tyrosine-sulfated peptide

ABSTRACT:

Plant parasitic nematodes pose a significant threat to global agriculture. These parasites invade plant roots and establish permanent feeding sites, which are their sole source of nutrients throughout their lives. To effectively manipulate the host's responses, they secrete effectors, such as phytohormones or peptides, that functionally or structurally hijack the host's cellular machinery. Plants produce *PLANT PEPTIDE CONTAINING SULFATED TYROSINE* (PSY)-family peptides to promote root growth through cell expansion and proliferation. Intriguingly, bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* has also been shown to produce a PSY-like peptide called RaxX (required for activation of XA21-mediated immunity X), which contributes to bacterial virulence. Our research has identified several genes in root-knot nematodes (*Meloidogyne* spp.) that are predicted to encode a group of secreted peptides called MigPSYS. These peptides resemble plant PSY peptide hormones and have been found to stimulate root growth in *Arabidopsis*. We observed that *MigPSY* transcript levels are highest during the early stages of infection in rice and tomato plants. Down-regulated expression of *MigPSY* results in reduced root galling and egg production, suggesting that the MigPSYs serve as nematode virulence factors. To further understand the roles of MigPSYs, I plan to characterize the mechanisms underlying their function and host perception in plants. This research is expected to provide valuable insights into the mechanism of nematode infection and may lead to the development of new methods for controlling plant-parasitic nematodes.

73. Efficiency of organic amendments and crop mulching on soil biodiversity in horticultural crops with low carbon stocks and reduced water availability at the Central area of the Iberian Peninsula

Jose Antonio Lopez Perez[1], Carlos García-Villarrubia[1], Sara Sánchez-Moreno[2]
1 Instituto Regional de Investigación y Desarrollo Agroalimentario and Forestal (IRIAF), Centro de Investigación Apícola y Agroambiental de Marchamalo, Spain
2 National Institute for Agricultural and Food Research and Technology (INIA), Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: crop management, nematode diversity, soil mulching, Mediterranean soils

ABSTRACT:
The use of organic amendments (OA) is a sustainable tool for poor agricultural soils and also useful for the control of soil pathogens. The combination of OA and soil mulching is especially adequate in low-carbon and low-moisture horticultural systems. An open field experiment was established in the CIAPA facilities (Guadalajara, Central Spain) to assess the effects of management on soil moisture and nematode diversity. The experimental design is a split-plot in which three mulches, a traditional polythene plastic mulch and two biodegradable covers (AgroPaper® and MATER-BI®), were randomly distributed in three blocks. Each block included five winter management systems based on different OAs: OV) Oats + vetch (75%-25%) cover crop sowed at 150 kg/ha, mechanically mowed in spring with no residue leftover, OVM) Same oats + vetch cover crop, mechanically mowed in spring, crop residues left on the soil, AC-HD) animal compost-high dose, made with 65% sheep manure and 35% crop residues, applied at 0.4 kg/m2, and AC-LD) same animal compost, applied at 0.2 kg/m2, CK) bare soil control. Along three years, soil samples were collected at 0-20 and 20-40 cm depth, and water content and soil nematode communities were studied. Our results showed that OV as cover crops reduced soil moisture for the next summer crop, and increased the presence of *Acrobeloides*, *Aphelenchus*, *Discolaimus*, Dorilaimida, *Helicotylenchus*, *Pratylenchus*, Tylenchidae and *Tylenchorhynchus*. Summer mulching affected differently soil moisture, and Agropaper® presented lower moisture values than the other mulches. Mulch incidence on nematode communities was less evident than winter management influence. Animal and plant-based OA affected differently the nematode community, which is also related to the presence of roots in the soil for longer periods along the year, OA roots during the winter and tomato roots during the summer. Further studies will contribute to determinate which soil properties are needed to enhance in order to obtain resilient soils with the ability of suppress pest and diseases, as well as stability to face future environmental changes.

74. Evaluation of five green manure species for the control of *Meloidogyne incognita* in a horticultural crop rotation under greenhouse at the Central area of the Iberian Peninsula .

Carlos Garcia-Villarrubia Bernabé[1], Marta María Moreno Valencia[2], José Antonio López-Pérez[1]

1 Instituto Regional de Investigación y Desarrollo Agroalimentario y Forestal de Castilla-La Mancha (IRIAF-CIAPA), Camino San Martín s/n 19180. Marchamalo. Guadalajara, Spain.

2 Universidad de Castilla-La Mancha (UCLM), Escuela Técnica Superior de Ingenieros Agrónomos. Rda. de Calatrava, 7, 13071 Ciudad Real, Spain.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: Biodisinfestation, galling index, organic amendment, sustainable tools.

ABSTRACT:

The use of green manure (GM) is a sustainable tool for agricultural soils and also useful for the control of soil pathogens. Its role as a cover crop and also their use as an organic plant amendment with biodisinfestation potential once incorporated into the soil, makes them a very complete management technique. Five GM species with different properties as soil improvers and biodisinfestants were evaluated in a crop rotation with Spanish cucumber and Swiss chard, common in greenhouses in Central Spain. The experiment was carried out in a greenhouse naturally infested by the root-knot nematode *Meloidogyne incognita*, located at the CIAPA facilities in Marchamalo (Guadalajara, Spain). The GM species tested were *Camelina sativa* L. cv. Beemelina (CS), *Raphanus sativus* L. Domin. cv. Melody (RS), *Pisum sativum* L. cv. Viriato (PS), *Brassica carinata* A. Braun. cv. Eleven (BC) and *Tagetes patula* L. cv. Helen (TP). Seeds were sown in late summer, the grown biomass incorporated, and left into de soil for biodisinfection during a period of seven weeks. The experimental design included a bare control and four replicates for each treatment and was carried out during three successive seasons. Swiss chard crop roots reduced their galling index (GI) values after the first application of GM regardless the GM applied, compared with results GI obtained the previous year. Biodisinfestation showed good control of *M. incognita*, with scarcely any root galls. Subsequently, the GI values increased slightly, being very low, with CS and RS showing the lowest and control the highest. There was no relationship between the amount of biomass amended, different for each GM, and galling production. Regarding Spanish cucumber, their roots experienced a generalized GI growth the second season, with control and BC treatments showing the highest infestation and RS the lowest root damage. Values decreased after the third season for all treatments, and CS and PS showed the least root damage at the end of the experiment. Root damage caused by *M. incognita* was reduced in the last stage of the experiment, after an increase in the GI in the first periods, with CS and PS green manures standing out as the most effective. GM showed that, in addition to their soil-improving properties, reduced the use of agrochemicals by reducing the damage caused by the root-knot nematode.

75. Heating tare soil for disinfestation from potato cyst nematodes: from laboratory to industrial implementation

Negin Ebrahimi[1], Kenny Couvreur[2], Nancy de Sutter[1], Nicole Viaene[1,3]
1 Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Merelbeke, Belgium
2 Department of Electromechanical, Systems and Metal Engineering, Ghent University, Ghent, Belgium
3 Department of Biology, Ghent University, Ghent, Belgium

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Globodera rostochiensis*, temperature, time, moisture, viability, prototype, screw heat exchanger

ABSTRACT:

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are quarantine nematodes posing significant challenges to potato cultivation. The transportation of potato tubers for trade and processing can be a major pathway for spreading PCN when contaminated tare soil is returned to other potato fields. Previous research revealed complete mortality of cyst contents in water when heated at 70°C for 2 minutes or 60°C for 20 minutes, dry cysts required more than 20 minutes at 70°C to die [1]. Building upon this foundation, we examined the effect of different heating conditions (temperature-residence time combinations) on cysts' viability using microwave technology and conduction/convection coupled with mixing and developed a heating installation for industrial implementation. We performed four laboratory tests, each involving 40 viable *G. rostochiensis* cysts mixed with 5 ml soil, sealed in cotton bags and exposed to various temperatures and residence times. In Test 1, cysts were added to washing and filter-press soils and subjected to different temperatures in a microwave with a continuous belt system. Test 2 employed static microwave heating in an autoclave for uniform temperature distribution. Test 3 subjected wet and dry soil to different temperatures and times in a water bath with continuous agitation. Test 4 aimed to determine precise time-temperature combinations for cyst elimination. Treatments with dry soil were subjected to 70°C for 5, 10, 15 and 20 minutes, while treatments with wet soil were subjected to 70°C for 5 minutes or 60°C for 10, 15, or 20 minutes. Complete cyst mortality was achieved in all treatments in the four tests, while untreated cysts (controls) showed 97% viability. Our findings highlight that 60°C for 10 minutes killed cysts in wet soil, while 70°C for 5 minutes achieved the same in soil with minimal moisture. Based on these findings, UGent, in collaboration with Deswarte, designed a prototype comprising two screw heat exchangers in series to attain temperatures of 70°C. We performed a validation test with a mass flow rate of 450-500 kg.h-1. After reaching steady state conditions with uncontaminated soil, 200 kg tare soil artificially contaminated with cysts was added to the heated screw conveyor. Soil was heated while being transported in the screw conveyer for at least 20 min. Despite a setback wherein temperatures reached 60°C instead of 70°C due to high moisture and soil adhesion to the screw conveyor's walls, all treated cysts were dead, regardless of residence time (5-20 min) after leaving the device. Heating proved an effective disinfestation technique for tare soil contaminated with PCN.

[1] Viaene et al., 2019. Asp. Appl. Biol. 142: 95-102.

76. Organic management of *Xiphinema index* in Franciacorta Vineyards, North Italy

Alessio Vovlas[1], Trifone D Addabbo[1], Alberto Troccoli[1], Elena Fanelli[1], Valentina Papeschi[2], Stefano Toninelli[2], Francesca De Luca[1]
1 Istituto per la Protezione Sostenibile delle Piante-CNR, SS di Bari, Via Amendola 122/D, 70126 Bari, Italy
2 Servizio Condifesa Lombardia Nord-Est, Via Malta 12, 25124 Brescia, Italy

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Xiphinema index*, vineyards, cover crops, organic amendments, drip irrigation

ABSTRACT:

Cover crops, organic amendments and plant-derived or microbial formulates could be useful tool for suppressing the dagger nematode *Xiphinema index* in organic vineyards. Annual cover crops and amendments were evaluated to contrast the infection. The aim of this study was to assess the suppressiveness of these techniques to *X. index* in high-value organic vineyards of Franciacorta region in Northern Italy. enhance suppression of. Two experimental approaches were conducted at different field sites. The first experiment was conducted from October 2020 to October 2023 by using cover crops with *Raphanus sativus*, *Sinapis alba*, mixed *Sinapis alba*, *Brassica juncea* and *Raphanus sativus* and mixed *B. nigra - B. juncea* and organic amendments with *Medicago sativa* pelleted biomass or neem cake. Treatments reduced the presence of *X. index* was by 40-50 %, depending on the experimental site, while *X. index* remained stable in the control soil. These observations emphasized overall the effectiveness of *Sinapis alba* and *B. nigra - B. juncea* cover crops and neem cake amendment with a *X. index* reduction of over 60%. The second experiment used drip irrigation approach with different formulations of plant products (garlic extract, quillaia extract, neem oil) and filamentous and mycorrhizal fungi. The most effective action was obtained with formulates of *Trichoderma* (-67%), neem oil (-52%) and garlic extract (-20%). These preliminary data offer useful indications to increase knowledge about environmentally friendly and eco-sustainable management of *X. index* in organic vineyards.

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77. Management of potato cyst nematodes using different organic amendments and cover crops

Javier Lopez Robles[1], Jose Antonio López Pérez[2]
1 Dpto. Química, Area Edafología y Química Agrícola, Facultad de Ciencias, Univ. Burgos, Spain
2 Centro de Investigación Apícola y Agroambiental (IRIAF) Guadalajara, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: soil biodiversity, biodisinfection, volatile fatty acids, ammonia, management, *Globodera*.

ABSTRACT:

The effect of different organic amendments and cover crops on *Globodera rostochiensis* populations was evaluated under laboratory and field conditions. Assays under laboratory conditions were conducted following the protocol developed by the Agroecology Department of Centro de Ciencias Medioambientales-CSIC, Madrid, Spain. Soils used came from fields under conventional potato production systems and contained large *G. rostochiensis* populations.

Tests were carried out with different organic amendments and cover crops, the organic amendments were liquid swine manure (LSM), solid swine manure (SSM), crop byproducts of strawberry, corn and mushroom, as well as sheep manure and sewage sludge, the cover crops were *Raphanus sativus*. Control microcosms received only distilled water to 100% of field capacity.

LSM was applied at: 0.4, 2.0, 4.0, and 6.0% (v/w) to 300 g nematode-infested soil under microcosms thermally sealed and opened, with four replicates per treatment. After 30 days at 30°C soil samples were analysed to determine changes in nematode communities and volatile fatty acids (VFAs).

All the treatments especially LSM significantly reduced *G. rostochiensis* populations and affected hatching activity while increasing soil fertility. A remarkable increase in VFAs level in the anaerobically treated LSM was observed on around day 20.

The main nematode trophic groups found in soil that are most relevant to this study are bacterial-feeders (Rhabditidae), fungal-feeders (Aphelenchidae), plant-feeders (Tylenchidae), and omnivores (Aporcelaimidae). Of these, the bacterial feeders always appeared as the major group present, at the other extreme there were the predators (Mononchidae), which were completely absents. Results suggest that soil disinfection with organic amendments and cover crops are an efficient non-chemical alternative for the management *G. rostochiensis* populations, especially when it is applied in closed microcosm (anaerobiosis). The treatment offers the additional advantages of avoiding organic residue accumulation and decreased contamination problems. The effectiveness of biodisinfection fundamentally depends on the method of application and not exclusively on individual factors such as doses, composition, or distribution of the organic matter in the soil. Successful biodisinfection requires retention of the gases produced during the biodecomposition process and accurate determination of the costs for the method employed to reduce doses and transport costs. The biodisinfectant material, also, stimulates biological activity of the soil acting as a bioimprover.

78. Tagetes species for root-knot nematode management in tomato agrosystems

Cliven Njekete[1,2], Anne-Violette Lavoir[1], and Caroline Djian-Caporalino[1]
1 INRAE, Université Côte d'Azur, CNRS, Sophia Agrobiotech Institute, Sophia Antipolis, France.
2 LIDEA, Lescar, France.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: host suitability, *Meloidogyne* species, *Tagetes*, nematicidal plant, poor host plant, non-host plant

ABSTRACT:

Root-knot nematodes (RKN) are difficult to detect and control, often causing important economic yield losses in vegetable agrosystems. *Tagetes* species could act as nematicidal plants to control RKN by being a non-host or poor host (allow penetration of RKN but starve them and prevent their prolific reproduction, thus trap). We conducted host suitability assays on six selected cultivars (by bibliography and participatory consultations with different stakeholders) from the three most commonly used *Tagetes* species. The assays were conducted in the climate chambers (n= 6+6, control: susceptible tomato and pepper) and assessed by counting the galls and egg masses after one RKN cycle. All the *Tagetes* species were nematicidal and non-host to *Meloidogyne incognita*, *M. arenaria*, and *M. enterolobii*, a newly emerging pest in vegetables and in quarantine status in Europe. Our complementary penetration tests suggested that *Tagetes* inhibit most of the *M. incognita* juveniles from penetrating the roots. Further, we studied the modes of action involved: compounds found inside the plant roots that are toxic to juveniles or compounds exudated in the soil that inhibit egg hatching or are lethal to juveniles or modify their behaviour through repellence or attraction. Root extracts (methanolic 50%:50% water) of *T. erecta* and *T. patula* had a larvicidal effect of up to 25% on *M. incognita* juveniles. *T. erecta* and *T. patula* root exudates inhibited 60% of egg hatching but had no larvicidal effects on the juveniles. Root exudates of *T. erecta* were repulsive to *M. incognita* whilst neutral when mixed with tomato root exudates in our chemotaxis assays. Hence, this mixture also dilutes the attractiveness of the tomato root exudates. Lastly, we chose two cultivars of two *Tagetes* species to intercrop with tomatoes at three densities in the greenhouse (one tomato: one *Tagetes*, one tomato: two *Tagetes*, and one tomato: three *Tagetes*). There was a reduction of *M. incognita* infestation post-one RKN cycle on the tomato crop when intercropped with *Tagetes*. For *Tagetes patula*, a high density of 3 *Tagetes*:1 tomato was necessary to control *M. incognita*. For *T. erecta*, a 1:1 density was enough to effectively reduce *M. incognita* on tomato as in the other higher intercropping densities. There is potential for *Tagetes* to be designed and in-cooperated into cropping systems for sustainable integrated RKN management.

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79. Diversity and composition of soil and rhizosphere microbial communities associated with a susceptible *Prunus* hybrid rootstock under a *Meloidogyne* root gradient infection

Ilenia Clavero-Camacho[1], Alba N. Ruiz-Cuenca[2], Carolina Cantalapiedra-Navarrete[1], Pablo Castillo[1], Juan Emilio Palomares-Rius[1]
1 Institute for Sustainable Agriculture, Córdoba, Spain
2 Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: metabarcoding, *Meloidogyne*, biological control, ITS, 16S rRNA

ABSTRACT:

Almond (*Prunus dulcis*) is a representative tree of the Mediterranean Basin and one of the most widespread crops in Spain. Several biotic and abiotic factors can limit the growth and yield of *Prunus* groves, including plant-parasitic nematodes (PPNs). Specifically, root-knot nematodes (RKN) of the genus *Meloidogyne* are recognised as one of the major PPNs damaging *Prunus* crops. Resistant/tolerant rootstocks and biological control agents (BCAs) are sustainable management strategies to reduce nematode populations. The knowledge of microbial communities associated with susceptible almond hybrid rootstock (GF-677) infested with *Meloidogyne* spp. is essential to stablish sustainable pest management strategies. This research aimed to characterize the soil and rhizosphere microbial community composition and diversity of a susceptible rootstock under a *Meloidogyne* root gradient infection, as well as determine the presence of nematophagous fungi and parasitized *Meloidogyne* eggs. Six commercial almond groves located in southern Spain and infested with different *Meloidogyne* population densities were selected to compare microbial communities between two levels of *Meloidogyne* population density [low (< 600 eggs per g of root) and high (> 600 eggs per g of root)]. The microbial communities were analyzed by high-throughput sequencing of Internal Transcribed Spacer 2 (ITS2) region of fungi and the V3-V4 region of bacterial 16S rRNA. The occurrence of target nematophagous fungi were determined by real-time qPCR, and fungi growing from *Meloidogyne* eggs were isolated to assess parasitism. The studied almond groves were classified as disturbed or degraded soil and showed a low soil quality according to nematode assemblies and fungal:bacterial ratio. Nematophagous fungi of *Meloidogyne* eggs were found in 56.25% of the samples. However, the percentage of parasitized eggs by fungi ranged from 1 to 8%. Three fungal species were isolated from *Meloidogyne* eggs, specifically *Pochonia chlamydosporia*, *Purpureocillium lilacinum* and *Trichoderma asperellum*. The diversity and composition of the microbial communities were more affected by the sample type (soil vs rhizosphere) and by the geographical location of the samples than by the *Meloidogyne* density, which could be explained by the vigorous hybrid rootstock GF-677 and a possible dilution effect.

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80. Europe as a secondary distribution hub in the worldwide invasion of the potato cyst nematode *Globodera rostochiensis*

Magali Esquibet[1], Eric Grenier[1], Josselin Montarry[1]
1 INRAE IGEPP, France

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords:

ABSTRACT:

The potato cyst nematode *Globodera rostochiensis* originates from the Andean Mountain region in South America and has unintentionally been introduced to all inhabited continents. Several studies have examined the population genetic structure of this pest in various countries by using microsatellite markers. However, merging microsatellite data produced from different laboratories is challenging and can introduce uncertainty when interpreting the results. To overcome this challenge and to explore invasion routes of this pest, we have genotyped 22 *G. rostochiensis* populations from all continents. Within populations, the highest genetic diversity was observed in the South American populations, the European populations showed an intermediate level of genetic diversity, and the remaining populations were the less diverse. This confirmed pre-existing knowledge such as a first introduction event from South America to Europe, but the less diverse populations could originate either from South America or from Europe. At the continental scale, STRUCTURE genetic clustering output indicated that North America and Asia have experienced at least two introduction events. Comparing different evolutionary scenarios, the Approximate Bayesian Computation analysis showed that Europe served as a secondary distribution centre for the invasion of *G. rostochiensis* into all other continents (North America, Africa, Asia and Oceania).

81. Soil nematode and microbiota evolution under different soil managements

Alba N. Ruiz-Cuenca[1,2], Ilenia Clavero-Camacho[1], Antonio Archidona-Yuste[1], Carolina Cantalapiedra-Navarrete[1], Pablo Castillo[1], Juan E. Palomares-Rius[1]

1 IAS-CSIC Instituto de Agricultura Sostenible-CSIC, Avda. Menendez Pidal sn, 14004, Córdoba, Spain

2 Departamento de Biologia Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus Las Lagunillas, Jaén, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology

Type: Oral

Keywords: nematodes, bacteria, fungi, biocontrol, soil structure, sustainable agriculture

ABSTRACT:

The aim of this research was to evaluate the changes in the composition of nematode communities and soil microbiota from an extensive to an intensive agricultural system in a field located at the Institute for Sustainable Agricultural (IAS-CSIC, Córdoba, Spain). To achieve this objective, we carried out a holistic study of the biological components and physico-chemical parameters of the soil and their interaction with four farming systems: i) no vegetation in winter, inorganic fertilization and summer crop under conventional management, ii) adventitious vegetation in winter and soil incorporation before the summer crop under organic management, iii) cover crop in winter and soil incorporation before the summer crop under organic management, and iv) cover crop in winter without soil incorporation (mulch), before the summer crop under organic management. The experimental field (300 m^2) was divided into four blocks, each with four random replications. The winter cover crop used was composed of a mixture of legume:cereal (*Vicia sativa* L., *Hordeum vulgare* L.), and the summer crop selected was tomato (cv. Roma). A total of 288 soil samples were collected at two depths (0-5 cm and 5-15 cm) during three sampling periods (T1: before sowing the tomato crop, T2: during the tomato harvest, T3: after the tomato harvest). So far, the results on the abundance of nematodes, fungi and bacteria have shown a clear difference between treatments, depth and over time. Abundance of soil fauna was higher at surface depth (0-5 cm), and higher for nematodes and fungi in the tomato crop early stages. For soil managements, the abundance of nematodes, fungi and bacteria, was lower in the conventional treatment (i) compared to the organic treatments (ii-iv). All organic treatments induced changes in the soil nematofauna and soil microbiota, however, the cover crop treatment without soil incorporation (mulch) showed the greatest increase in the abundance of nematodes, fungi and bacteria. Finally, in terms of tomato yield, the conventional management and organic management with cover crop incorporated into the soil, showed a higher production and there were no differences within the cover crop managements. It is concluded that the cover crop and the soil management have important implications for soil ecology and the successful design of sustainable management strategies.

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82. Investigating nematode biodiversity in the Irish boglands

Anusha Pulavarty[1], Tilman Klappauf[1], Douglas MacMillan[2], Thomais Kakouli-Duarte[1]
1 South East Technological University Carlow, Ireland
2 Green Restoration Ireland Ltd. GRI, Ireland

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: peatlands, boglands, nematodes, biodiversity, nematode communities, morphological analysis, molecular analysis

ABSTRACT:

Natural bogs and peatlands play a major role in reducing global warming, as they are natural carbon sinks. Approximately 1.2 million of Ireland’s 1.5 million hectares of peatlands are damaged to different degrees, disturbing the natural flora and fauna of these ecosystems. Sequestered carbon dioxide is released back into the atmosphere, thus contributing to climate change. Numerous rewetting and restoration programs have been implemented throughout Ireland and Europe with the goal of rehabilitating degraded bogs. The enterprise partner in this project, Green Restoration Ireland Cooperative (GRI), is directly involved in restoring these key components of Ireland’s natural heritage, helping fight climate change, and restoring ecosystem services in a way that helps farmers diversify their incomes. The contribution of the Molecular Ecology and Nematode Research Group of SETU to the project is to evaluate GRI’s peat restoration programme by studying and analysing nematode diversity and communities in restored and non-restored bogs. So far, five different peatland habitats have been sampled. A specific extraction procedure has been standardised to extract nematodes from the peat soils. The extracted nematodes have been permanently fixed to glass slides using formaldehyde, ethanol, and glycerine (Seinhorst, 1959). The fixed nematodes were then studied using a high-power light microscope to identify the families and genera present in Irish peatlands based on various morphological features of the nematodes. Simultaneously, molecular tools were used to sequence the nematode 18S V4 rRNA region using specific primer pairs on an Illumina paired-end platform. The healthy bog sites were found to be dominated by algal- and moss-feeding nematodes, belonging to the families Achromadoridae and Teratocephalidae. The degraded bog habitats were dominated by plant parasitic nematodes, with more than 50% belonging to the family Pratylenchidae. The wasted peat grassland habitats, on the other hand, had more omnivores belonging to Dorylaimidae. These preliminary findings provide a first view of the nematode taxa prevalent in various Irish bog habitats. The study is in progress, and these initial observations will further help to evaluate the ecological status of the restored and non-restored bog sites.

83. A broad overview of the biodiversity in the ring nematode genus *Xenocriconemella* De Grisse and Loof, 1965 (Nematoda: Criconematidae) in the Iberian Peninsula

Antonio Archidona-Yuste[1], Carolina Cantalapiedra-Navarrete[1], Ilenia Clavero-Camacho[1], Alba Nazaret Ruiz-Cuenca[2], Guillermo León-Ropero[1], Jorge Martín-Barbarroja[1], Gracia Liébanas[2], Juan E. Palomares-Rius[1], Pablo Castillo[1]
1 IAS-CSIC. Instituto de Agricultura Sostenible-CSIC, Avda. Menendez Pidal sn, 14004, Córdoba, Spain
2 Departamento de Biologia Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus Las Lagunillas, Jaén, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: *Xenocriconemella*, cryptic species, species delimitation, morphological convergence, phylogeography

ABSTRACT:

The study emerged from the lack of baseline taxonomic information on a common and globally distributed nematode genus *Xenocriconemella*. This monospecific genus or, alternatively, the nominal species *X. macrodora*, has received special attention in the world nematological community, but with most studies simplified to basic morphology and/or with incomplete morphological and molecular data. Therefore, we conducted an extensive sampling in the Iberian Peninsula, not only in previously reported areas but also in the most important areas of *Quercus*-dominated forests given the close association with this host plant, to cross-check morphology and morphometric features with molecular data. As a novel outcome, we found a new cryptic species complex from the extensive distribution of the nominal species *X. macrodora* in the Iberian Peninsula, USA and probably Italy (only based on a ribosomal and a mitochondrial molecular markers). *Xenocriconemella macrodora*-species complex is defined here with the description of three new species applying a broad taxonomic framework including morphology, morphometric and molecular data. Additionally, our study claimed a model example of unequivocal cryptic species complex under *X. macrodora* since most of its members can only be recognized using molecular data. However, species delimitation by multivariate morphometry analysis revealed that Iberian populations reflect a high morphometric variation across space. Since descriptive morphology is expected to emerge from environmental forcing over long periods, we could hypothesize that this high morphometric variability may be driven by environmental gradients. Another exciting gap in nematode research is the analysis of the environmental factors that regulate the relationship between the morphological and genetic diversity. These novel research questions in soil nematodes are explored here in the context of the high environmental variability offered by the extensive distribution of the genus *Xenocriconemella* in the Iberian Peninsula, which could be a valuable starting point for further research on soil biodiversity.

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84. An update of regulated and recent discoveries of invasive alien plant-parasitic nematodes species in Canada

Qing Yu[1] and F. Sun[2]
1 Agriculture and Agri-Food Canada, Ottawa Research and Development Center, Canada
2 Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, K2H 8P9, Canada

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: alien invasive nematodes, regulated nematode pests, Canada

ABSTRACT:

With the most recent discovery of the invasive alien nematode species *Litylenchus crenatae* ssp. *mccannii* in Ontario, Canada, the etiological agent causing beech leaf disease, and the 2006 report of *Globodera* rostochiensis from potato in the Saint-Amable region, Quebec, it is time to provide updated information on the Canada-regulated nematode pests, and recent discoveries of invasive alien nematode species in Canada. Since 2012, the stem and bulb nematode *Ditylenchus dipsaci* has caused epidemic disease on garlic in Ontario and it was subsequently found in neighboring provinces. Around that time, *Ditylenchus destructor* was also discovered on garlic in Ontario. *Ditylenchus weischeri* was identified in yellow peas in the Prairie provinces not *Ditylenchus dipsaci* as first thought. In 2013, the soybean cyst nematode *Heterodera glycines* was deregulated. Other recently discovered invasive alien plant parasitic nematode species are: *Heterodera* carotae, *Meloidogyne naasi*, *Pratylenchus alleni*, *Pratylenchus thornei*, *Trichodorus primitivus*, *Xiphinema chambersi*. The current updated list of regulated nematodes in Canada includes: *Ditylenchus dipsaci*, *Ditylenchus destructor*, *Globodera pallida*, *Globodera rostochiensis*, *Longidorus* spp. (virus vector), *Meloidogyne chitwoodi*, *Trichodorus* spp. (virus vector), and *Xiphinema* spp. (virus vector).

95. A full-length SSU rRNA-based workflow for high-resolution monitoring of nematode communities reveals direct and indirect responses to plant-based manipulations

Robbert van Himbeeck[1], Sara Cazzaniga[1], Sven van den Elsen [1], Johnny Visser[2], Johannes Helder[1]

1 Laboratory of Nematology, Wageningen University, Wageningen, the Netherlands

2 Field Crops, Wageningen Plant Research, Wageningen University & Research, Lelystad, The Netherlands

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: nematode metabarcoding, Oxford nanopore sequencing, soil health indicators, cover crops, *Meloidogyne chitwoodi*

ABSTRACT:

Agricultural intensification has resulted in a decline in soil biodiversity, and concerns about the deterioration of the biological condition of soils prompted the development of measures to restore soil life. Due to the overwhelming biodiversity of soils, evaluation of such measures is not straightforward, and proxies are used to assess soil health. Nematodes have been proposed as a proxy for soil health due to their ecological diversity, their abundance, and their easy extractability from soil. However, the use of nematodes as soil health indicators is hampered by the very specific taxonomic expertise required for microscopy-based analyses of nematode communities, as well as by the intrinsically time-consuming nature of this type of analyses.

DNA-based tools could overcome these issues, but current high throughput DNA sequencing methods produce relatively short reads allowing for - in case of nematodes - a taxonomic resolution mostly until family level. Here we present a workflow that exploits the possibilities offered by Oxford Nanopore (ON) sequencing. It is noted that only the latest ON chemistry (R9.4.1. and newer) has a sequencing accuracy that is needed for DNA-based nematode community analysis.

To illustrate the potential of this method, we applied it in an experimental field setting in which the density of a high-impact plant-parasitic nematode, *Meloidogyne chitwoodi*, was manipulated by growing plants with varying host status. Subsequently, eleven cover crop treatments (ten cover crops and an unplanted control) were grown. After cover crop termination, potato - a main crop highly sensitive to *M. chitwoodi* - was cultivated. The effects of the cover crop treatments on the root-knot nematode species as well as on all other plant-parasitic and non-parasitic (‘free-living’) nematodes were determined before and after the main crop.

Analysis of full-length small subunit ribosomal DNA (SSU rDNA, approximately 1,700 bp) delivered a complete overview of all trophic groups present in the experimental field and allowed us to characterise nearly all nematodes until genus or species level. Furthermore, a comparison between microscopic counts of the main pathogen, *M. chitwoodi*, and sequence read data revealed remarkably similar trends with the same statistical support. This novel community analysis approach allowed us to map in detail direct and indirect effects of plants on nematodes: not only

effects of cover crops and the main crop (potato) on the plant-parasitic community were observed, but also indirect effects on bacterivores, fungivores, omnivores and predators.

Our research might facilitate the use of soil ecological information that is incorporated in nematode communities also in research settings with limited access to nematode taxonomic expertise. Next to this, we think that Oxford Nanopore-based SSU rDNA sequencing could trigger a substantial refinement of the use of nematodes as indicators for soil health.

96. Biomonitoring of microbial and chemical indoor air toxins with *C. elegans* nematodes

Sari Paavanen-Huhtala[1], Karunambigai Kalichamy[1], Anna-Mari Pessi[2], Sirkku Hakkila[2], Annika Saarto[2], Maria Andersson[3], Paivi Koskinen[1]
1 Department of Biology, University of Turku, Turku, Finland
2 Aerobiology Unit, University of Turku, Turku, Finland
3 Department of Civil Engineering, Aalto University, Espoo, Finland

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: *C. elegans*, biomonitoring, toxins, indoor air

ABSTRACT:

The *Caenorhabditis elegans* nematodes have been found to be suitable for biomonitoring of multiple types of environmental agents, as their chemosensory neurons can efficiently detect volatile odorants or soluble flavors. Thereby the animals can discriminate between beneficial and harmful substances in their living environment, find food and avoid pathogens, many of which are hazardous also for humans. This has prompted us to test the possibility that *C. elegans* could be used also to monitor indoor air quality.

Indoor air problems are far too common in schools and other public buildings, where they weaken the health, well-being and working capacity of exposed people. The current methods to evaluate indoor air health hazards are limited and mainly based on cellular assays. However, multicellular organisms, such as *C. elegans* may provide additional advantages to predict their effects on human tissues. Therefore, we have used transgenic *C. elegans* strains carrying stress responsive fluorescent reporters and evaluated their abilities to sense microbial or chemical toxins, especially those that are present in moisture-damaged buildings. In animals exposed to such agents, we have reproducibly observed time- and dose-dependent fluorescent responses, which could be quantitated by either microscopy or spectrometry. Furthermore, our results have correlated well with those obtained from cell-based assays. Thus, based on our recently published results (Paavanen-Huhtala et al., Pathogens 2023), the *C. elegans* nematodes offer an easy and comprehensive method to monitor overall indoor air toxicity, but further studies are needed to validate their ability to distinguish between healthy and harmful buildings also under field conditions.

97. Unveiling soil nematode traits to predict ecosystem functions in changing environments

Manqiang Liu[1]
1 Centre for Grassland Microbiome, State Key Laboratory of Grassland Agro-Ecosystems, College of Pastoral Agricultural Science and Technology, Lanzhou University, Lanzhou 730020, China
2 Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: Nematodes, functional traits, plant traits, ecological strategy, ecosystem functions, global change.

ABSTRACT:

Soil nematodes, the most abundant and diverse metazoans globally, exhibit significant variations in visible attributes that can be identified by taxonomist. They are well-classified based on life strategies (defined by colonizer-persister values) and play a crucial role in the soil food web structure by occupying all major trophic levels. The past three decades have witnessed remarkable advances in exploring the potential use of soil nematodes in soil health assessment through the development of various ecological indices. However, compared with counterparts such as plants and macro-organisms, research on soil nematode traits to predict ecosystem functions in changing environments has been largely ignored. To bridge this gap, trait-based approaches are gaining traction in describing nematode ecological strategies and assessing linkages with ecosystem functions. Here we first establish a unified trait framework to categorize nematode traits into morphological, physiological, life history and community clusters and propose a ‘nematode economics spectrum’ that enables a better understanding of nematode trait responses to global changes and their effects on ecosystem function. Then we introduce an empirical study that adopts our trait-based framework, illustrating the interactions among traits within nematode communities and between soil nematodes and plants and the coordination between plant and nematode traits impact carbon cycling across 3 vegetations including grass, shrub and forest in six regions of China. In conclusion, insights gained from a trait-based understanding of nematodes shed lights on their mechanistic roles in ecosystem function and advance belowground ecology.

98. Nematode functional trait response to climate change in semi-arid systems

Miguel Talavera[4], Jordi Moya-Laraño[2], Pilar M. Gavín-Centol[2], Paula Lillo[1], Antonio Archidona-Yuste[3], Diana H. Wall[5], Sara Sánchez-Moreno[1]
1 National Institute for Agriculture and Food Research and Technology, INIA-CSIC, Madrid, Spain
2 Arid Zones Experimental Station, EEZA-CSIC, Almería, Spain
3 Sustainable Agriculture Institute, IAS-CSIC, Córdoba, Spain
4 Institute for Agrofood and Fishery Research and Training, IFAPA-Alameda del Obispo, Córdoba, Spain
5 Colorado State University, Colorado, USA

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: functional traits, juveniles, males, climate change

ABSTRACT:

Soils are extremely complex living systems, responsible for ecosystem services such as climate regulation, water cycling, and carbon storage, which rely on soil biota. Among soil organisms, nematodes are extremely abundant and functionally diverse, and such functional diversity is used as a notable bioindicator of soil condition and to calculate Nematode-Based Indices (NBIs).

Besides NBIs, nematode functional traits might be used as indicators of soil biota response to environmental change. Anhydrobiosis, a state of dormancy in which nematodes lose their body water and metabolism halts due to desiccation, may influence the efficiency of nematode extraction from soils and affect NBIs in arid systems. In this research, we evaluated anhydrobiosis rates, functional groups, male to female ratio, juvenile to adult ratio, and nematode body mass to assess the effects of climate change stress on soil nematode communities in semi-arid agricultural systems.

We assessed nematode functional traits in nematode assemblages subjected to various desiccation times in nematode assemblages from two locations in Central and Southern Spain. We showed that anhydrobiosis is widespread across nematode taxa and trophic groups, and that anhydrobiotic potential significantly differs across nematode genera and functional groups. We calculated anhydrobiosis rates for soil nematodes and demonstrated that anhydrobiosis is a relevant functional trait in soil nematode taxa. We found that body size, and juvenile and male prevalence varied in response to environmental stress. These findings may help understand soil biota response and resilience to climate change.

99. Soil nematode communities under a simulated climate change scenario in a semi-arid cropland.

<u>Paula Lillo</u>[1,2], Dorota L. Porazinska[3], Lee Robertson[1], Sara Sánchez-Moreno[1]
1 National Institute for Agriculture and Food Research and Technology, INIA-CSIC, 28040 Madrid, Spain.
2 International Doctoral School, University Rey Juan Carlos, 28933 Madrid, Spain.
3 Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32611, USA.

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: nematode diversity, community assembly, metabarcoding, soil warming, drought

ABSTRACT:

Anthropogenic climate change (CC) is modifying the functioning of terrestrial ecosystems and increasing Earth s surface temperatures are projected to continue [1]. With the ongoing CC, the Mediterranean region is expected to experience altered precipitation regimes, including reduced rainfall and elevated drought frequency. Agricultural systems face increased vulnerability to CC due to frequent disturbances caused by management practices. This human-induced environmental change not only threatens food security, but also belowground biota that play a crucial role in providing and maintaining soil functions and ecosystems services [2]. In this study, we assessed the impact of predicted CC stresses 'warming and reduced rainfall' on soil nematode communities in a semi-arid arable system in central Spain. Future climatic conditions were simulated for three years using open-top chambers (OTC) (increased soil temperature 2.5 °C), rainout shelters (reduced rainfall by 30%) and their combination. In addition, these CC conditions were imposed on the wheat-vetch-wheat rotation crop fields managed under conventional as well as reduced tillage.

To provide quantitative and qualitative analyses of nematode assemblages, we used a combination of DNA metabarcoding and morphological approaches. The effects of climate treatments on nematode diversity and community composition were addressed. We also tested whether changes in the physical-chemical soil properties influenced nematode community diversity and structure. As expected, DNA metabarcoding resulted in a higher taxonomical resolution (species-genus) than morphology (genus-family). Estimated nematode abundances did not fully match across both methods with root-associated Tylenchidae showing higher and omnivory-predatory Dorylaimida lower relative abundances, in morphological than in the DNA metabarcoding approached.

Total nematode abundance remained stable under simulated CC. Warming, reduced rainfall, and their combination increased fungal feeders, while bacterial feeders decreased. Warming altered soil nematode diversity regardless of the methods, with significantly increased Shannon index but only in response to warming under reduced tillage. In terms of DNA data, plant parasites abundances were unaffected by CC treatments but decreased under reduced tillage. Distance-based redundancy analysis based on DNA data showed distinct nematode communities under reduced vs. conventional tillage clearly indicating the effect of management history but not CC. Our results indicate that predicted CC scenarios and agricultural management practices independently altered soil nematode fauna and potentially the functioning of entire soil food webs.

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100. Biodiversity Soil Resilience (BSR): A new index to assess resilience in environmentally stressed ecosystems by including entomopathogenic nematodes

Giambattista Maria Altieri[1], Josefina Garrido[2], Alcides Moino Junior[3], Cezary Tkaczuk[4], Vito Santarcangelo[5], Giuseppe Bari[1], Salustiano Mato[2], Eustachio Tarasco[1]
1 Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy
2 Department of Ecology and Animal Biology, University of Vigo, Vigo, Spain
3 Department of Entomology, Federal University of Lavras (UFLA), Lavras, Brasil
4 Department of Horticulture and Plant Protection, University of Siedlce, Siedlce, Poland
5 Department of Biosciences, Biotechnology and Environment, University of Bari, Bari, Italy

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: ecological indicators, soil quality, entomopathogenic nematodes, entomopathogenic fungi, microarthropods, earthworms

ABSTRACT:

Over the last century, natural habitats and semi-natural environments have been under increasing environmental pressure, leading to significant changes and a decline in biodiversity. Biodiversity is the variety of life forms in an ecosystem and is essential for preserving genetic resources and sustaining populations. The soil is fundamental to terrestrial ecosystem function and environmental health, contributing significantly to support and regulation services. To address these challenges, a new resilience index named Biodiversity Soil Resilience (BSR) is proposed. This index focuses on the intricate relationship between biodiversity and soil health. It involves a comprehensive analysis of soil biodiversity, with a particular emphasis on entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF), earthworms, and soil microarthropods. Numerous studies support the crucial roles of these components in ecosystem resilience, offering diverse benefits that contribute to environmental stability. For example, entomopathogenic nematodes (EPNs) and entomopathogenic fungi (EPFs) play a significant role in biological control, the food chain, nutrient cycling, adaptability, and interactions with soil. Earthworms enhance soil aeration, promote the mixing of organic and mineral material, increase microbial biomass, provide biological control against pathogens, and foster beneficial symbioses. These organisms exhibit adaptability to various environmental conditions, contributing to the ecosystems ability to withstand and recover from environmental stresses. Microarthropods, including mites and collembola, also play a crucial role in the BSR-Index by contributing to soil properties such as decomposition, nutrient cycling, soil structure, biodiversity, adaptability, and resilience. Additionally, they engage in fundamental interactions with vegetation and other microorganisms, further influencing ecosystem health. A key innovation in this research is the development of a dedicated app/software for real-time data collection in the field. This tool streamlines the gathering of information on specific organisms and contributes to a global database of soil biodiversity data. This collaborative effort facilitates worldwide monitoring and maintenance of soil health. The BSR-Index is envisioned as a monitoring tool for the scientific community and government authorities. It provides valuable insights for the implementation of

sustainable and effective environmental planning measures. The Index, incorporating environmental stresses, soil biodiversity, and soil biological quality (QBS-ar and QBS-e), supported by chemical and physical parameters, aims to enhance our understanding of ecosystem health and promote informed decision-making for a resilient and sustainable future.

101. Development of calcium alginate beads containing the nematode *Steinernema feltiae* (SB 12[1]) and plant growth promoting rhizobacteria *Pseudomonas ogarae* F113 GFP

Islam Ahmed Abdelalim Darwish[1], Daniel Martins[2], David Ryan[3], Thomais Kakouli-Duarte[4]
1 South East Technological University, Molecular Ecology and Nematode Research Group, enviroCORE, Department of Applied Science, Ireland
2 University of Essex, School of Computer Science and Electronic Engineering, United Kingdom
3 South East Technological University, enviroCORE, Department of Applied Science, Ireland
4 South East Technological University, Molecular Ecology and Nematode Research Group, enviroCORE, Department of Applied Science, Ireland

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: entomopathogenic nematodes, *Steinernema* feltiae, plant growth promoting rhizobacteria, *Pseudomonas ogarae*, alginate bead, biological control, IPM

ABSTRACT:

The use of conventional chemical pesticides in agriculture poses severe threats on human health and the environment, with soil biodiversity also being negatively affected. Entomopathogenic nematodes (EPN) and plant growth promoting rhizobacteria (PGPR) have considerable potential as biological control agents. Formulations of EPN can be enhanced by PGPR and provide a good protection to plants against insects promot plant health. Two research studies have demonstrated that the PGPR, *Pseudomonas* spp., are associated with the EPN *Steinernema feltiae*, and they play a role in the parasitic lifecycle of these nematodes (Ruiu et al., 2022, Ogier et al., 2020). In addition, these two groups of beneficial organisms have been shown to tolerate each other well in a previous enviroCORE project (Hurley, 2018). The current study aims to formulate a novel biological product consisting of a combination of Irish strains of *S. feltiae* (SB 12[1]) and of *Pseudomonas ogarae* F113 GFP. In this study, a novel calcium alginate bead formulation is being developed as a suitable medium to encapsulate EPN and PGPR simultaneously. Both *S. feltiae* (SB 12[1]) and *P. ogarae* F113 GFP were cultured prior to their incorporation into the formulation of calcium alginate bead. 18% glycerol is included in the calcium alginate bead formula to improve the retention of the EPN while being stored and until they are needed for application. Preliminary results showed that a 1.5% sodium alginate-glycerol concentration was the best to generate well shaped calcium alginate beads. currently to ensure nematode dormancy as well as good organismal viability and infectivity within the beads after short- and long-term storage. Following these experiments, molecular communication analyses and modelling will be carried out to better understand how those biological agents interact in their novel formulation to improve their efficiency in the final biological product.

102. Efficacy of entomopathogenic nematodes against *Chrysomela populi* and *Gypsonoma aceriana* in Iran

Reihaneh Gholami Ghavamabad[1], Seyedeh Masoomeh Zamani[1], Majid Pedram[2]
1 Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
2 Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: Tortricidae, Chrysomelidae, poplar, EPNs, biocontrol

ABSTRACT:

Poplar, as the most widely cultivated fast-growing tree species in the middle latitude plain of the world, possesses characteristics such as large biomass, diverse uses and easy adaptability. *Chrysomela populi* L. (Coleoptera: Chrysomelidae) is a significant pest that affects *Populus* (Salacaceae) in Europe and Asia. Both larvae and adults of the species feed on the leaf material, causing major economic losses when infested. *Gypsonoma aceriana* (Duponchel) (Lepidoptera: Tortricidae), is another important poplar pest found in Europe, North America, Canada, North Africa and Asia. The most severe damage caused by this species occurs in spring when larvae emerge from their cryptic overwintering habitats, climbing higher to bore into terminal shoots. Entomopathogenic nematodes (EPNs) are efficient biocontrol agents against many insect pests found in other than soil habitats like cryptic habitats. In present study, during a survey in the distribution areas of *G. aceriana* in the Botanical Garden of Kashan, Isfahan province and C. populi in Alborz Research Station, Alborz province, various stages of pests were collected. The efficacy of three Iranian native isolates of the EPNs were evaluated against the two aforementioned pests in laboratory conditions and greenhouse. The lethal concentration 50 (LC50) values for *Heterorhabditis bacteriophora* (isolate Kashan), *Steinernema* feltiae (isolate Tabriz) and *Steinernema borjomiense* (isolate Gilan) were as follows: 62.0, 142.1 and 189.0 infective juveniles (IJs) mL-1 on second instar larvae of *G. aceriana*, 247.98, 478.81 and 1849.25 IJs mL-1 on the third instar larvae of *C. populi*, and 911.1, 1084.5 and 2229.8 IJs mL-1 on adult of *C. populi*, respectively in the laboratory tests 96 h post-treatment at 25°C and 60% relative humidity. In a pot experiment under greenhouse conditions, *H. bacteriophora*, at a rate of 50000 IJs pot-1, demonstrated 69.4% mortality against larvae of *G. aceriana*, 55.2% mortality against larvae of *C. populi* and 50.6% mortality against adults of *C. populi*, after 6 days post treatment, showing it is the most virulent species. The results of this study indicate that foliar application of EPNs could control feeding larvae and adults of *C. populi* on leaves. Complementarily, applying these entomopathogens in soil and on grass turfs would help control overwintering adults, that is an open field for study in future. Additionally, the results suggest that these EPNs have good potential for controlling *G. aceriana* larvae before they enter cryptic overwintering habitats. However, future trials must be conducted to evaluate the efficacy of these native EPNs in controlling pests in an open-field situation.

Our finding revealed that native entomopathogenic nematodes can infect third instar larvae and adults of *C. populi* in laboratory and greenhouse experiments, observed in both leaf and soil

assays. Additionally, these EPNs show promising potential for controlling *G. aceriana* larvae. Our laboratory and greenhouse results showed that *Heterorhabditis bacteriophora* (isolate Kashan) caused the highest mortality, reinforcing the great potential of this EPN for control of both pests, followed by *Steinernema feltiae* (isolate Tabriz) and *Steinernema borjomiense* (isolate Gilan).

104. Update on biodiversity of entomopathogenic nematodes and their symbiotic bacteria in Italy

Eustachio Tarasco[1], Elena Fanelli[2], Alberto Troccoli[2], Giulia Torrini[3], Alessio Vovlas[2], Giovanna Curto[4], Leonardo Marianelli[3], Mirella Clausi[5], Diego Leone[5], Giancarlo Rappazzo[5], Pio Federico Roversi[3], Francesca De Luca[2]
1 Department of Soil, Plant and Food Sciences (DiSSPA) - University of Bari 'Aldo Moro' - Bari, Italy
2 Institute for Sustainable Plant Protection (IPSP), Consiglio Nazionale delle Ricerche (CNR) - Bari, Italy
3 CREA - Research Centre for Plant Protection and Certification (CREA-DC) - Florence, Italy
4 Plant Protection Service Emilia-Romagna, Bologna, Italy
5 Department of Biological, Geological and Environmental Sciences - University of Catania - Bari, Italy

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: ecological indicators, entomopathogens, earthworms, monitoring

ABSTRACT:

Due to the renewed interest in entomopathogens, EPNs in particular, and to important research projects in which we have been involved, in the last ten years several investigations were carried out on the distribution and biodiversity of steinernematid and heterorhabdtid entomopathogenic nematodes (EPN) in North and South Regions of Italy. More than 3000 samples were collected from 500 localities and more than 100 of them yielded EPN specimens. A mapping of EPN/symbiotic bacteria distribution in Italy was update with new indigenous EPN strains some of which are currently being studied in depth because they could be new species (which would be added to the 13 species already known for Italy: *Heterorhabditis bacteriophora, H. downesi, H. megidis, Steinernema feltiae, S. affine, S. kraussei, S. apuliae, S. ichnusae, S. carpocapsae, S. vulcanicum, S. arenarium, Oscheius onirici* and *O. myriophilus*. Steinernematids are more widespread than heterorhabditids and *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* are the most commonly encountered species. Sampling sites were grouped into 11 habitats: uncultivated land, orchard, field, sea coast, pinewood, broadleaf wood, grasslands, river and lake borders, caves, salt pan and moist zones. The habitat preferences of each species were assessed and, except for the three dominant species, *S. feltiae, S. carpocapsae* and *H. bacteriophora*, EPN's occurrence tends to be correlated with a specific vegetation habitat and soil type.

105. Domestication of a nematode for biological management of insect pests

Ralf-Udo Ehlers[1]
1 Biocontrol Biotech Consult, Duesternbrook 1, 24211 Rastorf, Germany

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: crisis of insect pest management, role of EPN, domestication, breeding, beneficial traits improved

ABSTRACT:

Insect management is at a cross road. Increasing insecticide resistance and limited innovation in synthetic insecticide development produce major problems in agricultural practice. In greenhouses pest control has almost completely shifted to biological measures. In future arable crops will also depend on the natural antagonistic potential. Entomopathogenic nematodes will play a major role in the shift to environmentally safe management strategies. Innovation in application technology and in vitro mass production have much contributed to the reduction of application costs of EPN. However, short shelf life even at low temperature storage and transport complicate application of EPN. Longevity and other beneficial traits can be improved by classical breeding technology assisted by molecular markers. *Heterorhabditis bacteriophora* is a nematode which is a perfect target for domestication. It permits production of inbred lines through self-fertilisation of the hermaphrodites and production of hybrids through crosses of second generation amphimictic adults. The genetic pool of wild type and inbred lines can be preserved by storage in liquid nitrogen. Low cost mass production is done in industrial scale bioreactors in liquid culture. EPN have a short life cycle allowing rapid progress by genetic selection. Genetic selection is successful because of high heritability of several traits. The improvements are maintained during mass production as Heterorhabditis spp. cannot mate in liquid media. Hence, reproduction is only through self-fertilising stable inbred lines. The use of well characterised inbred lines overcomes problems with trait deterioration when production is done in liquid media. Several traits have been improved, e.g. reproduction, longevity, field persistence and stress resistance to heat, desiccation, reactive oxygen species and low temperature survival. A large pool of sequence information is available for further improvements. Together with development of adapted application technology, the genetic improvements made possible the reduction of application density to 1 billion/ha, why *H. bacteriophora* can now be offered at application costs comparable to synthetic insecticides, for instance in the control of the corm rootworm.

EPN will play a major role in pest control, domestication of EPN will help to improve beneficial traits, together with improved application technology and in vitro mass production EPN can be offered at competitive costs

106. Elevational gradients and plant ontogeny as predictors of entomopathogenic nematode activity in natural oak (*Quercus*) populations

Rubén Blanco-Pérez[1], Andrea Galmán[2], Alicia Pou[3], Xoaquín Moreira[1]
1 Misión Biológica de Galicia, Pontevedra, Spain
2 Montana State University, Bozeman, Montana, USA
3 Instituto de Ciencias de la Vid y del Vino, Logroño, Spain

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: biocontrol agents, Fagaceae, Heterorhabditis, *Steinernema*, belowground tritrophic interactions

ABSTRACT:

Entomopathogenic nematodes (EPNs) play a pivotal role in trophic soil interactions. They are tiny roundworms that parasitize insects, including various herbivorous pests. In soil, they can infect and kill insect larvae by entering the insect s body through natural openings or piercing the cuticle. A classic ecological assumption poses that insect herbivory increases in warmer, stable climates associated with lower elevations. However, the generality of this paradigm has been strongly questioned as it overly simplifies the ecological complexity in which plant-herbivore interactions are embedded, particularly along elevational clines. In particular, recent studies have demonstrated that differences in tri-trophic interactions may explain inconsistent patterns reported thus far in elevational gradients in insect herbivory. Elevational differences in the diversity, abundance, and activity of herbivore natural enemies such as EPNs could lead to variation in enemy top-down pressure, which then shapes concomitant variation in plant-herbivore interactions. At the same time, recent findings also highlight the role of plant ontogeny in its vulnerability to herbivory. For herbivores, adult plants are more apparent and thus more susceptible than saplings. Yet, this difference in herbivore vulnerability might decline at higher elevations, where detectability has less impact due to the already low herbivore pressure. However, whether elevational gradients in patterns of herbivore predation are contingent on the plant ontogenetic stage remains unknown.

In this study, we tested elevational differences in the activity of EPNs on 10 oak species (*Quercus*, Fagaceae) across various landscapes of the Iberian Peninsula and compared the observed patterns for saplings and adult trees. We collected soil samples under canopies of naturally occurring populations of each oak species found at low-, mid-, or high-elevation sites. We then estimated EPN activity through insect baiting with the last instars of *Galleria mellonella* (Lepidoptera: Pyralidae). Our findings showed a four-fold increase in EPN activity at lower elevations than at higher elevations. Moreover, plant ontogeny significantly affected EPN activity, with a five-fold increase in EPN activity on the surrounding soil of adult trees compared to those surrounding saplings. Finally, we found a non-significant interaction between elevation and plant ontogeny on EPN activity, indicating that EPN activity consistently increased at lower elevations for adult trees and saplings. Overall, our study contributes to a deeper understanding of the ecological processes

that lead to tri-trophic interactions in forest ecosystems and underscores the importance of considering multiple environmental and biological factors when assessing soil ecosystem health.

107. From soil to plant: strengthening carrot defenses against *Meloidogyne incognita* with vermicompost and arbuscular mycorrhizal fungi biofertilizers

Aasha Rana[1], Lukman Ahamad[1], Aashaq Hussain Bhat[1], Harendra Kumar[1], Md. Nurul Hasan[1], Ishtiaq Ahmed[1], Shakoor Ahmed[1], Ricardo AR Machado[1], Fuad Ameen[1]
1 Madhav University, Rajasthan, India

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: arbuscular mycorrhizal fungi, *Daucus carota*, disease management, *Meloidogyne incognita*, vermicompost

ABSTRACT:

Sustainable agricultural practices for controlling crop pests are urgently needed to reduce the reliance on chemical pesticides, which have long-term detrimental effects on ecosystems. In this study, we assessed the effectiveness of arbuscular mycorrhizal fungi (AMF) and vermicompost (Vc) supplementation, alone and in combination, in mitigating the negative impacts of *Meloidogyne incognita* infestation on carrot (*Daucus carota* L.) growth, development, and physiology. Different plant growth parameters such as plant height and biomass accumulation, several plant physiological parameters such as the levels of photosynthetic pigments, phenolics, and the activity of defense enzymes includes peroxidases and polyphenol oxidases, and evaluated the severity of *Meloidogyne incognita* nematode infestation on plants treated or not treated with vermicompost (Vc) and/or arbuscular mycorrhizal fungi (AMF). The findings show that *M. incognita* significantly affects plant growth, biomass accumulation, and photosynthetic pigment and carotenoid content. The incorporation of Vc and AMF into the soil, either individually or in combination, significantly alleviates the negative effects of nematode infestation on carrot plants. This was accompanied by the induction of phenolic compounds and defense enzymes such as peroxidases (+15.65%) and polyphenol oxidases (29.78%), and by a reduction in the severity of nematode infestation on Vc and AMF-treated plants compared to nematode-infested plants. Principal component analysis (PCA) shows significant correlations between various of the studied parameters.

In particular, we observed negative correlations between the application of AMF and Vc alone and in combination and disease severity, and positive correlations between plant growth, photosynthetic pigments phenol content, and activity of defense enzymes. Relevance of cultural practices and beneficial microorganisms for the sustainable and environmentally friendly management of agricultural pests.

108. *In vitro* parasitism of isolates of the nematode-trapping fungus *Arthrobotrys conoides* against *Meloidogyne incognita*

Cafer EKEN[1], Gulsum UYSAL[2], Dudu DEMIR[1], Selda CALISKAN[2], Emre SEVINDIK[3], Kardelen CAGLAYAN [1]

1 Isparta University of Applied Sciences, Department of Agricultural Biotechnology, Faculty of Agriculture, Isparta, Türkiye

2 Bati Akdeniz Agricultural Research Institute, Plant Health Department, Antalya, Türkiye

3 Aydin Adnan Menderes University, Department of Agricultural Biotechnology, Faculty of Agriculture, Aydin, Türkiye

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: biological control, fungus, root-knot nematode, tomato

ABSTRACT:

Root-knot nematodes (*Meloidogyne* spp.) are one of the important pest groups that cause economically significant yield losses in cultivated plants worldwide. Negative effects of chemicals on the environment and human health, the fact that it is difficult and expensive to apply has made it inevitable to investigate biological control possibilities in the management. In developed countries, many biopesticide preparations have been developed and used successfully in the biological control of pests and diseases. Nematode-trapping fungi that are among the fungi used in the biological control of nematodes and that create traps for nematodes have been identified as potential biological control agents against root-knot nematodes. In this study, soil samples were taken from the rhizospheres of tomato plants at 223 different greenhouses located in Antalya, Burdur and Isparta provinces in the West Mediterranean Region of Türkiye and nematode-trapping fungi were isolated using the soil sprinkling method. Seventy-two isolates of *Arthrobotrys conoides* were isolated from soil samples, and morphological and molecular methods were used to identification. The frequency of these isolates in the total samples was 32.29% (72/223). The in vitro experiments of effect of isolates of nematode-trappping fungus *A. conoides* on the root-knot nematode *Meloidogyne incognita* were conducted. After the isolates were developed in Petri dishes, 100 second-stage juveniles (J2s) of *M. incognita* were added to these Petri dishes and the petri dishes were incubated at 25°C (±1). Then petri dishes were examined under a stereo microscope 72 hours after inoculation and the number of live and dead J2s, the number of traps formed and the % activity of the trap fungus isolates were calculated. Experiments with each fungal isolate were completely randomized, and four replicates were maintained per isolate. As a results of the *in vitro* parasitism, it was determined that there were significant differences between the isolates. Seven isolates of *A. conoides* (A-A18-11-75%, A-K14-21, A-K14-3, A-K16-3, B-C8-3, B-G1-2 and I-Ge1-3) showed over 60% parasitism to *M. incognita* J2s.

109. Interactions of plant-parasitic nematodes with plant pathogenic soil fungi

Rik Peters[1]
1 Wageningen University Research, Field Crops, Lelystad, the Netherlands

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: plant parasitic nematodes, plant pathogenic fungi, interaction

ABSTRACT:

Plant-parasitic nematodes (PPN) and plant pathogenic soil fungi (PPF) are persistent, soil-bound problems and, under the right conditions, very harmful to the yield or quality of flower bulbs and field crops. When interacting, PPN and PPF may synergistically increase crop damage.

Through an extensive review of scientific literature, an overview of all currently known interactions between PPN and PFF, interaction type and potential interaction mechanisms has been made, structured by nematode group.

Three different types of interaction can be distinguished. Antagonistic: The pathogens involved in the interaction together cause less damage than when they occur individually on the plant

Additive: The pathogens involved in the interaction together cause the sum of the damage they could cause individually. Synergistic: The pathogens involved in the interaction together cause more damage than the sum of the damage they could cause individually

In temperate zones, cyst nematodes, root-knot nematodes and root-lesion nematodes most often engage in interactions with PPF. Interactions with *Fusarium*, *Rhizoctonia* and *Verticillium* are most common. Interactions led to delayed crop development, increased infection and increased rotting of affected plant parts in most cases. Some interactions led to enhanced multiplication of the nematodes in addition to the above effects.

In (sub)tropical zones, cotton root-knot nematode (*Meloidogyne incognita*), sugarcane nematode (*M. javanica*) and soybean cyst nematode (*Heterodera glycines*) most often engage in interactions with PPF. Interactions with *Fusarium* spp. and *Verticillium* and *Pythium* spp. were most common. Breakthrough of resistances was more common among these interactions.

The mechanisms through which PPN and PPF interacted with one another are the following:
1) Physical damage, 2) Disruption of cell function, 3) Formation of abnormal cells or plant parts, 4) Induction of growth of additional lateral roots, 5) Induction of plant defenses that weaken the plant, 6) Suppression of plant immune system through secretion of effectors and miRNA`s, 7) Suppression of plant immune system through metabolic substances, 8) Increased efflux of root exudates, 9) Additional efflux of substances from dead cells, 10) Adaptation of the microbiome in the rhizosphere and 11) Stimulation of pathogenicity in saprophytes.

In addition, the following factors played a role in their interaction:1) Influence of infection sequence, 2) Influence of infection site, 3) Influence of temperature, 4) Influence of damage to root system, 5) Influence of population density on interaction type and 6) Mechanism of resistance.

110. *Clonostachys rosea* as a biological control agent for *Meloidogyne incognita*

Paul Dahlin[1], Andrea Caroline Ruthes[1]
1 1. Entomology and Nematology, Plant Protection, Agroscope, Müller-Thurgau-Strasse 29, 8820 Wädenswil, Switzerland.
2 Mycology, Plant Protection, Agroscope, Route de Duillier 60, 1260 Nyon, Switzerland.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Meloidogyne*, *Clonostachys*, biocontrol

ABSTRACT:

In search of a suitable strain to serve as a bio-based alternative to chemical nematicides for the control of the root knot nematode, *Meloidogyne incognita*, an applied study was conducted on the widely distributed saprophytic fungus *Clonostachys rosea*. This sedentary endoparasitic nematode is common in southern regions and has become widespread in Swiss greenhouses. It causes significant agricultural damage, by causing severe root galling in plants. In order to identify a potentially effective strain of *C. rosea* against *M. incognita*, a total of twenty strains were subjected to a small in planta evaluation. From these, the three strains with the lowest gall index were further evaluated for their nematicidal activity *in vitro*. The most promising *C. rosea* strain, PHP1701, was additionally tested in laboratory and greenhouse trials to assess its ability to control *M. incognita*. The results showed that the PHP1701 strain had a significant effect on the control of *M. incognita* in soil, with the efficacy depending on the concentration used. A significant reduction in tomato root galling compared to untreated plants was observed in small-scale trials. In a larger greenhouse tomato trial, when PHP1701 was applied monthly, control was observed only in early and mid-season, with no effect towards the end of the growing season when all roots were severely galled. However, the tomato yield was higher than the untreated *M. incognita*-infected control. A large tomato field trial showed similar reduction in root galling. Initial and interim evaluation of tomato rootstocks showed a significant reduction in root galling compared to untreated plants infected with *M. incognita*.

111. Bacterial cell cultures: effects on the infectivity of *Meloidogyne hapla*

Carlos Cruz[1], Adriana S. Mata[2], Paula V. Morais[2], Diogo N. Proença[2], Isabel Abrantes[1], Isabel *Luci* Conceição[1]
1 University of Coimbra, Centre for Functional Ecology - Science for People and the Planet, Associate Laboratory TERRA, Department of Life Sciences, Coimbra, Portugal
2 University of Coimbra, Centre for Mechanical Engineering, Materials and Processes (CEMMPRE), Advanced Production and Intelligent Systems (ARISE), Department of Life Sciences, Coimbra, Portugal

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Meloidogyne hapla*, bacterial consortium, pest Management, PGPB

ABSTRACT:

The increasing frequency of extreme weather events is affecting ecosystems and threatening food production. Therefore, to reduce the impacts of climate change and ensure sustainable agricultural practices, it is vital that the use of chemical pesticides decrease and eco-friendly alternatives are explored. PPN are a significant threat to crop production worldwide and, among them, *Meloidogyne* spp. are the most damaging. Conventional nematicides have been the best alternative to control PPN but their indiscriminate use has raised environmental concerns and led to the development of nematicide-resistant nematode populations. Hence, innovative coupled with sustainable strategies should be developed to control and mitigate their impact, such as plant-growth promoting bacteria (PGPB). This research seeks to explore the potential of strains from *Bacillus* and *Pseudomonas* genera, as biological control agents, against RKN. Tomato plants cv. Coração de Boi, susceptible to RKN populations, were selected to assess the nematode infectivity carried out in pots (7 cm diameter, ca. 118.37 g soil). The assay was split into three treatments, with five replicates, including (i) control without the bacteria and inoculated with 300 J2 of *M. hapla*, (ii) inoculated with 1 mL of each consortium bacterial strain (adjusted to OD??? = 0.6, equivalent to 1×10^8 CFU mL-1) and, after 30 min, inoculated with 300 J2 of *M. hapla* and (iii) inoculated with 300 J2 of *M. hapla* and, after 30 min, inoculated with 1 mL of each consortium bacterial strain. After one week, the plants were uprooted, and the roots were carefully washed out of debris and stained with acid fuchsin. The RKN infectivity was determined by counting the number of stained nematodes inside the roots under a stereomicroscope. The bacterial consortium significantly reduced the infectivity of *M. hapla*, being the infectivity in the control 3 times more in average than in the treatments with consortium. Considering the two treatments, with the bacterial consortium, there were no significant differences, although the treatment with the consortium before inoculation of the nematodes was more effective to reduce the infectivity of RKN. In a previous study was shown that this consortium affected RKN motility and mortality but did not affect *Caenorhabditis elegans*. These findings revealed that this bacterial consortium has the potential to be a new tool for RKN management.

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(CFE) and Project ReNATURE - Valorization of the Natural Endogenous Resources of the Centro Region (Centro2020, Centro-01-0145-FEDER-000007).

112. Pochonia chlamydosporia a biocontrol agent, biofertilizer, plant vaccine or abiotic plant stress protectant?

Luis Vicente López-Llorca[1]
1 Plant Pathology Laboratory, Faculty of Sciences, Department of Marine Sciences and Applied Biology, University of Alicante, 03690, Alicante, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: nematophagous fungi, *Pochonia chlamydosporia*, VOCs, secondary metabolites, plant growth promotion, priming, biotic and abiotic stress protectant

ABSTRACT:

The original paradigm coined in the beginning of the biocontrol era has changed. Nematode antagonists are no longer just the magic bullets to replace chemical synthesis nematicides. Most of them such as Nematophagous fungi have a multitrophic mode of action. One of them, *Pochonia* chlamydosporia (Pc), it is not only a pathogen of nematodes (eg. PCN, CCN, RKN) parasitic (PPN) on food security crops. The Clavicipitaceous evolutionary origin of the fungus has left traces in its genome leaving a highly enriched secondary metabolism. These molecules (including many volatile organic compounds) display high biological activity against pests and pathogens. The fungus is a true cell factory. The endophytic phase of the fungus has a huge potential to promote growth of crops. Besides that, Pc enhances crop development and induces plant defenses vs. biotic stresses, including pathogens other that PPN such as wilt or necrotrophic fungi. Recent discoveries show that Pc can also help crops withstand Climate Change derived factors such as soil salinity or water shortage. We will present The Nematode Destroying Fungi, of the genius George Barron, under a XXI century new and fascinating perspective.

113. Investigation of fluazaindolizine as a potential novel tool to manage *Xiphinema index*

Emmanuel Tzortzakakis[1], **Tim Thoden**[2], **Anastasia Chatzaki**[1]
1 Institute of Olive Tree, Subtropical Crops and Viticulture, Department of Viticulture, Vegetable Crops, Floriculture and Plant Protection, ELGO-DIMITRA, Heraklion, Crete, Greece
2 Corteva Agriscience™, Riedenburger Str. 7 , 81677 München, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: dagger nematodes, Salibro, oxamyl, Vydate, fluopyram, Velum PRIME, fig

ABSTRACT:

Salibro® (500 SC) is a novel nematicide containing the active ingredient (a.i.) fluazaindolizine (Reklemel™ active). In *in vitro* tests, its effect on the motility and the mobility of *Xiphinema index* were evaluated at 0.5 to 100 ppm a.i. in comparison to Vydate® 10L at 12.5 ppm a.i. (a.i. oxamyl) and Velum PRIME® SC at 1 ppm a.i. (a.i. fluopyram). Salibro at 5 to 100 ppm a.i. as well as both other nematicides strongly reduced the motility of *X. index* compared to the control at all incubation times (24 to 72 hours). However, if the pre-exposed nematodes were rinsed and challenged to migrate through a 1 cm layer of coarse sand (mobility test) only the longest pre-exposure period of 72 hours caused a significant effect at 25-100 ppm of Salibro a.i. This indicates some level of reversibility. In addition to the *in vitro* experiments, pot tests were run on fig trees with various pot sizes (0.250 to 1.5 L), different timings of inoculations as well as different environmental conditions (indoors vs outdoors) to evaluate the impact of Salibro on *X. index* reproduction. Throughout the course of those experiments, it became clear that only the higher rates of Salibro (with 1 to 2 kg ai/ha) would qualify for sufficient and reliable control of *X. index*. Still Salibro performed significantly better and more consistently, than both of the other nematicide standards. Currently long fallow periods are recommended after grape vine uprooting and before replanting to eliminate *X. index*. In such replanting programs, one or more applications of Salibro around the newly planted vines may have the potential to prevent the subsequent build-up of any residual nematode population.

114. Host suitability of *Cucumis metuliferus* and *Citrullus amarus* to *Meloidogyne chitwoodi*, *M. enterolobii* and *M. luci*

Aïda M Fullana[1], Carla Maleita[2], Duarte Santos[3], Isabel Abrantes[3], Francisco Javier Sorribas[1], Ariadna Giné[1]

1 Department of Agri-Food Engineering and Biotechnology (DEAB), Barcelona School of Agri-Food and Biosystems Engineering (EEABB), Universitat Politècnica de Catalunya, BarcelonaTech (UPC), Castelldefels, Spain

2 University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Rua Sílvio Lima, Pólo II - Pinhal de Marrocos, 3030-790 Coimbra, Portugal

3 University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Associate Laboratory Terra, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: histopathology, plant resistance, root-knot nematodes, rootstock

ABSTRACT:

Meloidogyne chitwoodi, *M. enterolobii*, and *M. luci* are present in some EU countries with a restricted distribution. Plant resistance can be a valuable tool for managing these *Meloidogyne* spp. Two pot experiments were conducted under controlled conditions, for 56 days, to assess the host suitability of two potential rootstocks, *Cucumis metuliferus* BGV11135 and *Citrullus amarus* BGV5167 to one isolate of each nematode species. For comparison, the susceptible cucumber, *Cucumis sativus*, cv. Dasher II, watermelon, *Citrullus lanatus*, cv. Sugar Baby and tomato, *Solanum lycopersicum*, cv. Coração-de-Boi were included. Additionally, a histopathological study using confocal-laser microscopy was conducted 15 days after nematode inoculation. In the pot test, the rootstocks showed lower numbers of galls, egg masses, and eggs per plant than their susceptible ones. The reproduction index of the rootstocks varied from immune to moderately resistant, depending on the isolate-rootstock combination. In the histopathological study, *M. enterolobii* and *M. luci* induced a similar number of giant cells (GC) per feeding site in all germplasms. However, the GCs volume and the number of nuclei in rootstocks were lower than in susceptible germplasms. GCs induced by *M. chitwoodi* were only detected in susceptible cucumber. These findings not only underscore the potential of *C. metuliferus* BGV11135 and *C. amarus* BGV5167 as effective, eco-friendly strategy for managing root-knot nematodes but also illuminate complex host-pathogen interactions.

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115. Integrated *Globodera pallida* Management as part of Integrated Crop Management (ICM). The only route to sustainable potato production and maintaining the seed potato export market

Leendert Molendijk[1], **Johnny Visser**[1]
1 Wageningen University and Research, Field Crops, The Netherlands

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: INM, Integrated Nematode Management, *Globodera pallida*

ABSTRACT:

The Potato Cyst Nematode is a recurring problem in the Netherlands. Over the past 30 years, the PCN situation has improved considerably, especially through the cultivation of resistant varieties. It is now clear that we are dealing with the development of virulent populations that require a reflection on the control strategy.

A Nematode Control Strategy has been promoted in the Netherlands since the end of the 1990s . This NCS strategy was developed to reduce the use and dependency on chemical nematicides and proved to be successful. Since 2020 the NCS has been redesigned and developed further into Integrated Nematode Management (INM). Next to nematode management also fungi, insects and weeds are managed following the same approach which is an advanced IPM+, taking into account the whole crop rotation and their connected pests and diseases. This integrated approach is called Integrated Crop Management (ICM).

The Dutch arable sector organisation asked Wageningen University in 2023 to design a new INM scheme (Integrated Globodera Management) to help growers and advisers to develop a tailor-made *Globodera pallida* control strategy for their own farms. Communication with Dutch growers now follows the INM concept.

INM consists of five pillars : monitoring and evaluation, crop diversity in time and space, Cultivar choice and cropping strategy, Soil management and Targeted control

In this contribution the principles of ICM and INM will be e*luci*dated using the Integrated *Globodera* Management as an example. Newest research results on tools within the pillars will be presented. Information on the concept of INM can be found in the in 2022 published book of Sikora, A. et al. Integrated Nematode Management.
https://www.cabidigitallibrary.org/doi/book/10.1079/9781789247541.0000

116. Integrated *Meloidogyne* management, possibilities within the crop rotation to manage *Meloidogyne chitwoodi* and *M. fallax*

Johnny Visser[1], Misghina G Teklu[2], Leendert Molendijk[1]
1 Lelystad, Wageningen University & Research, Business unit Field Crops
2 Wageningen, Wageningen University & Research, Plant Sciences Group

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Meloidogyne chitwoodi*, *M. fallax*, Integrated Nematode Management, host status, crop rotation

ABSTRACT:

Meloidogyne chitwoodi and *M. fallax* are important nematode problems in arable farming in The Netherlands ever since their detection in the 1980th. Their quarantine status and the damage inflicted on product quality in important cash crops such as potato, carrots, black salsify and gladiolus has increased the need for integrated nematode management strategies that prevent yield and quality losses and further spreading. A control strategy, as part of an integrated crop management approach, has been promoted in The Netherlands since the end of the 1990th. This Integrated Nematode Management strategy was developed to reduce the use and dependency on chemical nematicides and is based on five major pillars: monitoring, crop rotation, varieties selection and cropping strategy, soil management and targeted control. In this integrated management strategy nematicides are only applied when necessary, as a last resort. Crop rotation, involving the use of non-hosts or resistant cultivars, is one of the main tools of this INM and should be the first and foremost practice of any Integrated Nematode Management strategy. To establish a 'smart' rotation, reliable data on host-status of crop are required.

In the Dutch project 'smart crop rotations' pot and field experiments were performed, at a range of initial population densities to evaluate host-status and resistance of crops developed by Dutch breeders. Seinhorst's model was used to study the population dynamics of *M. chitwoodi* and *M. fallax* on some important arable and cover crops and two parameters, the maximum multiplication rate (a) and the maximum population density (M) for each crop were estimated.

The results provided new insights on the host-status of some (cover) crops, revealed variation between cultivars and demonstrated that for some crops the host status for *M. chitwoodi* differ from the host status for *M. fallax*. The host-status ranged from poor-host (chicory) up to very good host (black oat) and resistance level of a new sugar beet variety was (> 99%) relative to the susceptible variety .

Based on results of the pot experiment sugar beet could be classified as a poor host for *M. chitwoodi* and maintenance host when tested in the field in which the later is unusual and difficult to comprehend type of host plant status. Results of this research project were made available to farmers and extension organisation via the nematode tool within www.Best4Soil.eu. Further methods and results will be presented and discussed.

117. The IRAC International Nematode Working Group and Nematicide Mode-of Action Classification and Labelling

John A Wiles[1], Bruno Lovato[2], Julia Huggins[3], Tim C Thoden[1], Ursel Collienne[4], Ralf Nauen[4], Andrew Crossthwaite[5], Ekaterini Riga[6], Matthias Gaberthueel[5], Russell Eldridge[7]
1 Corteva Agriscience
2 Adama Agricultural Solutions
3 BASF
4 Bayer CropScience
5 Syngenta AG
6 FMC Corporation
7 Valent BioSciences

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: nematicide, mode of Action, chemicals, biologicals

ABSTRACT:

The IRAC Nematode Working Group is the most recently established IRAC International 'Working Group'. The initial aim of the working group was to develop a similar international MoA classification scheme for nematicides, as for the insecticides (and indeed fungicides FRAC and herbicides HRAC). Several documents have been created (see the IRAC website - https://www.irac-online.org/teams/nematodes/) and continue to be updated. The 'Nematicide MoA Classification scheme' incorporates a wide range of active ingredients, organisms, conventional chemical nematicides, fumigants and agents of biological origin that have demonstrated nematicidal activity. The classification follows the same principles as other schemes, and the group names start with the letter N to denote 'nematicide/nematode control agent'. The proposed groupings include, Carbamates (Group N-1A), Organophosphates (Group N-1B), Avermectins (abamectin, Group N-2), Pyridinylmethyl benzamides and Phenethyl pyridineamides (fluopyram, cyclobutrifluram, Group N-3), Tetramic acids (spirotetramat, Group N-4), a group of novel compounds (Group N-UN) with unknown MoA's, including products such as fluazaindolizine, fluensulfone and furfural and fumigants (Group N-UNX). The various biologicals for nematode control have been divided into three groups: Bacteria (Group N-UNB), Fungi (Group N-UNF), and botanical/animal Extracts (Group N-UNE). As with other MoA Classification schemes, when new information is submitted to the Working Group, the group evaluate it and consider updating the nematicide MoA Classification scheme accordingly. The overall aim is to encourage companies/suppliers and regulators around the world to use this classification code and associated icon on nematicide product labels to inform the user/grower as to what type of nematicide they are using. The team is also developing an 'app' to make the scheme available via electronic devices. This presentation will provide an update on recent activities of the Working Group, including how submissions for additions are submitted and evaluated.

118. Reklemel active: a novel tool for integrated nematode management? Key learnings from a global nematicide development project

Thoden Tim[1], Yannis Stamatas[2], Raquel Abad[3], John Wiles[4]
1 Corteva Agriscience, Germany
2 Corteva Agriscience Hellas, Greece
3 Corteva Agriscience Iberica, Spain
4 Corteva Agriscience UK Limited, UK

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: fluazaindolizine, nematode management, soil health, nematicides

ABSTRACT:

Reklemel active (fluazaindolizine) is a proprietary novel soil applied nematicide that has been developed by Corteva Agriscience and is currently under commercialization in various geographies around the world. The development of this chemistry was started more than 10 years ago and during its global development, more than 3800 field studies have been performed to test it under different edaphic and climatic conditions in numerous crop-nematode combinations. During our talk, we will present both, key biological attributes (e.g. mode-of-action, spectrum, soil behavior) of this novel chemistry as well as some novel learnings regarding its soil health compatibility.

119. Understanding excreted/secreted products in *Heterorhabditis bacteriophora*: Activation material insights

Sara Sreibr[1], Jana Ilgova[2], Pavel Dobes[1], Jacek Marciniak[1], Jiri Vorel[2], Jana Hurychova[1], Martin Kasny[2], Pavel Dobes[1]

1 Department of Experimental Biology, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic

2 Department of Botany and Zoology, Faculty of Science, Masaryk University, 625 00 Brno, Czech Republic

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: entomopathogenic nematode, excreted/secreted products, *Heterorhabditis bacteriophora*, activation materials

ABSTRACT:

Entomopathogenic nematodes (EPNs) are insect parasites commonly used in the biological control of various insect pests. EPNs release biologically active compounds known as excreted/secreted products (ESPs) that play a crucial role in interaction with the host s immune system. This interaction enhances infection efficiency, thereby increasing the chances of EPNs survival and successful reproduction. Unravelling the composition and function of ESPs is essential for understanding the mechanisms of nematode pathogenicity and optimizing their effectiveness as biocontrol agents. In this study, we aimed to evaluate the protein spectrum of *Heterorhabditis bacteriophora* ESPs released in relation of different activation materials. We identified the infection specific molecules by comparing ESP profiles between infective juveniles treated with materials prepared from insects and controls.

We optimized the *in vitro* activation process of *H. bacteriophora* and selected three fractions of activation materials from *Galleria mellonella* larvae, each prepared differently, along with two control fractions: water or phosphate buffer. We collected ESPs from all treatments and subjected them to LC MS/MS analysis. For protein identification, we conducted searches against a protein database originating from the *H. bacteriophora* reference genome.

The results recorded using the LC MS/MS analysis revealed significant differences in the composition of ESPs based on the materials used for nematodes' in vitro activation. Within these ESPs, we identified numerous proteins with potential to play significant role in nematode insect interaction, including serpin domain containing protein, metalloendopeptidase, ShTK domain containing proteins and fatty acid retinoid binding proteins. Furthermore, our findings indicate that the choice of activation materials significantly influences both the abundance and diversity of collected ESPs. This implies that the way of nematode activation may play a crucial role in modulating the virulence of the EPNs, suggesting an increase in the effectiveness of biocontrol.

This study was supported by grant GA-R 23 06457S06457S.

120. Genomics of *in vitro* dauer juvenile recovery of *Heterorhabditis bacteriophora* in monoxenic liquid culture with *Photorhabdus laumondii*

Zhen Wang[1], Christopher Ogaya[1], Manoj Dhakal[3], Francisco Garcia[1], Verena Dörfler[2], Mike Barg[2], Bart Vandenbossche[2], Olaf Strauch[2], Ralf-Udo Ehlers[1], Carlos Molina[2]
1 Kiel University, Germany
2 e-nema GmbH, Germany
3 Ghent University, Belgium

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: *Heterorhabditis bacteriophora*, EMS-mutagenesis, DJ recovery, *Photorhabdus* bacterial supernatant, SNPs, RNA-seq

ABSTRACT:

The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora*, symbiotically associated with *Photorhabdus*, is a biological control agent against insect pests. The dauer juvenile (DJ) is a free-living and developmentally arrested stage that carries cells of the symbiont, seeks and invades the insect host. The sequence of the DJ perceives the haemolymph food signal, exits from the dauer stage and develops into the fertile adult stage is called DJ recovery. In monoxenic liquid cultures, DJs depend on unknown bacterial food signals to trigger the recovery. A rapid, synchronized, and high DJ recovery is a major key factor for commercial production of EPN. Understanding the DJ recovery in *H. bacteriophora* is thus crucial to improve the mass production of EPN.

Concerning the DJ recovery variability, we developed a predictor bioassay based on *Photorhabdus* supernatant to evaluate the DJ recovery phenotype of several *H. bacteriophora* wild type (WT) and more than 160 EMS mutant lines. More than 150 single nucleotide polymorphisms (SNPs) were characterized within the mutants via high throughput genotyping, and four SNPs are robustly associated with the DJ recovery. Sets of mutants with contrasting reproducible DJ recovery phenotype were revealed.

Furthermore, we carried out a detailed geno- and phenotypic characterization of 14 *Photorhabdus* strains and evaluated their influence on the DJ recovery in a set of *H. bacteriophora* materials. It was evidenced that the bacterial component plays a subordinate role, whereas the nematode genetic pre-disposition is a main factor in the regulation of the DJ recovery in this species.

As final approach, we conducted a genome-wide RNA-seq analysis of the early DJ recovery stages (0.5 - 6 h) in two nematode mutant lines with contrasting phenotype (M31 & M88). The expression of more than 14,000 gene models was analysed and the information was combined with functional databases (Gene Ontology) considering the homologies with *Caenorhabditis elegans*. As outcome, nine gene models are postulated as potential targets for future approaches.

121. SlugBot: Developing an autonomous monitoring and biocontrol system for slugs

Jenna Ross OBE[1], Archita Barua[1], Faye McDiarmid[1], Tom Ashford[2]
1 Crop Health and Protection (CHAP), 1.34, Innovation Centre, Innovation Way, Heslington. York. YO10 5DG, UK
2 Rothamsted Research, West Common, Harpenden AL5 2JQ, UK

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: slugs, biocontrol, nematodes, robotics, AI

ABSTRACT:

Slugs are major economic pests in agricultural and horticultural crops in the UK. Historically, methods of slug control have been reliant on chemical molluscicide pellets, containing metaldehyde or Iron (Ferric) phosphate, however due to the negative impact on UK water systems, as well as on non-target organisms, metaldehyde has subsequently been banned in the UK. Bio-molluscicides are also commercially available as in the form of nematode-based products, however these are not economical for use in arable and oilseed rape crops. Therefore, the slug control toolbox in the UK is limited. The aim of this project was to develop an innovative game-changing prototype technology for autonomous slug monitoring and precision bio-molluscicide treatment. This presentation will provide high level results of the development and testing of the aforementioned technology, as well as introducing the follow on project code-named: SLIMERS.

122. Exploring the influence of entomopathogenic nematodes on aerial pest control in grape volatile composition

Mar Vilanova[1], Miguel Puelles[1], Bianca S. Costa[1], Elisabet Vaquero[1], Javier Manzanares[1], Raquel Campos-Herrera[1]
1 Instituto de Ciencias de la Vid y del Vino, Spain

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: aerial application, grape composition, must volatile compounds, *Steinernema*

ABSTRACT:

Entomopathogenic nematodes are recognized as effective biological control agents against various soil-dwelling pests. Recent formulations and application systems developments have expanded their use to target aerial pests. However, this approach brings nematodes into contact with new plant areas, which can be applied even close to harvest. Vineyards, crucial for global economic, social, and heritage reasons, often face challenges from various insect pests, such as the European Grape moth, *Lobesia botrana* (Lepidoptera: Tortricidae), which affect grape quality. Introducing new management tools can enhance viticulturists pest control strategies. Recent studies indicated entomopathogenic nematodes (EPNs) as potential biological control agents for managing this aerial pest, with specific recommendations for application during sunset and warm temperatures (15-25 ºC). Yet, limited knowledge exists regarding their impact on grape quality. This study explores the hypothesis that EPNs do not compromise grape quality while acting as biocontrol agents aboveground. Our objective was to assess the impact of EPN application for controlling *L. botrana* on the volatile composition of grapes under insect attack, comparing efficacy with a *Bacillus thuringiensis* (Bt) (Bacillales: Bacillaceae), another commercially available biological control agent. First, in a laboratory study, grapevines were exposed to EPNs *Steinernema feltiae* and *S. carpocapsae*, alone or with the adjuvant Maximix, using water and the adjuvant only as controls. The impact of *L. botrana* presence was also examined. After 4- and 24-hour exposures, grape must was collected and analyzed for free and glycosylated fractions of volatile compounds using solid-phase extraction/gas chromatography-mass spectrometry (SPE/GC-MS). Results suggested a tendency for increased concentrations of free volatile compounds when *L. botrana* was present, possibly induced by stress as a defense mechanism. However, the presence of the EPN alone or combined with Maximix did not affect the must composition. In a subsequent semifield experiment, the efficacy of biocontrol agents (nematodes and bacteria) was assessed. Larval mortality after six days and feeding damage after two days were significantly reduced only in the bacteria treatment. The glicosidically bound fraction was notably affected, with increased concentrations observed in *Lobesia* + *S. feltiae* and *Lobesia* + *S. carpocapsae* treatments compared to the control and Lobesia + B. thuringiensis. In detail, by chemical groups, glicosidically bound C13-norisoprenoids and phenol volatiles increased in *Lobesia* + *S. feltiae* and *Lobesia* + *S. carpocapsae*, respectively. This study underscores the importance of comprehending the impact of EPN application on fruit quality, highlighting potential variations in volatile compound composition based on different treatments.

123. Effects of chromium VI on the attraction, percentage penetration and reproduction of the entomopathogenic nematode *Steinernema feltiae* in *Galleria mellonella*

Anique Godjo[1]
1 South East Technological University, Department of Applied Science, enviroCORE, Kilkenny Road Campus, Carlow, R93 V960, Ireland

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: entomopathogenic nematodes, wax worms, EPN, heavy metal, Cr VI, ecotoxicology

ABSTRACT:

Hexavalent chromium (Cr VI+) is dangerous for human and environmental health. This element occurs naturally in agricultural soils (5-250 mg/Kg) in Ireland. In an effort to understand the effects of this potential toxicant on soil biota, a study was initiated using the entomopathogenic nematode (EPN) *Steinernema feltiae*. Investigating the effects of a toxicant on a bioindicator organism provides essential information on the effects of this pollutant on the local ecosystem. *Steinernema feltiae* is naturally present in Irish soil and thus was chosen to risk assess soil Cr VI+ pollution. Using *Galleria mellonella* larvae, an Irish strain SB 12(1) of *S. feltiae* was cultured and studied for their host penetration percentage and reproduction, when exposed to Cr VI+ at various concentrations (50-300ppm in increments of 50). In addition, nematode attraction to its insect host was studied using different lengths of PVC tubes (5, 10, 15, 20 cm,) filled with Cr VI+ contaminated play sand (50-300ppm in increments of 50), with 3-fold replication and counting *G. mellonella* mortality at the end of the experiment. Positive control tubes received nematodes but no Cr VI+ and negative control tubes received no nematode and no Cr VI+. Results showed that the percentage host penetration and reproduction of *S. feltiae* were highly positively correlated. These experiments have been repeated using the same Cr VI+ concentrations under the same laboratory conditions to ensure reproducibility of data related to these toxicity endpoints. Both distance between nematode application point and host and Cr VI+ concentration significantly affected *S. feltiae* attraction to the host. Higher insect mortality of 100% and 88.89% were recorded at low Cr VI+ concentrations (positive control and 50ppm respectively) over 5 cm. No significant differences were recorded between insect mortalities at 150-300ppm Cr VI+ and the negative control suggesting that the threshold of Cr VI+ effect on *S. feltiae* attraction to its insect host is between that range of metal concentration. Overall, *S. feltiae* showed great potential for soil Cr VI+ risk assessment and represents a good candidate for developing a protocol for international use and accreditation.

124. Defining climatic parameters for optimal timing of EPN application against apple codling moth

Bart Vandenbossche[1], Mike Barg[1], Verena Dörfler[1], Hartmut Kaiser[2], Nikolina Grabovac[3], Thorsten Rocksch[3], Ralf-Udo Ehlers[4,5]
1 e-nema GmbH, Schwentinental, Germany
2 Department of Biology, Christian-Albrechts-University Kiel, Germany
3 Department of Biosystems Engineering, Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt-Universität zu Berlin, Germany
4 Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany
5 Department of Biology, Ghent University, Belgium

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: *Cydia pomonella*, decision support systems, *Steinernema feltiae*, temperature, humidity

ABSTRACT:

The product nemapom® contains entomopathogenic nematodes *Steinernema feltiae* for the biological control of overwintering larvae of the apple codling moth *Cydia pomonella*. Nematodes are sprayed in autumn or spring on the tree bark. To secure success of the application, it is recommended to 'keep the bark moist for approximately 4 hours'. This rather sketchy recommendation is not appropriate for modern fruit production. The aim of the NemaSens Project is to define the range of the climatic parameters (e.g. humidity, temperature, radiation, wind etc.), at which the nematodes can find and kill the host insects and under which conditions EPN ultimately die. To investigate the survival on bark, we developed a bark spraying assay and collected the nematodes 1,2 and 4 hours after application. Two and four hours after spraying, 85% and 51% of the nematodes had survived on bark, respectively. Low temperature activity of *S. feltiae* was tested in incubators at 5, 6, 7,5 and 8°C. *S. feltiae* infected and killed *C. pomonella* larvae at temperatures as low as 5°C, but after 14 days. Although the time to kill the larvae is long, this low temperature activity is advantageous as the larvae overwinter in the bark for several months. These data together with field data will be used to produce decision support systems for the farmer to decide when optimal conditions prevail long enough to obtain high control results.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL).

125. Advances in the isolation and characterization of endophytic fungi for the biological control of root-knot nematodes in vegetables in Mexico

María Gabriela Medina-Canales[1], Manuel Silva-Valenzuela[2], Alejandro Tovar-Soto[1], Reyna Isabel Rojas-Martínez[2], Rosa Helena Manzanilla-López[3]
1 Departamento de Parasitología, Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional (ENCB-IPN), México
2 Fitosanidad-Fitopatología, Colegio de Postgraduados, Montecillo, México
3 Centro de Desarrollo de Productos Bióticos (CEPROBI-IPN), Yautepec, Morelos, México

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: endophytic Fungi, biological control

ABSTRACT:

The cultivation of vegetables for local consumption and export contributes significantly to the economy of Mexican farmers. Damage caused by root-knot nematodes (*Meloidogyne* spp., Nacobbus aberrans sensu lato) and cyst nematodes (*Globodera* spp., *Heterodera* spp.) is common in vegetables such as tomato, potato, chilli, carrot, lettuce and beet, and in legumes such as beans causing important losses in production. As part of an agroecological and integrated approach to manage root-knot nematodes, the search for potential biological control agents has been increased due to local demand, the importance of export agriculture and need to develop a national biotechnology that can enable quality standards for production, formulation, application and validation tests in both laboratory and field for the safe use of nematophagous endophytic fungi. To achieve this aim, the correct identification of the isolates and studies on their effectiveness in the parasitism or antagonism to nematodes, as well as their response to different physicochemical factors, is necessary. Notable among the nematophagous and endophytic fungi that have been identified are some isolates of *Pochonia chlamydosporia* var. chlamydosporia, *P. chlamydosporia* var. mexicana, *Echria macrotheca*, *Stagonospora trichophoricola* and *Chaetomium globosum* that were isolated from horticultural areas in the states of Puebla, Sinaloa, and Mexico City, either as parasites or antagonists of root-knot nematodes. In the present work, the rhizocompetence and response of selected isolates of *P. chlamydosporia* var. mexicana to physicochemical factors (temperature, pH, osmotic stress) are compared. Some of the molecular mechanisms involved in the antagonism of *E. macrotheca*, *S. trichophoricola* and *C. globosum* towards *Meloidogyne* spp. and *N. aberrans* s.l. are described as well as management strategies that could improve fungal parasitism on root-knot nematodes.

126. The Biochemical Diversity of the Second-stage Juvenile Surface Coat: its Origin, Role and Interaction with *Pasteuria* Endospores

Keith Davies[1]
1 Keith G Davies, University of Hertfordshire UK
2 Sharad Mohan Indian Agricultural Research Institute, India
3 Victor Phani College of Agriculture West Bengal, India
4 Arohi Srivastava DY Patel Biotechnology Institute Pune, India

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: cuticle, surface coat, *Pasteuria* endospores, stem cells, seam cells,

ABSTRACT:

A major challenge in the commercial development of *Pasteuria penetrans* as a biological control agent to control root-knot nematodes is its host specificity, one population of the bacterium can attach to and infect one population of plant-parasitic nematode but not another of the same species. A specificity model that is now over 10 years old is that a *Velcro*-like attachment process was involved that relied on a fibrous hair-like nap of the surface of the endospore interacting with a receptor on the second-stage juvenile cuticle. The model suggested that the hair-like nap on the endospore surface was made from collagen-like proteins and this interacted with mucin-like molecules to which the collagen-like proteins attached. Here we review our understanding of the genetics behind the molecular processes. Our review will focus on the nature of the bacterial adhesins and how they interact with the nematode cuticle receptors by exploiting new insights gained from studies of bacterial infections of *Caenorhabditis elegans*. A new *Velcro*-like multiple adhesin model will be proposed in which the cuticle surface coat, which has an important role in endospore adhesion, is a complex extracellular matrix containing glycans originating in seam cells. Recent research suggests that these genes are associated with these seam cells and appear to have a dual role by retaining some characteristics of stem cells.

127. Dual nematode infection in *Brassica nigra* affects shoot metabolome and aphid performance in distinct contrast to single-species infection

Jessil Ann Pajar[2], Stefanie Döll[1], Nicole M. van Dam[1]
1 Leibniz Institute for Vegetable and Ornamental Crops (IGZ) e.V., Großbeeren, Germany
2 Max Planck Institute for Chemical Ecology, Jena, Germany
3 Molecular Interactions Ecology, German Centre for Integrative Biodiversity Research (iDiv), Jena-Halle-Leipzig, Germany
4 Institute of Biodiversity, Friedrich-Schiller-University, Jena, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: flavonols, glucosinolates, hydroxycinnamic acids, metabolomics, plant-herbivore interaction, root-shoot interactions, simultaneous herbivory

ABSTRACT:

Previous studies showed that aphid performance was compromised on *Brassica nigra* infected by root-lesion nematodes (*Pratylenchus penetrans*, Pp), but less, or positively influenced by root-knot nematode (*Meloidogyne* spp., Mi) infection. These experiments were on single-nematode infections, yet in nature roots are infected with several nematode species simultaneously. We performed greenhouse assays to assess the effects of single (Mi, Pp) and concurrent (MP)-nematode infections on aphid performance. Using targeted biochemical analyses and untargeted profiling of leaf and phloem metabolomes, we addressed the question how single- and concurrent-nematode infections affect leaf and phloem metabolomes, and elucidated their consequence on aphid performance. We found that the metabolic response towards double infection is different from single-species infections. Moreover, Mi- and Pp-infections triggered discrete changes in *B. nigra* leaf and phloem metabolic profiles. Both Pp and MP-infection reduced aphid survival, suggesting that the biological effect was primarily dominated by Pp-induced changes. This concurred with increased indole glucosinolates and hydroxycinnamic acid levels in the leaves, in particular the involvement of salicylic acid-2-O-D-glucoside. Our study provides evidence that concurrent infection of different nematode species, as is common in natural environments, distinctly alters aboveground plant metabolomes, thereby affecting the performance of aboveground herbivores.

128. Cyst nematodes are a rich source for the discovery of new nematode antagonistic fungi producing a multitude of new bioactive secondary metabolites

Samad Ashrafi[1], Jan-Peer Wennrich[2], Marc Stadler[3], Wolfgang Maier[4]
1 Institute for Crop and Soil Science, Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants Braunschweig Germany
2 Department of Microbial Drugs, Helmholtz Centre for Infection Research GmbH (HZI), Braunschweig, Germany
3 Department of Microbial Drugs, Helmholtz Centre for Infection Research GmbH (HZI), Braunschweig, Germany
4 Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Braunschweig, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: cyst nematodes, metabolites, nematophagous fungi

ABSTRACT:

Nematode parasitic fungi are a functional group that can antagonise especially plant parasitic nematodes. We have recently reported several new egg parasitic fungi including *Ijuhya vitellina*, *Laburnicola nematophila*, *Niesslia gamsii*, *Polydomus karssenii* and *Polyphilus sieberi*. The fungal strains obtained were isolated from the symptomatic eggs of the cereal cyst nematode *Heterodera filipjevi*. The symptomatic eggs exhibited unusual discolouration caused by the fungal colonisation. All fungal isolates fulfilled Koch's postulates. Some of these fungi exhibit a bi-functional lifestyle as endophytes and nematode antagonists. Among them the strains of the three dark septate endophytes *Laburnicola nematophila*, *Polydomus karssenii* and *Polyphilus sieberi* were isolated from either plant roots or nematode eggs. Their hyphae are highly melanised, hence they can colonise the developing juveniles inside the eggs and render the nematode cysts and eggs dark brown. They could also colonise plant roots without damaging the plants, and develop microsclerotia or monilioid cells inside the plant cells as typical structures for this group of endophytic fungi.

All fungal species mentioned above were also intensively studied for their secondary metabolites. For this purpose, over 400 extracts were prepared upon variation of culture media and subjected to screening for known and new compounds and subsequently subjected to a broad biological characterization procedure. Several new natural products with different biological activities (e.g., antimicrobial, nematicidal, or cytotoxic) were identified. *Polydomus karssenii*, for example, produces the new lipodepsipeptide ophiotine, which showed moderate nematicidal activity. *Polyphilus sieberi* could produce the known metabolite ophiocordylongiiside A that showed significant effect against Caenorhabditis elegans. As another example, the new compounds including omnipolyphilins, talaroderxine and derivatives of aphtho-pyranone were also obtained from *Polyphilus sieberi* and showed potent cytotoxicity and antimicrobial effects. These ecologically interesting nematophagous fungi discussed here display an inter-kingdom host interaction parasitising nematode eggs and colonising plant roots as endophytes. We thus defined them as endophytic nematode antagonistic fungi.

129. The use of *Bacillus thuringiensis* Cry5 protein to control root-knot nematodes

Yolanda Bel[1], Magdalena Galeano[2], Mireya Baños-Salmerón[2], Baltasar Escriche[1]
1 Universitat de Valencia, Instituto BIOTECMED, Departamento de Genetica. Calle Dr. Moliner 50. 46100-Burjassot (Valencia) Spain
2 Koppert España, S.L. R&D Department, Paraje Piedra Rodada, nº470. 04738-Vícar (Almería) Spain

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Meloidogyne incognita*, *Meloidogyne javanica*, bioassay, toxicity, in vitro, in planta

ABSTRACT:

Plant-parasitic nematodes cause severe damage to global crop production. In the Mediterranean area, root-knot nematodes are particularly prevalent causing substantial economic losses ranging from 15 to 60%. Nowadays, there is a worldwide need to increase the sustainable protection of crops against agricultural pests while preserving the environment. In this scenario, biological pesticides such as the entomopathogenic bacteria *Bacillus thuringiensis* (Bt), represent an excellent alternative to traditional chemical pesticides. Bt is a Gram-positive bacterium that produces parasporal crystals composed of highly specific pesticidal proteins. Notably, eight Bt crystal proteins have been described as toxic for diverse types of nematodes, namely Cry5, Cry6, Cry13, Cry14, Cry21, Cry31, App6, and Xpp55.

In the present study, the toxicity of Cry5A or, alternatively, the toxicity of spores and crystals of Cry5-producing strains, was evaluated against two root-knot nematodes belonging to the genus *Meloidogyne* (*M. incognita* and *M. javanica*), isolated from tomato and pepper orchards in south eastern Spain. The solubilized Cry5A protein was toxic to *M. incognita*, consistent with previous reports, and was also found to be toxic to *M. javanica*. Besides, in order to explore the potential use of Bt strains to field control of root-knot nematodes, in planta assays were conducted, using a standard Bt strain producing Cry5A and a wild-type strain producing Cry5 along with other Cry proteins. Bt strains without crystals were assayed as strain background controls. The infestation/Bt watering conditions were set up using cucumber plants, infesting with *M. javanica* J2, and subjecting them to one or two irrigations with Bt spores or Bt spore+cristal mixtures. After 8 weeks, the nematode egg masses, number of J2, and foliar weight were recorded. The results showed that Bt irrigations (one or two) did not reduce the egg masses or the J2 individuals per plant or did not exhibit bio-fertilizer activity regarding the respective controls, irrespective of the irrigation method used. Therefore, the high toxicity displayed by the solubilized Cry5 protein could not be translated into effective nematode control when using Bt as a conventional bio-pesticide.

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130. *Pochonia chlamydosporia* and chitosan: A new biocontrol strategy to manage plant parasitic nematodes.

Federico López-Moya[1]
1 Plant Pathology Laboratory, Faculty of Sciences, Department of Marine Sciences and Applied Biology, University of Alicante, 03690, Alicante, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: Chitosan, Plant Immunity, Nematophagous fungus, chitooligosaccharides

ABSTRACT:

Nematophagous fungi have been employed in biological control to protect crops of interest because of their ability to manage plant parasitic nematodes sustainably. *Pochonia* chlamydosporia, a worldwide fungal parasite root knot nematodes (RKN), is responsible for natural suppression of soils to plant parasitic nematodes. This fungus is also a true endophyte in both mono and dicot crop plants. *P. chlamydosporia* can modulate plant local and systemic defenses. Extracellular depolymerases of nematophagous fungi reflect their parasitic, endophytic, and saprophytic traits. Cell wall degrading enzymes play a key role during fungus-nematode interaction. RKN eggshell is enriched in chitin and glycoproteins. *P. chlamydosporia* a highly expanded family of hydrolases related with chitin modification. Chitosan is a highly deacetylated form of chitin. Most biocontrol fungi are resistant to chitosan. Genomes of most isolates of P. chlamydosporia from worldwide origin show genes encoding chitin deacetylases and chitosanases. These enzymes are overexpressed during Pc infection on RKN eggs. Most of these isolates display high parasitism to nematode eggs and degrade chitosan. Chitin perception is a key component of the Plant Immune System (PTI). Chitin shielding/deacetylation in fungi is a way to circumvent plant defenses. Plant chitinases show less affinity for chitosan than chitin. Besides, chitosan is a less efficient plant defense elicitor than chitin. We propose that chitosan metabolism allows endophytic biocontrol fungi such as *P. chlamydosporia* for evading plant defenses in the rhizosphere and allows them to parasitize efficiently endoparasitic nematodes embedded in root tissues.

131. Agronomy matters! Improving the performance of solanaceous trap crops for the management of potato cyst nematodes (*Globodera* spp.)

Matthew Back[1], Katarzyna Dybal[1], James Lee[2], Richard Griffiths[2], Tom Eyles[2], Ivan Grave[3], Alexander McCormack[4], Graham Tomlin[5], William Watts[2]
1 Harper Adams University, Newport, Shropshire, TF10 9EY, UK
2 Produce Solutions, Greenvale AP, 7 The Forum, Minerva Business Park, Peterborough, PE2 6FT, UK
3 Curious Raven, 1 Betton Crossing, Brownhills, Market Drayton, Shropshire, TF9 4BE, UK
4 Crop Health and Protection Ltd., York Biotech Campus, Sand Hutton, York, YO41 1LZ, UK
5 VCS Potatoes Ltd., Burnt Cottages, Badingham Road, Framlingham, Suffolk, IP13 9HX, UK

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: trap crop, *Globodera*, *Solanum*, field experiments

ABSTRACT:

Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) are widely regarded as the most important biotic threat to UK potato crops. Under current legislation, there is zero tolerance for PCN in land used for seed production, and as result, land availability is reducing. Added to this, PCN feeding damage has the potential to cause yield loss in ware crops, as high as 80%. Chemical control options have been drastically reduced since losing ethoprophos and oxamyl, and the future of the few remaining nematicides is uncertain. Potato cyst nematode population densities can be lowered in rotations through the inclusion of solanaceous trap crops or brassica biofumigants. This is beneficial, as it reduces pressure on resistant varieties that lack tolerance to the pest. Solanaceous trap crops function by inducing PCN hatch but preventing the development of infective juveniles as they invade the roots or attempt to initiate syncytia.

Our paper will present key findings from a two year 'Innovate UK' project, which has been investigating the performance of solanaceous trap crops under different agronomic treatments. The objectives of the work are to 1. compare the efficiency of *Solanum sisymbriifolium* (sticky nightshade DeCyst), *S. scabrum* (African nightshade DeCyst Broadleaf) and *S. chenopodioides* (tall nightshade DeCyst Podium) for PCN lowering population densities under glasshouse and field conditions, 2. Evaluate establishment methods such as seed rate, drilling method and soil cultivation, and 3. Assess inputs such as nutrients and herbicides on trap crop establishment and performance.

132. The rice root-knot *Meloidogyne graminicola*: an evaluation of the aggressiveness level regarding rice and wheat cultivars mainly grown in France.

Killian Laurent[1], Maíra Grossi De Sá[1], Stéphane Bellafiore[3], Jamel Aribi[3], Luc Villain[2], Laurent Folcher[1]
1 ANSES - Plant Health Laboratory, Le Rheu, France
2 CIRAD - UMR DIADE, Institut Agro, Montpellier, France
3 IRD - Plant Health Institute of Montpellier, University of Montpellier, Montpellier, France
4 ITC - Research and Innovation Center, Phnom Penh, Cambodia

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: *Meloidogyne graminicola*, rice, invasive species, aggressiveness, resistance capabilities, risk assessment

ABSTRACT:

Climate change, especially global warming, increase of trade globalization and intensive agriculture pose increasing challenges to agrosystems, affecting species and communities, particularly their resilience to new tropical invasive species. In Europe, prioritizing sustainable practices amid reduced pesticide use necessitates alternative solutions. This research focuses on the rice root-knot *Meloidogyne graminicola*, a threat to rice crops. *M. graminicola* s recent introduction in Europe, notably Italy, jeopardizes economically rice production in Europe because of potential yield losses of up to 80% in tropical rice growing countries, and other vital cereal productions. This work focuses on the *M. graminicola* s intra-interspecific plasticity, but at a behavioral level, by evaluating its infection and dissemination capacities on different varieties of rice as well as on other crops of interest in France. Objectives include analyzing aggressiveness on various rice varieties from Camargue and replicating the experiment on France's economically significant crops like durum and common wheat. Four populations of *M. graminicola* were studied: a Vietnamese population as a control, based on previous studies, an Italian population recently identified in Lombardy, a Cambodian and a Bornean populations, both known for their aggressiveness. For rice varieties, in addition to the control (*Oryza sativa indica*), three Camargue cultivars were selected (*O. sativa japonica*). For wheat, the Anvergur (durum wheat) and Chevignon (common wheat) varieties were chosen due to their widespread production in France. Each trial replicate comprises 160 plants, 4 populations x 4 varieties repeated 10 times, guaranteeing statistical robustness. Those trials, reproduced 3 times and totaling 480 inoculated plants, were completed with 120 non-inoculated controls per variety. Each plant was inoculated with 100 second-stage juveniles *M. graminicola*. Two variables were observed for assessing nematode aggressiveness: reproduction rate and galls number after 30 days. Three variables were observed for measuring the susceptibility of rice varieties: roots, leaves and total dry mass. Same parameters were applied to wheat: 80 plants, 4 populations x 2 varieties repeated 10 times, grown in a sandy substrate. Although rice trials are completed, wheat analysis are pending. Still, emerging trends indicate varying aggressiveness among *M. graminicola* populations, with Cambodian and Bornean populations being more aggressive, unlike the Italian population, being

the less aggressive one, showing no significant differences between varieties. Wheat could also be susceptible to *M. graminicola*, indicating that transmission to other crops of interest is indeed possible. This work shows promising results and will shed light on the risks posed by *M. graminicola* to French cereal farming.

133. Organic Production – management of root-knot nematodes, *Meloidogyne* spp.

Rosa Guilherme[1], Isabel Abrantes[2], Hermínio C. de Sousa[3], Mara Braga[3], Carla Maleita[3]
1 Regional Directorate of Agriculture and Fisheries of the Center (DRAPC), Avenida Fernão Magalhães, 465, 3000-177 Coimbra, Portugal
2 University of Coimbra, Centre for Functional Ecology - Science for People & the Planet, Associate Laboratory TERRA, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
3 University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, 3030-790 Coimbra, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: agro-industrial residues, crop rotation, fallow, field experiment

ABSTRACT:

Combining healthier and a more sustainable production method, the Organic Production (OP) has been gaining importance, despite the demand for specialized technical knowledge. Data on main crop's enemies and auxiliaries, and sustainable soil and water management are crucial, as they will contribute to the adoption of protection strategies that promote ecosystem equilibrium. In biologically stable soils, populations of non-pathogenic nematodes are much more abundant than plant-parasitic nematodes (PPN) and the losses caused by PPN depend not only on their soil population densities, crop susceptibility, and soil temperature, but also on soil fertility and the balance in the nematode community. PPN management strategies, generally, involve the application of synthetic nematicides, which are not acceptable in OP. Therefore, there has been a growing need to define sustainable strategies based on the use of resistant cultivars, crop rotation, solarization, plants with nematicide properties, biological control, organic content/fertility of the soil (compounds organics, manure/composted organic waste, post-harvest crop residues), among others. In 2021, in two greenhouses (A and B) of the Coimbra Innovation Hub of the Regional Directorate of Agriculture and Fisheries of the Center, a high population density of root-knot nematodes (RKN) was detected, with impact on crop production. In order to find alternatives to nematicides, in accordance with OP guidelines, greenhouse A was divided into four plots with the following cultural schemes: 1) fallow (tomato-fallow), 2) brassica sideration (fallow-*Brassica juncea* L.-tomato), 3) manure incorporation (manure-fallow-lettuce-tomato): and 4) conventional (fallow-lettuce-tomato). In greenhouse B, agro-industrial residues with nematicide properties were applied at four concentrations considering the bioactive compound. After 1 year, in greenhouse A the number of RKN eggs/g root was lower in manure and the total tomato production/plot was similar, compared to conventional and in B, the RKN reproduction decreased 50% at the highest concentration of agro-industrial residues and production was similar compared to the control. The 2nd year is in progress to validate the two strategies that will protect the crops and also the environment.

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134. Population dynamics of stubby root nematodes, associated with ‘Docking disorder’ of sugar beet, in field rotations with cover crops

Nyambura Mwangi[1], Mark Stevens [2], Alistair Wright[2], Simon Edwards[1], Martin Hare[1], Matthew Back[1]
1 Agriculture & Environment Department, Harper Adams University, Edgmond, UK
2 British beet research organisation, Norwich Research Park, Colney Lane, Norwich. NR4 7UG, UK

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: suppression, endophytes, cover crops, reproduction, ectoparasites

ABSTRACT:

Nematodes in the family Trichodoridae (*Trichodorus* and *Paratrichodorus* spp.) are some of the most economically important parasitic nematodes of sugar beet crops in Eastern England. Their feeding on sugar beet roots causes death of the tap root and thickening of the lateral roots, resulting in a fangy/stubby root system, a condition known as ‘Docking disorder’. Commonly used nematicides, such as Oxamyl (Vydate), have been phased out leaving growers with limited management options. This study evaluated the effect of growing and incorporating cover crops (CC) on suppression of stubby root nematodes (SRN). The effect on yield and quality of follow-up crop, sugarbeet was also evaluated. Field experiments were laid out in two sites in England, Bury St. Edmunds, Suffolk and Docking, Norfolk. The CC evaluated were: Indian mustard (*Brassica juncea*), oilseed radish (*Raphanus sativus*), daikon radish (*Raphanus sativus* subsp. *longipinnatus*, endophyte infected (E+) and non-infected (E-) hybrid grass (*Festulolium loliceum*), Italian ryegrass (*Lolium multiflorum*), phacelia (*Phacelia tanacefolia*) and opium poppy (*Papaver somniferum*). At Suffolk, plots drilled with brassica CC’s: Indian mustard and oilseed radish had significantly lower SRN reproduction factor (Rf).

135. A transcriptomic perspective on the effects of temperature on tomato-*Meloidogyne enterolobii* interaction

Duarte Santos[1], Victor Souza[2], Isabel Abrantes[1], Carla Maleita[3], Sebastian Eves-van den Akker[2]
1 University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Associate Laboratory Terra, Departament of Life Sciences, Coimbra, Portugal
2 University of Cambridge, Department of Plant Sciences, Cambridge, United Kingdom
3 University of Coimbra, Chemical Engineering and Renewable Resources for

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: plant-nematode Interaction, degree day model, root-knot nematode, *Meloidogyne enterolobii*, comparative transcriptomics

ABSTRACT:

Understanding the dynamics of plant diseases involves unravelling a complex web of factors acting together. Environmental factors, particularly temperature, play a crucial role in disease outcomes. Heat stress is known to affect plant susceptibility to pathogens, but most studies address short-term exposures, leaving the long-term impact largely unexplored. To address this knowledge gap, we conducted an in-depth study, deploying a degree day model to synchronize the life cycle of the root-knot nematode *Meloidogyne enterolobii* and tomato plants, under two temperature regimes 20-26°C and 26-32°C. Through comprehensive transcriptomic analysis, the gene expression profiles of *M. enterolobii* and tomato plants were analysed in response to temperature. The transcriptomic data revealed substantial differences in the *M. enterolobii* gene expression patterns, under the two temperatures conditions, even though the nematodes were developmentally matches samples. Interestingly, our data also suggest potential similarities in gene expression patterns of tomato plants at 26-32ºC and those infected with *M. enterolobii* at 20-26ºC. These data open new avenues for understanding the relationship between environmental stress and pathogen-induced stress in plants. In summary, our study not only introduces a novel degree day model for studying long-term temperature effects on plant-pathogen *Meloidogyne* sp. interaction, but also significantly contributes to the understanding of how changing environmental conditions impact the dynamics of plant diseases. Our findings emphasize the significant impact of temperature on *M. enterolobii* gene expression and on plant-*M. enterolobii* interaction, providing valuable insights into the complex mechanisms at play in these processes.

136. Transcription factor WOX11 modulates tolerance to cyst nematodes via adventitious lateral root formation

Jaap-Jan Willig[1], Nina Guarneri[1], Thomas van Loon[1], Sri Wahyuni[1], Ivan E. Astudillo-Estévez[1], Lin Xu[2], Viola Willemsen[3], Aska Goverse[1], Mark G. Sterken[1], José L. Lozano-Torres[1], Jaap Bakker[1], Geert Smant[1]

1 Laboratory of Nematology, Wageningen University & Research, 6708 PB Wageningen, the Netherlands
2 National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China
3 Cluster of Plant Developmental Biology, Cell and Developmental Biology, Wageningen University & Research, 6708 PB Wageningen, the Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: tolerance, adaptation, root architecture plasticity, adventitious lateral root formation WOX11, cyst nematode, *Heterodera* schachtii, *Arabidopsis*

ABSTRACT:

A plastic root system helps plants to cope with biotic and abiotic stresses. In *Arabidopsis*, the transcription factor WUSCHEL-RELATED HOMEOBOX 11 (WOX11) initiates the formation of adventitious lateral roots upon mechanical injury of primary roots. Cyst nematodes also induce de novo root organogenesis leading to excessive root branching, but it is not known if this symptom of disease involves mediation by WOX11 and if it benefits the plant. We found with targeted transcriptional repression and reporter gene analyses in *Arabidopsis* that the beet cyst nematode *Heterodera* schachtii activates WOX11-mediated adventitious lateral rooting from primary roots close to infection sites. The activation of WOX11 in nematode-infected roots occurs downstream of jasmonic acid-dependent damage signaling via ETHYLENE RESPONSE FACTOR109, linking adventitious lateral root formation to nematode damage to host tissues. By measuring different root system components, we further found that WOX11-mediated formation of adventitious lateral roots compensates for nematode-induced inhibition of primary root growth. Our observations demonstrate that WOX11-mediated rooting reduces the impact of nematode infections on aboveground plant development and growth. Altogether, we conclude that the transcriptional regulation by WOX11 modulates root system plasticity under biotic stress, which is one of the key mechanisms underlying tolerance of *Arabidopsis* to cyst nematode infections.

137. Cellular dynamics underlying *Globodera pallida* effector RBP-1 recognition and function

Vera Putker[1], Qi Zheng[1], Ana Catarina Silva[1], Erik J. Slootweg[1], Maikel Zerdoner[1], Andelko Zivkovic[1], Octavina C.A. Sukarta[1], Aska Goverse[1]
1 Wageningen University and Research, The Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: plant-nematode interactions, cyst nematodes, plant immunity, virulence targets, host specific resistance

ABSTRACT:

One of the most studied effector genes in the field of plant parasitic nematodes is GpRBP-1 from the potato cyst nematode *Globodera pallida*. This is largely due to the discovery of its matching immune receptor Gpa2 which to date, remains the only matching effector-R gene pair identified for plant-nematode interactions. Although we have gained some understanding of how effector-triggered immunity by Gpa2 depends on the host factor RanGAP2, the cellular mechanisms underlying nematode effector detection by Gpa2 and subsequent activation of plant immune responses remain elusive. Moreover, no knowledge is available about the involvement of RanGAP2-related developmental processes in the subcellular localisation of Gpa2 or GpRBP-1, such as the Ran cycle. The Ran cycle is an energy driven process in which the small GTPase protein Ran is sandwiched between RanGAP2 and RanBP1 to convert to a different energy state, thereby dictating nucleocytoplasmic trafficking. By fusing nuclear and cytoplasmic targeting signals, we demonstrate that the subcellular localisation of the plant immune receptor Gpa2, its nematode effector GpRBP-1, and co-factor RanGAP2 has a precise cellular choreography essential for eliciting a Gpa2-mediated hypersensitive response. Moreover, we show that RanGAP2 inactive mutants have a reduced interaction with GpRBP-1 and that RanGAP2 activity is required for Gpa2-mediated cell death. From our data we present a compelling model wherein GpRBP-1 manipulates the Ran cycle to orchestrate the nucleocytoplasmic trafficking of host cell components, thereby modulating Gpa2-mediated immune responses.

138. G-protein Signalling Promotes the Susceptibility of Plants to a Biotrophic Plant Pathogenic Nematode.

Sharon Letia[1], Shamim M.D. Hasan[1], Florian M.W. Grundler[1]
1 Bonn University, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: G-Proteins, biotrophic nematodes, susceptibility genes, plant-nematode interactions, signalling

ABSTRACT:

The cyst nematode *Heterodera* schachtii is able to establish a syncytial feeding site in the vascular tissue of the host roots. This provides the developing nematode with nutrients for several weeks. Transcriptomic data describing the interactions between the sugar beet cyst nematode *H. schachtii* and *Arabidopsis thaliana* shows that a number of plant G-protein subunits are significantly upregulated in the early stages of *H. schachtii* parasitism. Plant G-proteins and their related elements are associated with abiotic and biotic stress responses that mediate tolerance and resistance to these stress factors. The role of the G-signaling pathway in plant-nematode interactions remains unclear. One of the differentially expressed subunits is the homolog of the alpha subunit, the so-called extra-large G protein 3 (XLG3). Characterization of the XLG triple mutant xlg123 revealed that disruption of these genes has a negative effect on parasitism of H. schachtii, as evidenced by a lower number of females per plant, smaller syncytia, and smaller female size. Analysis of reactive oxygen species (ROS) in the mutant revealed that xlg123 actually has less ROS compared to the wild type, which could explain the lower virulence of *H. schachtii*, as previous work has shown that this species cannot develop in RBOD/F mutants impaired in ROS production. However, infection tests conducted in xlg123 with the root-knot nematode *Meloidogyne incognita* also showed lower virulence of the pathogen, although this nematode is able to develop in mutants impaired in ROS production. While the G-signaling pathway has been shown to be a positive regulator of plant defense against necrotrophic pathogens such as bacteria and fungi, here we provide evidence that the same pathway, or at least some of its components, facilitate biotrophic parasitism of nematodes in *A. thaliana*.

139. Exploring genomic insights into draft genomes of *Heterodera* species

Akshita Jain[2], John Wainer[2], Jacqueline Edwards[2], Brendan Rodoni[2], Timothy Sawbridge[2]
1 School of Applied Systems Biology, La Trobe University, Bundoora, VIC 3083, Australia
2 AgriBio, Centre for AgriBioscience, Agriculture Victoria Research, Department of Energy, Environment and Climate Action (DEECA), Bundoora, VIC 3083, Australia
3 Australian National Insect Collection, National Research Collection Australia, CSIRO, PO Box 1700, Canberra, ACT 2601, Australia
4 Forest Science, NSW Department of Primary Industries, Parramatta, NSW 2150, Australia

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: cyst nematodes, genomics, whole genome sequencing

ABSTRACT:

Cyst nematodes constitute a significant group of plant-parasitic nematodes with a substantial global economic impact. Their defining feature is the female s capacity to retain numerous eggs within its body upon completing its life cycle. These nematodes are notorious agricultural pests and fall into eight genera, with *Heterodera* and *Globodera* being two of the most economically significant ones. Traditionally, the identification of cyst nematodes relied on time-consuming morphological techniques requiring expertise. Consequently, there has been a growing adoption of molecular diagnostic methods.

Whole genome sequencing (WGS) offers insights into unique molecular markers for species differentiation and the mechanisms underlying host invasion. Nonetheless, WGS for cyst nematodes presents challenges due to the difficulty of extracting DNA from individual juveniles. To overcome this challenge, millions of juveniles are combined to generate sufficient data for a high-quality sequenced assembly. So far, only six draft genomes have been sequenced, shedding light on the various biological processes associated with cyst nematodes.

Our research aims to sequence, assemble and annotate draft genomes of cyst nematodes belonging to the *Heterodera* genus recorded in Australia along with a couple of exotics. The primary objective of this genome sequencing initiative is to enhance existing genomic resources, delivering quality, usable data to the field of nematology. This will also enable the development of diagnostic assays for species identification and management purposes.

140. Detection of *Meloidogyne incognita* infection by measuring the root electrical capacitance

Renáta Petrikovszki[1], Fanni Bárányos[1], Gergely Boros[1], Amelita Gerda Molnár[1], Imre Cseresnyés[2]
1 Hungarian University of Agriculture and Life Sciences, Godollo2 Centre for Agricultural Research, Budapest, Hungary

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: chlorophyll content, in situ root methods, non destructive detection

ABSTRACT:

Nowadays, the success of targeted and effective plant protection could be increased by the early detection of pests. It is a key issue for pests in the soil with hidden life strategies, such as the southern root-knot nematodes (*Meloidogyne incognita*). Therefore, early damage of *M. incognita* was examined by non-destructive method in cucumber and tomato plants. The electrical capacitance, electrical conductance and dissipation factor of the root, the chlorophyll content of leaf were measured three times every two weeks, which was followed by the evaluation of the root damage (Mukhtar scale).

The electrical capacitance raised significantly after 2 weeks of *Meloidogyne* infection, which was likely due to the increased surface area due to giant cell formation. The dissipation factor and electrical conductance decreased for the sixth week, which could be caused by restricted root growth and solute uptake by *Meloidogyne* infection. However, there was no measurable change in leaf chlorophyll content.

To summarize, the dielectric measurement could be a promising method for early detection of *Meloidogyne* infection without plant damage, before visible aboveground symptoms.

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141. Nematodes of coffee in Kenya: Challenges and Prospects in the Identification Process

Joseph Maosa[1], Denis Gitonga[2], Zipporah Githinji[1], Huu Tien Nguyen[1], Marjolein Couvreur[1], Laura Cortada[3], Celestine Oduor[4], Danny Coyne[4], Wim Bert[1]

1 Nematology Research Unit, Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium
2 Department of Entomology and Nematology - Fort Lauderdale Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 3205 College Ave., Davie, FL 33314-7719, USA
3 VIB-International Plant Biotechnology Outreach (IPBO), Technologiepark 122, 9052 Ghent, Belgium
4 International Institute of Tropical Agriculture, ICIPE, 30772-00100 Nairobi, Kenya

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: characterisation, identification, integrative, molecular, morphological, nematode, survey

ABSTRACT:

Despite the importance of coffee to the Kenyan economy, and of its prominence across the country's agricultural landscape, the exploration of nematode diversity and the damage they cause within coffee farms remains surprisingly limited. Comprehensive surveys in nine coffee-growing counties in Kenya, involving 53 farms and comprising 158 soil and 156 root samples, revealed a total of 11 genera including *Meloidogyne*, *Tylenchulus*, *Paratylenchus*, *Pratylenchus*, *Rotylenchulus*, *Scutellonema*, *Trophotylenchulus*, *Xiphinema*, *Trichodorus*, *Aphelenchoides* and *Hemicycliophora*. *Meloidogyne* was the most prevalent genus, detected in 90% of the coffee farms with densities up to 346 juveniles 100 cm-3 soil in Kirinyaga and Kiambu counties. Morphological and molecular characterisation revealed several nematode species, the most important being *Meloidogyne javanica*, *M. hapla*, *M. africana*, *Rotylenchulus macrosoma*, *Helicotylenchus* spp., *Pratylenchus goodeyi*, and a putative new *Pratylenchus* species. In addition, this study generated the first 18S, D2-D3 of 28S rRNA, and ITS sequences for *Trophotylenchulus* obscurus. By delving deeper into the characterisation of *Trophotylenchulus* obscurus, a nematode of significant importance yet insufficiently examined, we present a compelling example that highlights both the challenges and opportunities inherent in integrating traditional morphological and molecular methods for nematode species identification. In conclusion, our research illuminates numerous gaps in nematode diversity studies within Kenyan coffee farms, underscoring the crucial importance of addressing this aspect of coffee production. Furthermore, our findings strongly advocate for the implementation of integrative approaches in nematode identification.

142. Comparison of two real-time PCRs to identify *Meloidogyne enterolobii*

Daniel Apolonio Silva de Oliveira[1], Paul Mooijman[1], Tim Warbroek[1], Aron van Duijnhoven[1], Robin du Pré[1], Anne Sophie van Bruggen[1], Evelyn van Heese[1], Gerrit Karssen[1]
1 Netherlands Institute for Vectors, Invasive plants and Plant Health (NIVIP), National Plant Protection Organization (NPPO), Netherlands Food and Consumer Product Safety Authority, The Netherlands

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: *Meloidogyne enterolobii*, molecular diagnostic, real-time PCR

ABSTRACT:

The polyphagous tropical root-knot nematode (RKN) *Meloidogyne enterolobii* is an European Union quarantine species with a high reproductive rate and capacity to break through plant-resistance. Since its description in China in 1983, *M. enterolobii* has been recorded in almost all continents. Prevention of further spread is of great importance. Diagnosis of *M. enterolobii* is challenging, especially because of its morphological resemblance to other common co-occurring tropical RKN species. Therefore, reliable and sensitive molecular identification methods are needed. As NPPO-NL we receive samples from around the world, and accurate diagnosis covering the diversity of *M. enterolobii* is crucial as the Netherlands is a very important entry point and trader of plants in Western-Europe. A validated SYBR green real-time PCR (SBg-RT-PCR) is used at the NPPO-NL to confirm the morphological identification of *M. enterolobii*. However, in 2023 we observed some inconsistencies between morphological and molecular results. Considering that samples may consist of mixtures of RKN species from multiple origins, three basic possible scenarios, but not limited to, are possible: 1) False negative - *M. enterolobii* is present but our SBg-RT-PCR does not cover the intraspecific molecular diversity of *M. enterolobii*, 2) False negative: The dominance of other *Meloidogyne* species masks the presence of *M. enterolobii* in the test, and/or 3) False positive: our current test cross-reacts with other *Meloidogyne* species. To investigate the abovementioned scenarios, pure RKN population cultures from the NPPO-NL collection were selected. Prior to molecular analysis, species identity was confirmed by isozyme electrophoresis. Subsequently, we tested our SBg-RT-PCR and a modified version of the probe-based RT-PCR (PB-RT-PCR) method of Kiewnick et al., 2015 for: 1) Specificity (inclusivity) and sensitivity - Six *M. enterolobii* populations from different origins were used to cover the intraspecific molecular diversity, with different numbers of individuals per PCR reaction (1, 2, 3, 5, 10), 2) Selectivity: Five co-occurring *Meloidogyne* species were used to create spiked DNA mixtures with *M. enterolobii* as background in a ratio of 9:1, to test whether the non-target species dominance would mask the presence of *M. enterolobii* and 3) Specificity (exclusivity): The same co-occurring non-target species were used to test for cross-reaction, causing a false-positive result. Our results and future prospects are discussed.

144. Tools to prevent the spread of root-knot nematode *Meloidogyne luci* in potato

Barbara Gerič Stare, Nik Susič, Uroš Žibrat, Melita Theuerschuh, Andrej Vončina, Janez Lapajne, Saša Širca
1 Agricultural Institute of Slovenia, Plant Protection Department, Hacquetova ulica 17, 1000 Ljubljana, Slovenia

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: RKN, detection, latent infestation, qPCR, hyperspectral imaging, remote sensing, *Meloidogyne luci*, *Meloidogyne ethiopica*, *Meloidogyne inormata*, potato

ABSTRACT:

Meloidogyne luci is a root-knot nematode (RKN) with a broad host range, including important agricultural crops such as potatoes. *M. luci* is known to occur in Brazil, Chile, Guatemala, Greece, Iran, Italy, Portugal, Serbia, Slovenia and Turkey. Due to the serious threat to agricultural production, *M. luci* has been included in the EPPO A2 list of pests recommended for regulation as quarantine pests. *M. luci* is closely related to and very similar to *M. ethiopica* and *M. inornata*, and the three species are collectively referred to as the *M. ethiopica* group. *M. luci* was found in potato fields in Portugal and reproduced in 16 commercial cultivars with high reproduction factor values. Severe damage by RKN species of the tropical group has already been observed in Europe, and due to climate change and rising temperatures, these pests could become a new problem in potato production in temperate regions. We have shown that all three species of the *M. ethiopica* group can parasitize potato plants in an experiment reflecting potato seed production technology. Typical RKN galls were observed on the roots, while some tubers showed surface galls, which look like small, pimple-like swellings on the tuber surface. Other tubers showed no visible symptoms on the surface, although females were detected under the skin of the potato tubers. These were defined as latent infections. The symptoms of potato infection caused by the *M. ethiopica* group were identical to those caused by the two quarantine species *M. chitwoodi* and *M. fallax*. Seed potatoes with latent infection that show no visible symptoms on the tuber surface could pose a major risk for the uncontrolled spread of *M. luci* in Europe. To prevent the spread of *M. luci* and also the closely related species *M. ethiopica* and *M. inornata* (to our knowledge, the latter two have not yet appeared in Europe), we have developed tools to detect the infection of potatoes using hyperspectral remote sensing and real-time PCR methods. Using hyperspectral imaging, it was possible to detect (and differentiate) *M. luci*, *M. ethiopica* and *M. inornata* in the aerial part of the plants (overall accuracy above 85 %) and in infected potato tubers with visible surface galls and in tubers without visible symptoms (i.e. latent infection) with a high success rate (overall accuracy for species identification above 85%). The real-time PCR method, molecular method similar to MeloTuber Test, enabled the detection of *M. luci*, *M. ethiopica* and *M. inornata* with high sensitivity in infected potato tubers with visible surface galls as well as in tubers without visible symptoms (i.e. latent infection). The real-time PCR method is not species-specific. To prevent further spread of *M. luci* in Europe, national pest surveys should be carried out and the tools presented here could be used.

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145. Management of plant-parasitic nematodes with cover crops in rotations with open-field vegetable crops

Lirette M. Taning[1], Louis Lippens[2], Ellen Formesyn[3], Sander Fleerakkers[4], Wim M.L. Wesemael[1]
1 Flander Research Institute for Agriculture, Fisheries and Food ILVO, Merelbeke, Belgium and Ghent University, Department of Plants and Crops, Gent, Belgium
2 Provinciaal Proefcentrum voor de Groenteteelt PCG, Kruisem, Belgium
3 Inagro, Rumbeke, Belgium
4 Proefstation voor de Groenteteelt PSKW, Sint-Katelijne-Waver, Belgium

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: *Meloidogyne chitwoodi*, *Pratylenchus* penetrans, crop rotation, host plant status

ABSTRACT:

Root-lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Meloidogyne* spp.) cause yield loss in many vegetable, arable and horticultural crops. In Flanders, Belgium *Pratylenchus penetrans* and *Meloidogyne chitwoodi* are major constraints for open field vegetable production. Increased use of cover crops without knowledge on how to use them when these nematode species are present can result in population build ups. Moreover, other plant-parasitic nematodes can also benefit and create new problems. Therefore, management using cover crops starts with knowledge on the host plant status. This study evaluated the reproductive potential of *P. penetrans* and *M. chitwoodi* on different cultivars of cover crops in pot tests and field experiments. Twenty-three different cultivars were evaluated against *P. penetrans* and *M. chitwoodi* at low (10 *P. penetrans* or 10 second-stage juveniles (J2) of *M. chitwoodi* per 100 cm^3 soil) and high (100 *P. penetrans* or 100 J2 of *M. chitwoodi* per 100 cm^3 soil) initial inoculum density in a pot experiment under glasshouse conditions. Fallow was used as control. Based on their reproductive factor (Rf) after 8 weeks, they were classified under five different categories: Non-host, Poor host, Maintenance host, Good host, and Excellent host. At both low and high initial inoculum density (Pi) of *M. chitwoodi*, fodder radish 'Maximus', 'Contra', 'Dacapo' and 'Defender', Italian ryegrass 'Meroa', rye 'Matador' and bird's foot trefoil 'Barguay', 'Franco' and 'Lotar' were considered poor hosts reducing the *M. chitwoodi* population. At low Pi for *P. penetrans*, fodder radish 'Defender' and 'Doublet' and birds foot trefoil 'Lotar' were poor hosts. At high Pi for *P. penetrans*, birds foot trefoil 'Lotar' and 'Franco', fodder radish 'Doublemax', 'Maximus' and 'Contra', black oat 'Pratex', phacelia 'Natra', Italian ryegrass 'Meroa' and 'Fedra', rye 'Dukato' and 'Matador' reduced the *P. penetrans* population. Subsequent field experiments showed that the host plant status depends on rotations in which the cover crops are implemented, weather conditions, previous crop grown, the growing period and initial population densities of plant-parasitic nematodes. However, successful rotations were developed to reduce both *P. penetrans* and *M. chitwoodi* populations and these will be discussed.

146. Efficacy of TYMIRIUM® as different soil application methods against plant parasitic nematodes on tobacco, citrus, vegetables, and potato, banana in Africa and the middle east.

Hany Monir[1], Tia Ferreira[2], Driekie Fourie[2]
1 Syngenta Crop Protection AG, Kaha Research Station, Qalubiya, Egypt.
2 Syngenta Crop Protection AG, Block C Westend Office Park, 254 Hall St, Die Hoewes, Centurion, 0157, South Africa

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: TYMIRIUM® technology, Vaniva®, tobacco, citrus, vegetables, banana, potato, nematicide, plant-parasitic nematodes

ABSTRACT:

Africa will contribute more to global population growth than any other region. There is no doubt about the need to increase agricultural efficiency and productivity. To achieve this, cropping systems must become more intensified while improving water-use efficiency, managing elevated pest and disease threats, and boosting the quality and use of external inputs. It is crucial to emphasize the need to address the obstacle of nematodes if world hunger is to be alleviated. Crops are stapled with a designated nematode problem. Feeding by nematodes creates root deformation necrosis and death in spots with high nematode pressure, this leads to root damage and weakened plant anchorage. Infected root systems result in yield loss but when roots become severely damaged, their ability to support plant growth and health in translocating water and nutrients is jeopardized, during adverse climatic conditions such damaged plants topple over with a total loss of yield.

Now, Syngenta has innovated the newest and latest nematicide technology TYMIRIUM® which has been evaluated during the past 10 years in the world and especially in African countries. This soil-applied nematicide showed high efficacy, ranging between 72 to 94%, in reducing population densities of the key nematode pest species in tobacco, citrus, vegetables, and potato under both semi-controlled (tunnel or micro-plot) and naturally occurring environmental conditions (field). In addition, TYMIRIUM® technology controls key fungal diseases like *Fusarium* spp.

147. Plant pathogenic nematodes behave badly: Uncovering the first case of field-relevant nematicide resistance

Christoph Andreas Braun[1], Ralf Nauen[1], Marc Rist[1]
1 Bayer AG, Crop Science Division, R&D, Pest Control, 40789 Monheim am Rhein, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: Plant pathogenic nematodes, nematicide resistance

ABSTRACT:

Chemical nematicides have been widely used to control plant-parasitic nematodes in agricultural fields. To date, there are no substantiated examples of resistance of plant-parasitic nematodes towards commercial agricultural nematicides under natural field conditions. This presentation aims to report the first case of field-relevant nematicide resistance, focusing on the impact of this resistance on nematode management. By identifying and understanding the underlying mechanisms of nematicide resistance, this research seeks to provide valuable insights for the development of integrated pest management strategies and the sustainable use of nematicides in agriculture.

148. Efficacy of TYMIRIUM® technology in protecting row crops against key nematode pests

Benedikt Kurtz[1], Hany Monir[2], Driekie Fourie[3], Tia Ferreira[3]
1 Syngenta Crop Protection AG, Stein Research Station, Switzerland
2 Syngenta Crop Protection AG, Kaha Research Station, Egypt
3 Syngenta Crop Protection AG, Centurion, South Africa

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral

Keywords: TYMIRIUM® technology, Victrato®, corn, wheat, soybean, nematicide, plant-parasitic nematodes

ABSTRACT:

Infection and parasitism by economically important plant-parasitic nematode genera such as root-knot (*Meloidogyne*), lesion (*Pratylenchus*) and cyst nematodes (*Heterodera*) adversely impact global grain production. To mitigate yield and quality losses of row crops, mostly grown in rotation in African cropping systems, is challenging due to the wide host and high pathogenicity levels of particularly root-knot and lesion nematodes, concomitant occurrence of nematode-microbe complexes, and adverse climatic conditions, amongst others. Addressing the need to develop a nematicide with superior action against key nematode pests of row crops coupled with a favorable profile to the environment and non-target organisms, TYMIRIUM® technology acts through contact and feeding activity and potentially above-ground nematodes through systemic feeding activity. It has been evaluated during the past 10 years in major row crops producing areas of the world and especially in African countries. TYMIRIUM® technology showed high efficacy, in reducing population densities of the key nematode pest species in maize (corn), soybean and wheat under both semi-controlled (tunnel or microplot) and natural occurring environmental conditions (field). In addition, the adverse effects of TYMIRIUM® technology on key fungal diseases were also recorded. Results about the efficacy of TYMIRIUM® technology as a nematicide, as well as added benefits as a fungicide, and how it can be used to sustainably produce grain crops by not impacting negatively on soil health will be presented.

149. Development of integrated *Meloidogyne* spp. management with digital support: a case study

Josep Izquierdo Casas[1]
1 Bayer Crop Science S.L Av. Baix Llobregat 3-5. 08970 Sant Joan Despi (Barcelona)

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: protected vegetables, degree days, biological nematicides, solarization, Nematool, seasonal management program

ABSTRACT:

The management of nematodes, basically *Meloidogyne* spp, in protected vegetables in the EU-Mediterranean countries is challenged by the loss of conventional tools currently used by farmers, such as chemical nematicides and fumigants. The present EU regulatory scenario calls for innovation in the European nematode management. There is a lot of knowledge and potential alternative tools available, but how can these be used to significantly promote an evolution of the commercial cropping practices to a highly integrated & sustainable nematode management - maintaining the competitiveness of the European farmers on the global market' With its innovative synthetic and biological based nematicides, respectively Velum® and BioAct®, Bayer is an active player in nematode management and development of tailored solutions, combining chemical and biological products for higher sustainability and reduction of the environmental impact.

Soil temperature is a key factor driving the dynamics of the *Meloidogyne* cycle. Based on degree day models Bayer developed and paired autonomous soil probes with a user-friendly digital app called Nematool® to direct optimal timing of nematode management measures at field level. Nematool® is commercially available in several Mediterranean countries and supports the farmers in understanding their problems in the soil, as well as in optimizing solarization management and the positioning of the timing of nematicide applications.

Combining nematode management measures (innovative nematicides, solarization, crop resistance, microbiome management & biostimulation...) with this data driven decision system of Nematool® leads to a win-win situation for both the farmer and the environment, maximizing yields, as well as improving sustainable crop management.

150. Complex plant-microbe interactions determine the performance of root-knot nematodes on plants

Olivera Topalovic[1], Mette Vestergaard[2], Stefan Geisen[3], Flemming Ekelund[1]
1 Department of Biology, University of Copenhagen, Copenhagen, Denmark
2 Department of Agroecology, Aarhus University, Slagelse, Denmark3 Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: root-knot nematodes, microbiomes, *Pseudomonas protegens* strain CHA0, amplicon sequencing, gene expression, plant species

ABSTRACT:

Plants are in a continuous communication with the rhizosphere which is comprised of plant-beneficial and plant-deleterious organisms. Among these are plant-parasitic nematodes as the most devastating plant parasites which are extremely difficult to control. Nonetheless, plants rely on its microbiome in the rhizosphere whose composition can highly influence how plants interact with plant-parasitic nematodes. Here, we followed whether the bacterium *Pseudomonas protegens* strain CHA0 affects the performance of the root-knot nematode *Meloidogyne incognita* by modulating the microbiome in the rhizosphere and endosphere of tomato plants or by interfering with the transcriptional response of the plants. We found that *P. protegens* CHA0 significantly induced plant growth and nematode performance as seen through improved invasion and reproduction of nematodes in the treatments with *P. protegens* CHA0. Using amplicon sequencing of the 16S rRNA and ITS genes, we showed an enrichment or depletion of specific microbial taxa including *Bacillus*, *Pseudomonas*, *Algoriphagus*, *Sphingomonas* and others in tomato rhizosphere and endosphere in response to *P. protegens* CHA0 and *M. incognita*, suggesting that complex plant-microbe interactions take place and affect nematode performance on plants. In addition, we recorded a higher expression of plant PTI-responsive genes, TFT1 and GRAS, in response to *P. protegens* CHA0 compared to its absence, which may indicate a trade-off between plant growth and defense against *M. incognita*. We also used amplicon sequencing to study which microbiomes are present in the galls of *M. incognita* on plant species including tomato, cucumber, soybean, salad, and cabbage. We aimed at deciphering which microbial taxa associate with nematodes on different plant species and whether some taxa are shared among good and bad plant hosts. Overall, our studies show new insights into plant-nematode-microbe interactions and contribute to the better understanding of microbial role in the life cycle and performance of root-knot nematodes.

151. Soil bacterial, fungal, and nematode community structures and co-occurrence network structures in coniferous plantations and broadleaves secondary forests

Yudai Kitagami[1], Yosuke Matsuda[1]
1 Graduate School of Bioresources, Mie University, Mie

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: co-occurrence network, *Cryptomeria japonica*, metabarcoding, *Quercus serrata*

ABSTRACT:

Nematodes are a major group of soil microfauna playing crucial roles in ecosystem processes, such as facilitating soil carbon and nitrogen cycling by feeding bacteria and fungi. In forest ecosystems, the alteration of management programs from coniferous plantations to broadleaves secondary forests are likely affect the structure and diversity of soil nematode communities and interactions with other soil organisms. The network analyses have been proven to be a powerful tool for understanding the ecological characteristics of co-occurrence patterns, unveiling potential interactions of nematodes with other soil organisms. This study aimed to determine the effects of forest types on soil bacterial, fungal, and nematode community structure, biodiversity, and co-occurrence networks. Surface soils (10 cm in depth) were collected from 1 ha areas at neighboring two forest types: coniferous plantations composed of 50-years-old *Cryptomeria japonica* (Cupressaceae) and broadleaves forests dominated by *Quercus serrata* (Fagaceae). The soil bacteria, fungi and nematode taxa were determined using a high throughput sequencing of the MiSeq system. As a result, the community structure of bacteria, fungi and nematodes was significantly different between the forest types. Bacterial diversity was not significantly different between the forest types, but the diversity in fungi and nematodes was significantly higher in coniferous plantations. The co-occurrence network structures of bacteria, fungi and nematodes in broadleaves forests had higher edge numbers than those of coniferous plantations. Moreover, the network patterns of fungi and nematode showed that saprotrophic fungi and symbiotic ectomycorrhizal fungi were mainly co-occurred with fungivorous nematodes between the coniferous plantations and broadleaves forest, respectively. These results suggest that bacteria, fungi and nematodes were interlinked, which shaped unique network structures between coniferous plantations and broadleaves forests.

152. The biocontrol strategy based on the hatching stimulation of *Heterodera* carotae is influenced by the soil microbiota selected by previous crops

Marine Biget[1], Nathan Vannier[1], Kévin Gazengel[1], Bruno Marquer[1], Juliette Linglin[1], Christophe Langrume[1], Catherine Porte[1], Sylvain Chereau[1], Didier Fouville[1], Sylvain Fournet[1], Lionel Lebreton[1], Josselin Montarry[1]
1 Igepp - Inrae - Le Rheu, France

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: cyst nematodes, suicide hatching, soil microbiota, carrot, agronomic system

ABSTRACT:

Cyst nematodes are major crop pests, causing severe economic losses for farmers. Among the pest management alternatives, the suicide hatching strategy, which aims to mislead the nematode by using host specific root exudates, has already proved its efficiency on several cyst nematodes including *Heterodera carotae*. However, previous studies have highlighted several constraints, one of which being the impact of soil microbial communities on suicide hatching efficiency. Indeed, plants secrete root exudates to recruit beneficial microorganisms which provide major ecological functions and help the plants to face biotic and abiotic stresses. Root exudates are thus a signal recognized by microorganisms in the soil but also by cyst nematodes to begin their parasitic cycle. In this study, we assessed the efficiency of the suicide hatching strategy depending on its positioning in the agronomic system. Indeed, potential variations in efficiency are expected due to the specific composition of the soil microbiota selected by the different plant species likely to be cultivated in rotation with the carrot. To do so, we first grew for 3 cycles of 3 weeks, under controlled conditions, three plants (leek, turnip and barley) usually cultivated with the carrot, in 2 distinct soils (sandy and muddy soil). Bags containing 12 *H. carotae* cysts were added to these microbial-enriched soils before applying the biocontrol solution (carrot root exudate) at two doses and in the presence or absence of the corresponding plant to compare the hatching efficiency of an application of the product during or following the cultivation. At the end of the experiment, we calculated the hatching rate and analysed the soil microbiota composition and structure using a metabarcoding approach targeting bacteria and fungi. We clearly demonstrated a specialisation of the soil microbiota depending on the rotation plant at the end of the 3 cycles of growth. Moreover, in the sandy soil, we observed clear patterns with a greater hatching rate when the plant is present compared to the bare soil and the highest rate when the barley is cultivated. Further statistical analyses should enable us to attribute or not the hatching rate variations to the microbial communities and to identify favourable and/or unfavourable microorganisms specific to the barley or to the leek, respectively. Taken together, our results will allow us to propose the best positioning of the biocontrol product in the technical itinerary by considering the soil microbiota.

153. On the remarkable richness of antagonists in a conventional arable soil, and why it doesn't translate yet into increased plant-parasitic nematode suppression

Sara G. Cazzaniga[1], Sven van den Elsen[1], Carin Lombaers[3], Johnny Visser[2], Leendert Molendijk[2], Joeke Postma[3], Liesje Mommer[4], Johannes Helder [1]
1 Laboratory of Nematology, Wageningen University & Research, Wageningen, The Netherlands
2 Wageningen Research, Field Crops, Wageningen University & Research, Lelystad, The Netherlands
3 Biointeractions and Plant Health, Wageningen University & Research, Wageningen, The Netherlands
4 Forest Ecology and Forest Management Group, Wageningen University & Research, Wageningen, The Netherlands

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Meloidogyne chitwoodi*, *Pseudomonas*, *Arthrobotrys*, nematophagous microorganisms, plant-parasitic nematodes

ABSTRACT:

Plant-parasitic nematodes are among the most harmful pathogens of cultivated crops causing important yield losses. Most root-knot nematodes (*Meloidogyne* spp.) are highly polyphagous endoparasites and are notoriously hard to control. Introduction of nematode antagonists has been considered a potential strategy to curb root-knot nematode proliferation, but - unless they are applied in a highly localized manner (for instance in seed coat) - this strategy often failed due to the highly competitive nature of this habitat. An alternative approach would be to stimulate indigenous nematode antagonists.

To test this, we performed a field experiment in which three densities of the pathogenic Columbia root-knot nematode *Meloidogyne chitwoodi* were generated. We mapped the effects of 10 cover crop treatments against these backgrounds on both the resident (DNA) and the active (RNA) fraction of the bacterial and fungal communities, with a specific focus on those referred to in literature as nematode antagonists. Generally, cover crops had a stronger impact on microbial genera harbouring nematode antagonists than *M. chitwoodi* density. Among the eight bacterial and 26 fungal genera known from literature to harbour antagonists of plant-parasitic nematodes, respectively five and thirteen were detected in our experiment. Among the five bacterial genera, four included bacterial species for which nematode antagonism has been documented. The fungal genera included facultative nematode parasites (e.g., six *Arthrobotrys* species), endophytes strengthening host defences (e.g., four *Acremonium* species), as well as multiple obligatory nematophagous species including *Haptocillium balanoides*, *Hirsutella vermicola*, and *Drechmeria coniospora*.

This study revealed that conventionally managed arable field mght harbour an unexpected diversity of nematode antagonists. However, the richness in putative nematode antagonists did not translate in *M. chitwoodi* suppression presumably because most antagonists have a facultative nematophagous lifestyle and will only predate nematode under poor nutritional conditions. Recruitment of this native antagonistic potential would require a lowering of the nutritional status

of the soil, or - alternatively - a manipulation of antagonists in a way that they *perceive* their environment as being low in (especially) nitrogen.

154. Unraveling the influence of earthworms, cutaneous excreta, and entomopathogenic nematodes on the plant-soil ecosystem: a holistic approach

Maryam Chelkha[1], María del Toro Hernández[2], David Labarga[3], Rubén Blanco-Pérez[3], Alicia Pou[3], Kyle Wickings[1], Raquel Campos-Herrera[3]

1 Department of Entomology, Cornell University, Cornell AgriTech, 15 Castle Creek Drive, Geneva, 14456, NY, USA

2 Genomics & Bioinformatics Core Facility, Center for Biomedical Research of La Rioja (CIBIR), C/ Piqueras 98, 3rd floor, 26006 Logroño, Spain

3 Instituto de Ciencias de la vid y del Vino (ICVV), Gobierno de La Rioja, CSIC, Universidad de La Rioja, Finca La Grajera Crta. Burgos Km. 6 Salida 13 Lo-20, Logroño, 26007, Spain

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: microbiome, next-generation sequencing, *Steinernema*, *Eisenia*, tomato, sustainable agriculture, biocontrol

ABSTRACT:

Entomopathogenic nematodes (EPNs) belonging to Steinernematidae and Heterorhabditidae effectively suppress various insect pests, making them invaluable components of sustainable agricultural practices. Earthworms are crucial to soil ecosystems due to their burrowing and feeding activities, improving soil aeration, enhancing porosity, and aiding in nutrient cycling. These activities are essential for maintaining soil structure, fertility, and the overall well-being of terrestrial environments. Our understanding of the interactions between EPNs and earthworms remains limited, with varying effects observed across different species and populations. This has implications for the overall functioning of plant-soil-biocontrol systems, underscoring the need for further investigation. This study sought to investigate the impact of earthworms and their cutaneous excreta (CEx), individually or in combination with EPNs on the soil-plant system. Using a mesocosm approach with tomato plants and field soil, we evaluated the influence of earthworms, their CEx, and EPNs at varying application times. Our methodology encompassed next-generation sequencing to analyze bacterial communities, bait tests to evaluate EPN infectivity, and soil analyses to assess nutrient availability. Furthermore, we monitored the physiological parameters of the plants to capture a holistic perspective. Our results revealed an increase in the infectivity four weeks post-treatment and a positive effect on the larva-producing offspring. However, while none of the treatments significantly influenced the soil microbial population, we found a significant impact of *Steinernema feltiae* and the combination *S. feltiae* and *E. fetida* on plants fresh weight. Moreover, there was a negative effect on soil nutrient dynamics, particularly magnesium for the *S. feltiae* and CEx combined treatment. In our study, we highlight the timing post-inoculation as one of the crucial factors to consider when assessing the specific earthworm/EPN interaction within the plant-soil system.

155. Long-term suppression of *Meloidogyne* in organic fields in north-eastern Spain

Ariadna Giné[1], Mar Cendra[1], Alejandro Expósito[1], Aida M. Fullana[1], Heuer Holger[2], Francisco Javier Sorribas[1]

1 Department of Agri-Food Engineering and Biotechnology (DEAB), Barcelona School of Agri-Food and Biosystems Engineering (EEABB), Universitat Politècnica de Catalunya, BarcelonaTech (UPC), Castelldefels, Spain

2 Department Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut-Federal Research Centre for Cultivated Plants, Braunschweig, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: suppressive soils, *Meloidogyne*

ABSTRACT:

Soils contain a diversity of nematode antagonists able to suppress root-knot nematodes. In 2012, a soil sampling was conducted through northern Spain to detect fungal egg parasites of *Meloidogyne* spp. (RKN) in 40 vegetable sites conducted under organic and integrated standards. Fungal egg parasites were identified in all organic and 73.3% of the integrated sites. The egg parasitism exceeded 40% in some fields being greater in organic than integrated soils. Among all the identified fungal species, *Pochonia* chlamydosporia was the most frequent and the only one positively related to the percentage of parasitism. Then, two organic fields were chosen to characterize RKN suppressivity. The fluctuation of RKN densities and the fungal egg parasitism during three cropping seasons were determined and pot experiments were conducted to certify the soil suppressiveness. Both soils showed high levels of fungal egg parasitism, basically due to P. chlamydosporia. Using DGGE, genetic microbial profiles differentiated suppressive from conducive soils. Due to these results, more fields under organic and integrated standards were monitored and fungal egg parasites were recovered from all of them, being P. chlamydosporia the only fungal species isolated in 5 out of 6 of the six sites, the same one that showed their suppressiveness in pot experiments. The microbiome of these soils associated with the infective RKN stage was studied by DGGE. A deep amplicon sequencing of two organic soils with similar community profiles, one of them strongly suppressive towards RKN, indicated a difference in their microbiomes. Four fungal OTU of the family Plectosphaerellaceae and one assigned to *Malassezia globosa* were much more abundant on the nematode from the most suppressive soil and only one bacterial OTU, most similar to *Neorhizobium galegae*. Under these results, six fields subjected to regenerative techniques for over 5 years without RKN history were studied. Soil suppressiveness in pot experiment inoculated with RKN was confirmed in 3 out of 6 soils. In the two most suppressive soils, the microbial activity and the soil microbiome were studied. Some microbial taxons and their abundance were affected by the different regenerative practices. Regarding this finding, growers will be aware of the enhanced antagonistic potential of the soil against RKN with the transition of agricultural practices to more sustainable and regenerative production systems.

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156. Biopesticides for plant parasitic nematodes: Screening essential oils and volatiles

Jorge M S Faria[1], Pedro Barbosa[3]

1 INIAV, I.P., National Institute for Agrarian and Veterinary Research, Quinta do Marquês, 2780-159 Oeiras, Portugal ,

2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

3 MED, Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Évora University, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Oral
Keywords: plant parasitic nematodes, volatiles, nematicides, plant health, sustainable pest management

ABSTRACT:

Essential oils (EOs) are complex mixtures of low molecular weight terpenes and phenylpropanoids that have been often tested against plant parasitic nematodes (PPN) due to their advantages for formulating more sustainable biopesticides. They are generally easily accessible, show high activities, lower environmental impacts due to their biodegradability, and are subjected to less strict regulatory approval mechanisms. Plant parasitic nematodes are responsible for large economic and ecological losses in modern agriculture and forestry. Pest control is commonly performed by resorting to synthetic pesticides, however, these chemicals can be extremely harmful to non-target organisms and have been phased-out, due to serious environmental and public health concerns. Thus, as a contribution to the development of effective biopesticides based on EOs, the present work reviews the existing literature on the chemical composition of the EOs with the highest direct biological activity against the most damaging PPNs, e.g., the root-knot nematodes (RKNs), the plant cyst nematodes (PCNs) or the pinewood nematode (PWN). Reports on the activity of EOs on the RKNs and PWN are more numerous, while data on PCNs is scanter. The highest activities were reported for EOs rich in phenylpropanoids, e.g., cinnamaldehyde and derivatives, and monoterpenoids, e.g., carvacrol, citronellal, citronellol, geranial, geraniol or neral. Chemicals with electronegative elements, e.g., oxygen (O) or sulphur (S), seem to impart a higher nematicidal potential to the EO. Further research will allow uncovering EOs and volatiles with directed activities against different PPNs and, thus, outlining *greener* pest management strategies.

157. The potential use of terpenes for the control of *Meloidogyne javanica*

Eleni Nasiou[1], Ioannis Giannakou[1], Dionysios Dinokas[1], John Vontas[1]
1 Agricultural University of Athens, Athens, Greece

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: botanical nematicides, terpenes, root-knot nematodes, nematodes management, mode of action

ABSTRACT:

Root-knot nematodes *Meloidogyne* spp. are a major problem reducing greenhouse vegetable's production in Greece. The last two decades many experiments have been conducted using either essential oils or their main constituents, such as terpenes, against different genera of plant parasitic nematodes. The present study aims to summarize the findings of a series of trials to record the nematicidal activity of four terpenes (carvacrol, thymol, geraniol, and eugenol) on different life stages of the root-knot nematode *Meloidogyne javanica* at concentrations of 30 to 1000 µl/L. Overall the percentage of J2s paralysis was irreversible and dose dependent. All tested terpenes considerably inhibited eggs differentiation (from 35 to 77%) at a concentration of 1000 ppm. Also, a substantial hatching inhibition was recorded at concentrations of 125 to 1000 ppm. Additionally, terpenes have shown a nematicidal activity against J2s in soil, reducing juveniles' survival up to 99% at the concentration of 1000 ppm. However, no vapor action was recorded for any of the terpene tested. Carvacrol was the most effective terpene followed by geraniol, thymol and eugenol in pot trials using tomatoes. Different mixtures of terpenes were tested showing some interesting results as far as effectiveness against J2s is considered. Particularly the mixtures of binary terpenes exhibited higher nematicidal activity in lower doses compared to the individual activity of each terpene alone at the same doses. We believe that terpenes could be a valuable tool for nematode control in an IPM program but, based on our results, we could conclude that determining the most efficient rate and dose of terpenes is not an easy task and further experimental work is needed. In addition to the effectiveness trials, we are currently evaluating the mode of action of these terpenes against *Meloidogyne* sp., using comparative transcriptomic analyses on nematode samples subjected to treatments with sublethal doses and the results will be discussed.

159. An overview of new nematicides against nematodes

Fariba Heydari[1]
1 Department of Plant Protection, Faculty of Agriculture, Isfahan University of Technology, Iran

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords:

ABSTRACT:

Nematodes cover about 90% of all multicellular organisms, and they represent a total biomass of approximately 0.3 gigatons. The parasitic forms infect almost all living creatures. The plant parasitic nematodes (PPNs) are a group of pests known as the pests, hard to manage. Nematicides are widely used in agriculture, however, there are several issues linked to their use. First, many drugs lose their effectiveness after repeated use because of resistance. Second, the impact of nematicide treatment at large scale is huge on the long term. Thus, it is urgent to develop safer and more effective new nematicides. New nematicides that exhibit lower toxicity to vertebrates have been developed. These include fluopyram, fluensulfone, fluazaindolizine and cyclobutrifluram. Fluopyram was first developed as a fungicide and inhibits the function of succinate dehydrogenase (SDH), an enzyme that is essential to the mitochondrial respiratory chain in nematodes. Fluensulfone was shown to reduce root infection and plant penetration by PPNs. Although tested for their efficacy against PPNs, some of the new nematicides such as fluazaindolizine require additional studies to characterize the mode of action. Furthermore, fluazaindolizine, did not show any deleterious effects on different stages of C. elegans and Drosophila melanogaster at examined concentrations, but exhibited good activity on plant-parasitic nematodes. Regarding cyclobutrifluram, in personal observations we found the impact of cyclobutrifluram on the reproduction and development of C. elegans but also the previously unknown molecular action of this nematicide that was not known so far. This study will contribute to further evaluating the potential resistance of nematodes against nematicides and to plan the elaboration of new drugs against PPNs. Also in RNA sequencing, genes encoding cytochrome P450s were also shown to be deregulated in *Meloidogyne incognita* upon treatment with various nematicides, including fluopyram the same as cyclobutrifluram. Some nematicide resistant C. elegans strains were generated in the lab by mutagenizing the worms. However, these mutations could potentially also arise in PPNs living in treated soil. Overall, published and personal observations indicate that these new nematicides are generally more effective against C. elegans as well. Due to recent advances in genomic and proteomic studies, the nematicide development process may change from screening chemical libraries in vitro to computerized molecule screening and design for some target nematode species. Such a change might reduce the time and cost of development and increase the nematode-control efficacy, and may even lead to the ideal nematicide.

160. Spent coffee grounds extract repels second-stage juveniles of *Meloidogyne* species and inhibits their infection

Yuji Oka[1]
1 Gilat Research Center, Negev, Israel

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: control, *Meloidogyne*, nematode repellent, spent coffee grands

ABSTRACT:

Second-stage juveniles (J2) of *Meloidogyne* species are known to be repelled by several compounds. Although such repellents could be a possible means for nematode control, effective, practical, and sustainable repellents have yet to be discovered. The present study tested the repellency effect of a methanolic spent coffee grounds (SCG) extract against J2 of three *Meloidogyne* species, *M. javanica*, *M. hapla*, and *M. marylandi*, on an agar plate. The infection-inhibitory effect of the extract was also evaluated with lettuce seedlings using *M. javanica*, *M. hapla*, and *M. incognita*. The SCG extract repelled *M. javanica*, *M. hapla*, and *M. marylandi* J2 on the plate. When the SCG was mixed with a chemical J2 attractant, its attractiveness for *M. javanica* and *M. hapla* J2 was reduced. The attraction and infection of *Meloidogyne* J2 to lettuce seedlings were tested in an 8.5cm Petri dish by adding the SCG extract near the seedling roots on the agar plate, while control seedlings on the same plate were not received. The SCG extract deposited near lettuce seedling roots reduced the number of *M. javanica*, *M. incognita*, and *M. hapla* J2 attracted to the root tips and the infection rates of the seedlings, and increased the root length compared to control seedlings grown on the same agar plate. The SCG extract mixed into the agar plate in a 5.5 cm Petri dish reduced the number of *M. javanica*, *M. incognita*, and *M. hapla* J2 attracted to lettuce root tips and the lettuce infection rate and increased the root length of the seedlings compared to control seedlings. No nematicidal or immobilizing effect on *Meloidogyne* J2 was found in the extract at concentrations used in the experiments. The results suggest that the SCG extract has repellent and infection-inhibitory activities on the *Meloidogyne* J2 and is a potential control means for *Meloidogyne* species.

161. Plant-parasitic nematodes use SWEETs to obtain their resources

Chris Bell[1]
1 School of Biology, Faculty of Biological Sciences, University of Leeds, UK

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: *Globodera pallida*, feeding, resources

ABSTRACT:

Plant-parasitic nematodes have evolved sophisticated mechanisms to enable successful feeding from root tissue. For sedentary nematodes, feeding is enabled by the formation of specialised feeding sites. Plant Sugars Will Eventually be ExporTed (SWEET) proteins facilitate the movement of carbohydrates with recent studies reporting their role in resourcing feeding sites. Here, we have shown that *Globodera pallida* express an array of SWEETs, with GpSWEET3 expressed within the intestine and during feeding stages. We report an increase in the expression of GpSWEET3, a likely hexose transporter, in nematodes that are receiving a reduced supply of carbon-based resources, as a possible ameliorating mechanism. SWEET3 is present in all major plant-parasitic nematode genomes. Knockdown of this gene reduced nematode growth and development up to 28dpi. Additionally, RNAi increased the hexose content of the nematodes, possibly by reducing sugar export out of the intestine and subsequent breakdown. HBL1 is a transcription factor that has spatial and temporal expression overlap, and interacts with GpSWEET3, potentially through several identified promoter motifs. Knockdown of GpHBL1 elevated expression of GpSWEET3, indicating a negative regulatory function. Overall, this suggests that these nematodes utilise SWEETs to enable assimilation of root-derived solutes and that their expression is negatively regulated through HBL1 and responsive to food composition.

162. Shared and distinct host pathways to nematode infection through multi-omics

Peter DiGennaro[1], **Shova Mishra**[2]
1 University of Wisconsin-Madison, USA
2 University of Florida, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: genomics, parasitism, genes,

ABSTRACT:

Advances in -omics technologies have made large scale studies more accessible, and an increase in available bioinformatic tools have rendered such big data more tractable. Transcriptomic data analyzed independently, at the experimental level only, often provides near-sighted inferences into gene-trait associations, such as generating lists of candidate genes for a specific system under explicit parameters. Further, thousands of plant parasitic nematode genes have no shared homology within protein databases, leaving inferred biology from single -omics datasets inherently flawed. Here, we gathered numerous RKN-host transcriptome datasets from the public domain and developed algorithms that specifically tackle cross-species interactions between host and nematode. After assessing the structure and distribution pattern of all the data, we curated the datasets for quality and generated data formats required for multi-omics downstream analyses. E*luci*dating commonly induced plant pathways among different hosts could be a target for nematode management while distinct pathways could help describe nematode host ranges. Differentially expressed plant and nematode genes were identified and cross-species gene networks were developed to identify novel gene pathway associations.

163. Nematodes need a water film to move in the soil? Wrong, a misinterpretation of experiments on nematode movement in soil monolayers

Ralf-Udo Ehlers[1]
1 Biocontrol Biotech Consult, Duesternbrook 1, 24211 Rastorf, Germany

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: nematode movement, water potential, pores, relative humidity

ABSTRACT:

All textbooks tell us that nematodes need a water film to move in the soil. This talk will show that this is a wrong conclusion of experiments conducted in the 1960s. Movement of nematodes in the soil depends on their biology, mobility, tolerance to environmental factors (soil moisture, temperature), the edaphic parameters and the soil water dynamics. The free-living stage of EPNs, for instance, is the 3rd juvenile stage, the so-called dauer larva (DL). Due to its diameter of 25-43 µm (depending on the species), it can only move through coarse soil pores (defined at > 10 µm diameter). Considering that nematode can only move when these pores are lined with water, movement would be impossible once these pores are dry. These pores are dry at a water potential (pF) < 2.5. Would DJs only be able to move on a water film, infestations of insects by EPN would be impossible when coarse pores are dry. However, results of field trials show that control was obtained at lower values. The talk will explain why the assumptions based on previous experiments are wrong, based on a misinterpretation of observations of nematode movement in soil particle monolayers. Up to the permanent wilting point of plants the relative humidity in pores is above 98%, enough for DL to move. Whether this conclusion is also applicable to nematodes, like PPN that do not form dauers needs to be investigated.

Nematode do not need a water film to move, movement is possible even in dry pores as relative humidity is at 98% up to the permanent wilting point of plants.

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164. Defining a tissue-independent response to nematode parasitism.

Olaf Kranse[1], Siyuan Wei[1], Beatrice Senatori[1], Jie Zhou[1], Ji Zhou[1] and Sebastian Eves-van den Akker[1]
1 The University of Cambridge, UK

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Oral
Keywords: aerial infection, susceptibility

ABSTRACT:

Cyst nematode parasitism is established partially through the alteration of gene expression in a host cell, resulting in physiological alteration of root tissue. A specific pattern of expression alteration defines the feeding site, some of which will be the ‘core’ to parasitism (i.e. susceptibility genes). Understanding the biology of susceptibility genes has intuitive routes to application in agriculture.

In *Arabidopsis thaliana*, ~ 5,500 genes are differentially regulated over the course of infection by the model cyst nematode *Heterodera schachtii*. To screen mutants in all these genes for susceptibility, using the current state-of-the-art susceptibility screening protocol, would take at least seven years.

In this work, we exploit for utility the under-appreciated fact that these nematodes can also induce feeding site formation in aerial parts of the plant. By cross-referencing RNA-seq data from infected roots and infected shoots we define a tissue-independent response to parasitism, substantially reducing the number of putative susceptibility genes.

To accelerate screening further, we developed a novel phenotyping platform combining low-cost and open-source 3D printed hardware with computer vision and deep-learning software. We use this novel capacity to conduct an exhaustive screen of mutants in genes that are differentially regulated by the parasite, independent of tissue. Susceptibility genes identified in this way represent attractive targets to engineer resistance using genome.

165. Sensory choices may be influenced by host imprint in the root-knot nematode *Meloidogyne incognita*

Sophie Mantelin[1], Victoria Faivre[1], Olivier Pierre[1], Martine Da-Rocha[1], Mathis Pochon[1], Karine Robbe-Sermesant[1], Silène Lartigue[1], Cyril Van-Ghelder[1], Bruno Favery[1]
1 INRAE-Université Cote d Azur, Sophia Antipolis, France

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: chemotaxis, host imprint, *Meloidogyne incognita*, perception, plant parasitic nematodes

ABSTRACT:

Soil-born plant pathogenic nematodes infest roots, causing severe damage to crops. Nematodes need their host to feed and/or reproduce. Finding the plant is therefore the first and essential prerequisite for those pathogens to establish parasitism. Infective nematode larvae can perceive plant-derived chemical signals in soil and follow the gradients towards the roots. This chemotactic response must be conditioned by the sensory capacity of the nematodes. Using acclimatised populations of nematodes based on experimental evolution of the model *Meloidogyne incognita* on two distant hosts (tomato and fig tree) and a dynamic chemotaxis monitoring system, we observed that the rearing host plant deeply impacts the nematode behaviour towards root extracts. Concomitantly, RNAseq analysis of these populations indicated that the host significantly imprints the nematodes’ transcriptional landscape. This host effect may modulate the nematodes’ sensory capacity and parasitism efficiency. In the long term, one can wonder how building knowledge of nematode chemical ecology can benefit the development of control strategies towards these pests in the field.

166. From genes to galls: exploring molecular mechanisms of plant-root knot nematode interactions

Cynthia Gleason[1]
1 Washington State University, USA

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: *Meloidogyne*, callose, PTI, potato

ABSTRACT:

Meloidogyne chitwoodi is a root-knot nematode found in potato growing areas of the Northwestern USA. It is a nematode that can infect both potato roots and tubers, and in the case of tuber infections, it causes small pimple-like blemishes on the skin, giving the potato a rough, bumpy appearance. Our goal has been to identify the molecular mechanisms that allow *M. chitwoodi* to initiate and maintain infection in the host. By using combinatorial approaches in studying effectors and plant defense, we want to develop more sustainable control measures and establish new ways to protect potato crops and maintain value for producers. Here we will present the status of research on innovative potato control measures being developed for root-knot nematodes, including *M. chitwoodi*, in the USA. In addition, we will present the results of whole juvenile and esophageal gland transcriptome analyses of pre-parasitic *M. chitwoodi*. This data, combined with in situ hybridization data, has allowed us to home in on gland-localized genes that are specific to root-knot nematodes, including the effector gene *Mc265*. Transgenic *Arabidopsis* plants constitutively expressing *Mc265* were more susceptible to nematode infections and had significantly lesser PTI-based callose deposits. To investigate the relationship between callose deposition and plant susceptibility to root-knot nematodes, we treated wild type *Arabidopsis* plants (Col-0) with 2-deoxy-D-glucose (DDG), an inhibitor of callose synthesis, and found the plants were more susceptible to nematodes compared to the untreated controls. We then expanded our approach to potato and found that DDG treatments significantly enhanced its susceptibility to nematode attack. Further, we focused on the potato Glucan Synthase gene 5 (*StGSL05*) because of its putative role in PTI-based callose synthesis in potato. Two *stgsl05* potato knockout lines using RNAi-based gene silencing were created. We have two knockdown lines (stgsl05-1.9 and stgsl05-3.32), which do not produce callose deposits in leaves upon elicitor treatment. These knockdown lines exhibited a significant increase in the number of juveniles in the roots and increased levels of galling. The data suggest that callose is produced as part of the basal defense response triggered by root-knot nematodes and decreasing this response makes plants more susceptible to infection.

167. Phylogenomics resolve the timing and pattern of nematode evolution

Qing Xue[1], Miles Zhang[2], Sidi Sun[1], Mohammed Ahmed[1], Wen-Sui Lo[4], Oleksandr Holovachov[5], Wim Bert[6], Hongmei Li[1]
1 Nanjing Agricultural University, China
2 University of Edinburgh, UK
3 Liverpool University, UK
4 Northwest A&F University, China
5 Swedish Museum of Natural History, Sweden
6 Ghent University, Belgium

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: nematodes, worm, phylogeny, genome sequencing, whole genome amplification, diversity

ABSTRACT:

Nematode parasites threaten the health of plants (e.g. *Meloidogyne* spp., *Heterodera* spp.), animals and humans (e.g. ascariasis, trichuriasis, and hookworm disease, filariases.). Free-living nematodes occupy all trophic levels in the food web and play a pivotal role in carbon cycling and energy flows (Ferris, 2010, Ingham et al., 1985, Thomas et al., 2022, van den Hoogen et al., 2019). The Caenorhabditis and Pristionchus are two popular nematode models in molecular and evolutionary biology. In this study we newly sequenced 54 genomes from terrestrial and marine environment covering 8 orders of phylum Nematoda, and provided the first genome representatives for 6 orders (Dorylaimida, Mononchida, Monhysterida, Chromadorida, Triplonchida, and Enoplida). Using the newly obtained genome together with publicly available genomes, we reconstructed the first phylogenomic tree for phylum Nematoda. The well-resolved phylogeny suggested the vertebrate parasitism has arisen independently at least four times, with threadworm (*Strongyloides* spp.) and hookworm (*Ancylostoma* spp. and *Necator* spp.) probably evolved from bacterivore ancestors. The plant-parasitic nematodes appeared in four lineages, and they were likely evolved from mycetophagous ancestors. The devastating plant-parasitic stomatostylet bearing nematodes (including root-knot nematode *Meloidogyne* spp. and cyst nematode *Heterodera* spp., *Globodera* spp.) are monophyletic, with frequent transition among fungivores, facultative plant-parasitic, and entomopathogenic at early-branching clades. The invertebrate parasites have arisen independently for at least four time, with a possible ancestor of fungivores, bacterivore and predator. The identification of frequent habitat transitions at a low taxonomic level suggests phylum Nematoda has an unusual ecological and trophic flexibility, and thus contributed to their evolutionary success of nematodes. The molecular dating suggested the first major diversification event within the Nematoda probably took place between the Silurian to Ordovician, resulting in the subclass Enoplia and the Dorylaimia+Chromadoria. The genera Trichinella and Trichuris were earliest arisen vertebrate parasitises (between Devonian to Carboniferous) while threadworm and hookworm were appeared more recent between Jurassic and Cretaceous. The plant-parasites arisen between Triassic to Jurassic, far late after the appearance of the first vascular plants. The clade of root-knot nematode was split from cyst

nematode in Jurassic, and sedentary feeding habitat was only established in Cretaceous. The analysis of genome sizes revealed majority nematodes were less than 200 MB, while genomes were significantly expanded towards vertebrate parasites. Besides, we tested the performance of genome amplification in acquiring sufficient DNA for Illumina sequencing, and demonstrated it is a promising strategy in phylogenomic of microscopy eukaryotes. The presented nematode phylogenomic is important to understand the origin of parasitism and transition of lifestyles, from basic evolutionary biology to pathogen control and predict new anthelmintic drug targets.

168. DNA barcoding, phylogeny and phylogeography of the cyst nematode species of the *Schachtii* group from the genus *Heterodera*

Sergei A. Subbotin[1], **Tatiana V Roubtsova[2]**, **Richard M. Bostock[2]**, **Zahra Tanha Maafi[3]**, **Vladimir N. Chizhov[4]**, **Juan E. Palomares-Rius[5]**, **Pablo Castillo[5]**
1 PPDC, CDFA, 95832 Sacramento, CA, USA
2 University of California, Davis, CA 95616, USA
3 Iranian Research Institute of Plant Protection, AREEO, Tehran, Iran
4 Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, 117071, Moscow, Russia
5 IAS, CSIC, Campus de Excelencia Internacional Agroalimentario, 14080 Córdoba, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: biogeography, *Heterodera betae*, *Heterodera* glycines, *Heterodera medicaginis*, *Heterodera schachtii*, Mediterranean basin biodiversity hotspot, molecular clock, Sino-Japanese floristic region

ABSTRACT:

Cyst-forming nematodes of the genus *Heterodera* are highly derived and economically important plant parasites. The *Schachtii* group of this genus is one of the largest ones with a total of 18 species parasitising dicotyledons. Species of the *Schachtii* group are characterised by a lemon-shaped cyst having ambifenestrate cone, well-developed bullae, strong underbridge and long vulval slit and differentiated from each other in morphometrics of the second-stage juveniles and cyst structures. In this study, we provided comprehensive phylogenetic analyses of several hundred COI and ITS rRNA gene sequences of selected species from the *Schachtii* group, including *H. betae*, *H. cajani*, *H. ciceri*, *H. galeopsidis*, *H. glycines*, *H. medicaginis*, *H. mediterranea*, *H. schachtii*, *H. sonchophila* and *H. trifolii*, using Bayesian inference, maximum likelihood, and statistical parsimony. One hundred and twenty-four new COI, 57 ITS rRNA and eight hsp90 gene sequences from 81 nematode populations collected in 19 countries were obtained. Our study showed that the ITS rRNA gene has limited discrimination power compared to the COI gene. However, our analysis also revealed that partial COI gene sequences were identical for *H. trifolii*, *H. betae* and *H. galeopsidis*. Based on the results of phylogeographical analysis and age estimation of clades with a molecular clock approach, it was hypothesised that the majority of the Schachtii group species originated and diversified in the Mediterranean Basin biodiversity hotspot during the Pleistocene and then dispersed from this region across the world. The Sino-Japanese Floristic Region is likely one of the centers of diversification for the soybean cyst nematode, which showed distinct population structure. The sequence analyses indicated that hybridisation events might have occurred within the *Schachtii* group. Future whole genome sequence datasets for the Schachtii group species will allow testing the proposed hypotheses and give a more distinct pattern of evolutionary relationships between species.

169. A decade in plant-parasitic nematode barcoding: key messages and challenges

Wim Bert[1], Marjolein Couvreur[1], Joseph Maosa[1], Rolish Singh Phougeishangbam[2], Huu Tien Nguyen [3], Toon Janssen[4], Xue Qing[5]
1 Nematology Research Unit, Department of Biology, Ghent University, Ghent, Belgium
2 Fera Science Ltd., York, United Kingdom
3 Institute of Ecology and Biological Resources, Vietnam Academy of Sciences and Technology, Hanoi, Vietnam
4 Center for Medical Genetics, UZ Brussel VUB, Brussels, Belgium
5 Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: taxonomy, molecular barcoding, phylogeny, microscopy

ABSTRACT:

Taxonomic expertise is on the decline, contrasting with the rapid growth of sequence-based identification, commonly known as molecular barcoding. Regardless of the current or future (meta)barcoding method used, it is essential to establish a link between traditionally defined taxonomic units, such as species, and corresponding DNA sequences. The primary challenges in achieving this linkage include: 1) low interspecific variation, 2) high intraspecific variation, 3) the quest for a universal barcode, and 4) connecting DNA sequences to genuine species. This presentation provides an overview of our research spanning the last decade, addressing the challenges faced and presenting our key findings. A critical aspect is establishing a robust link between morphology and DNA sequences to prevent or minimize misidentifications based on sequences. The initial step involves sequencing morphologically vouchered individual, thereby reducing errors in public databases and averting potential misinterpretations. In the case of root-knot nematodes, isozyme characterization may still be indispensable. However, in many instances, collecting topotype material becomes necessary to associate previously described morphospecies with their respective DNA sequences. Dealing with species exhibiting high intraspecific sequence variation requires a tree approach for accurate interpretation. Unfortunately, developing a universal barcode for nematodes, comparable to COI in most other animals, proves challenging, if not impossible. For instance, tropical root-knot nematodes necessitate the utilization of the rapidly evolving Nad5 mtDNA region. For most other plant-parasitic nematodes, based on molecular species delimitation analyses, we argue that a combination of ribosomal D2-D3 of 28S rDNA and COI mtDNA barcodes emerges as the most optimal choice.

170. Single specimen free-living nematode genomes rebalance the nematode phylogeny

Erna King[1], Lewis Stevens[1], Witold Morek[1], Oleksandr Holovachov[2], Christopher Laumer[3], Mark Blaxter[1]
1 Tree of Life, Wellcome Sanger Institute, Hinxton, CB10 1SA, UK
2 Swedish Museum of Natural History, SE-104 05 Stockholm, Sweden
3 Natural History Museum, London, SW7 5BD, UK

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: genome, phylogeny, evolution, free-living

ABSTRACT:

Molecular analyses have significantly revised the phylogeny of Nematoda, but single-gene analyses are unable to robustly dissect deep relationships. Current practice is to move to datasets that encompass many loci from across the genome. The majority of nematode genome sequences generated to date have been derived from a limited diversity of culturable free-living species and parasites few representatives of free-living Chromodorea or Enoplea, leading to imbalance across nematode phylogeny. Using a new method, we generated over 60 high-quality genomes for single specimens of wild-isolated, free-living nematode species from 13 under-sequenced orders across the phylum, including the first representatives for seven orders. Four of these genomes were scaffolded to produce the first chromosome-level assemblies for species in the basal class Enoplea and for free-living marine species deep within Chromadorea. In addition to providing better resolution of nematode phylogeny, these chromosomally-complete genomes have permitted the first exploration of the pattern and process of evolution of nematode ancestral linkage groups. We find support for the extension deep into Chromodorea of the model of seven ancestral linkage groups ('Nigon elements') inferred to exist at the base of Rhabditida. Whilst there are still some missing branches to be targeted, our more complete sampling supports and expands on the existing genome-based phylogeny and sheds light on genome evolution across Nematoda.

171. *Globodera pallida* as a species complex : what did we learn so far on some atypical South American populations

Eric Grenier[1], Laurent Folcher[2], Nicolas Mariette[2], Nicolas Parisey[1], Sylvain Fournet[1], Stefan Van De Ruitenbeek[3], Mark Sterken[3]

1 INRAE, Institut of Genetic, Environment and Plant protection, Domaine de la Motte au Vicomte, F-35653 Le Rheu, France.

2 ANSES, Plant Health Laboratory, Nematology Unit, Domaine de la Motte au Vicomte, F-35653 Le Rheu, France

3 Wageningen University and Research, Laboratory of Nematology, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands.

Area: Taxonomy, Systematics and Phylogeny
Type: Oral
Keywords: *Globodera*, taxonomy, cryptic species, new species

ABSTRACT:

Cryptic species should not be ignored as they are important for a number of applied reasons regarding in particular food security, risk assessment or non-chemical management technologies. The question of whether *G. pallida* should be considered a species complex rather than a single species has been raised by several authors and strong suspicions of cryptic species within *G. pallida* were presented by Thevenoux et al. (2019) regarding a group of population called '*pallida* Chilean-type'.

To determine whether this group should be considered different from *G. pallida*, we carried out a greenhouse test to reveal differences in host range. The test was carried out with 19 *Globodera* populations representing the species *G. pallida, G. rostochiensis, G. mexicana* and *pallida* Chilean-type and five host plants (8 replicates/plant) including tomato, Peruvian groundcherry, tobacco, pepper and potato. The number of cysts produced and the percentage of hatchings in cysts used as inoculum were recorded. Results showed that the *pallida* Chilean-type populations are clearly different from *G. mexicana*, but can develop on the same plants as *G. pallida* and *G. rostochiensis*. However, certain differences were observed in relation to *G. pallida*: firstly, the average number of cysts produced on potatoes is lower in the *pallida* Chilean-type than in *G. pallida* and *G. rostochiensis*, and ground cherry seems to induce a higher hatching percentage in the *pallida* Chilean-type than in *G. pallida* and *G. rostochiensis*. To complement these biological investigations, we also used whole-genome sequencing data produced in partnership with the Wageningen nematology laboratory to construct genome assemblies of *G. mexicana* and the *pallida* Chilean-type. Using effector sequences conserved in these assemblies, we also showed that the *pallida* Chilean-type is phylogenetically closer to *G. mexicana* than to *G. pallida*. Finally, using novel methods of automatized digitalisation, we created a tool capable of exploring the morphological variability within the *Globodera* species and distinguish species already described. Between 30-40 individuals of each *Globodera* species were photographed and used to build an automatized method using up-to-date image processing.

Using such image analysis, we were able to accurately detect anatomical parts, predict simple metrics and correctly distinguish *G. pallida* from *G. rostochiensis* in 83% of the comparisons made and *G. pallida* from the *pallida* Chilean-type in 63% of the comparisons made, a score similar to that obtained in the interspecific *G. pallida* - *G. mexicana* comparison.

172. Epigenetic signatures associated with nematode adaptation to plant resistance

Rahim Hassanaly-Goulamhoussen[1], Julia Truch[1], Cyril van Ghelder[1], Bruno Favery[1], Martine da Rocha[1], Corinne Rancurel[1], Etienne GJ Danchin[1], Philippe Castagnone[1], Christoph Grunau[2], Pierre Abad[1], Laetitia Perfus-Barbeoch[1]
1 Institut Sophia Agrobiotech, Sophia Antipolis, France
2 IHPE, Univ Perpignan Via Domitia, CNRS, IFREMER, Univ Montpellier, Perpignan, France.

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: plant resistance, virulence, apomixis, epigenetics, histone post-transcriptional modifications

ABSTRACT:

Plant parasitic nematodes of the genus *Meloidogyne* are crop pests of global importance. As such, *M. incognita* is the emblematic species as it is present all over the world with a wide range of species. Currently the use of resistant plants is the most effective to control nematodes. However, there is an increase in the emergence of virulent lines of *M. incognita* worldwide, able to bypass the resistance of the commonly used tomato Mi1-2 gene, despite their asexual mode of reproduction (obligatory mitotic parthenogenesis). It has been suggested that epigenetic modifications may be involved in the virulence character, with most of the strongest evidence so far coming from research conducted on *M. incognita* isolates using isofemale lineage analysis. In a simplified definition, epigenetics is the study of biological mechanisms that turn genes on and off in a reversible manner, for example in response to any external stimulus. Using high-throughput sequencing technologies to characterize epigenomes, we investigated whether exposure to the Mi1-2 resistance gene affects epigenetic marks. Our analysis of virulence character in *M. incognita* isolates from different geographical origins revealed that the avirulent and virulent lineages differ in their epigenomes and that the virulent lineages share common epigenetic signatures. Some of these findings point to previously identified genes, such as effectors.

173. Application of hyperspectral signatures for the detection of virulent populations of the potato cyst nematode *Globodera pallida*

Djaafar Babaali[1], Matthias Daub[2], Niels Lakämper[3], Heike Gerighausen[3], Kai Schmidt[4], Sebastian Kiewnick[1]

1 Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen, Institut für Pflanzenschutz in Ackerbau und Grünland (JKI-BS-A), Messeweg 11/12, 38104 Braunschweig, Germany

2 Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen, Institut für Pflanzenschutz in Ackerbau und Grünland (JKI-ELS-A), Dürener Str. 71, D- 50189, Elsdorf, Germany

3 Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen, FLFForschungszentrum für Landwirtschaftliche Fernerkundung am Institut für Pflanzenbau und Bodenkunde (JKI-BS-PB), Bundesallee 69, 3, Germany

4 Nemaplot In den Weingärten 31, 53127 Bonn, Germany

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: early/latent infestation, emsland, hyperspectral signature, potato cyst nematode

ABSTRACT:

The Potato cyst nematode (PCN) *Globodera pallida* is responsible for major yield losses in the production of industrial, seed, and staple potatoes. Resistant potato cultivars play a key role in the control of PCN. However, since the first reports of *G. pallida* populations of the new virulence type ´´Emsland´´ in Germany in 2014, the durability of PCN resistance in current potato cultivars has become a matter of concern. Thus, rapid and reliable *G. pallida* detection is vital for surveillance and control. Overcoming current limitations in early detection through innovative technologies is necessary. The project Hypall [funded by the Federal Ministry of Food and Agriculture, Project Number: FKZ 28A8704A19.] aims to develop a method for detection using hyperspectral sensor data, focusing on identifying early/latent infestations that lead to significant population increases without visible symptoms. The detection system is based on a hyperspectral camera (©SPECIM, Spectral Imaging LTD.) operating within the range of 400 - 1000 nm. Different potato cultivars (susceptible or resistant) were challenge inoculated with different *G. pallida* populations. Hyperspectral images were taken at various time points and nematode development was determined by destructive sampling and staining of roots. As an additional model system, tomato plants were inoculated with either *Meloidogyne incognita* or *M. enterolobii* juveniles to induce rapid stress to the test plants. It could be demonstrated that plant stress can be detected by hyperspectral imaging, but nematode induced stress is often masked by external factors. The factors include heat stress by H4 lights in a closed box, but also different cultivars. Therefore, to exclude external factors masking plant responses to nematode invasion and development, LED light sources provide a valuable alternative for the identification of latent *G. pallida* infections by virulent populations under controlled conditions.

174. Screening of lettuce cultivars for resistance to root-knot nematodes *Meloidogyne incognita* and *M. luci*

Gökhan Aydınlı[1], Şevval Barut[2], Sevilhan Mennan[2]

1 Ondokuz Mayıs University, Bafra Vocational School, Department of Crop and Animal Production, 55400 Samsun, Türkiye

2 Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, 55139 Samsun, Türkiye

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Lactuca sativa* L., management, resistance, root-knot nematodes,

ABSTRACT:

Lettuce (*Lactuca sativa* L.), a leaf vegetable essential to the human diet, is grown in both open fields and greenhouses. Root-knot nematodes (*Meloidogyne* spp.) are one of the most destructive pests infecting vegetable crops and are commonly distributed in greenhouses of Türkiye. The use of resistant cultivars is one of the most cost-effective methods. However, the knowledge on resistance reactions of commercial lettuce cultivars to *Meloidogyne* species is highly limited. The study aimed to evaluate the resistance of eight lettuce cultivars against *M. incognita* and *M. luci* in pot experiments. Each *Meloidogyne* species-plant cultivar combination was replicated five times and the experiment was repeated once. Pots were arranged in a completely randomized design in a greenhouse. Sixty days after nematode inoculation with 3000 eggs per plant, gall index (GI, 0-5 scale) and reproduction factor (Rf) were detected.

Resistance responses of lettuce cultivars varied according to *Meloidogyne* species. In response to *M. incognita* inoculation, root galling was observed on all lettuce cultivars and GI ranged from 2.4 on Partition and Davidole to 4.4 on Gorone. *Meloidogyne incognita* reproduced (Rf>1) on all cultivars, ranging from 1.07 (Funtime) to 26.25 (Gorone). All lettuce cultivars would be considered susceptible to *M. incognita* (GI>2 and Rf>1), however, lettuce cv. Funtime had a significantly lower Rf value ($P < 0.05$) than the other cultivars. GI on the lettuce cultivars inoculated with *M. luci* ranged from 1.1 to 4.5. The Rf values of *M. luci* varied between 0.41 and 26.30. Lettuce cv. Vaidosa had the highest GI and Rf values, while the lowest values were recorded on Bonus, which was significantly different ($P<0.05$) from other cultivars. This cultivar responded as resistant to *M. luci*. The cultivars that allow little root galling and nematode reproduction would be good choices for *Meloidogyne*-infested soil because they will increase yield and suppress the nematode population for succeeding crops. Future work is needed to evaluate the yield potential of these cultivars in *Meloidogyne* infested soils.

175. Potato cyst nematodes, resistance, tolerance and the future of potato crops in Scotland

James A. Price[1], Ingo Hein[1], John T. Jones[1], PCN Action Scotland[2]
1 The James Hutton Institute
2 www.pcnhub.ac.uk

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: potato cyst nematode, *Globodera*, Scotland, resistance, tolerance

ABSTRACT:

The UK is the 5th largest potato producer and exporter in Europe with an industry worth approximately £4.5bn. Almost 80% of seed potatoes used in Great Britain originate from Scottish farms. However, this industry is under threat from potato cyst nematodes (PCN) which have been spreading across many UK potato growing areas for decades. Recent predictions suggest that PCN will cause the end of the Scottish seed potato industry by 2050, potentially only 5 rotations away.

Following a report in 2020, a Scottish Government PCN working group was initiated (PCN Action Scotland, www.pcnhub.ac.uk). This group, consisting of over 50 government, academic and industry partners, has now received government funding across 9 work packages to provide practical solutions for the Scottish PCN problem. These work packages include, economics, decision support system, resistance, dihaploid breeding, tolerance, groundkeeper control, integrated pest management, knowledge exchange and policy. The PCN working group is currently the largest PCN-focussed collaborative research group in the UK.

Natural host resistance is the best method for reducing PCN populations. Potato breeders typically use molecular markers to genotype PCN resistance in new varieties before phenotypic screens. With a greater understanding of PCN resistance genes, PCN Action Scotland have produced new markers for genotyping resistance. These KASP markers are less prone to false positive results than previously available markers and can also provide copy number information. To date, improved markers have been developed that are associated with the H1, GpaV and Grp1 resistance sources. These markers will be made available to breeders and researchers to facilitate incorporation of multiple resistance sources in future varieties.

Varietal tolerance is another key trait for growers but is extremely difficult to phenotype in breeding programmes. Tolerant genotypes are commercially valuable as they continue to yield under heavy PCN infections. However, when not combined with resistance, tolerant varieties allow PCN populations to rapidly increase. Unlike resistance, our understanding of PCN tolerance is extremely limited. This project is investigating the genetic background of tolerance and the feasibility of developing markers to assist breeding.

176. Using novel bioinformatic approaches to identify candidates for the Potato Cyst Nematode resistance genes *Gpa5* and *H1*

Thomas M. Adams[1], Yuhan Wang[2], Lynn H. Brown[2], Xinwei Chen[1], Yuk Woon Cheung[2], Vanessa Young[3], Drummond T. Todd[3], Micha M. Bayer[4], Ingo Hein[1]
1 Department of Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Scotland, DD2 5DA, UK
2 Division of Plant Sciences, University of Dundee, Dundee, Scotland, DD1 4HN, UK
3 James Hutton Limited, James Hutton Institute, Invergowrie, Scotland, DD2 5DA, UK
4 Department of Information and Computational Sciences, James Hutton Institute, Invergowrie, Scotland, DD2 5DA, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: potato cyst nematode, resistance, R genes, SMRT-AgRenSeq-d, Gpa5, H1, *Globodera pallida*, *Globodera* rostochiensis, -omics

ABSTRACT:

Potatoes (*Solanum tuberosum*) are an important food crop, with approximately 375 million tonnes produced globally in 2022 (FAOSTAT, 2023). However, potato crops are threatened by numerous pathogens, including the potato cyst nematodes *Globodera pallida* and *G. rostochiensis*, which are estimated to cause annual yield losses of approximately 9% worldwide (Jones et al., 2013). Importantly, legislation in many jurisdictions requires seed potatoes to be produced on land where the presence of PCN species has not been detected. In Scotland, the amount of infested land area is doubling every seven years (PCN Action Scotland, 2023), threatening a crucial industry.

Resistant genes for both these species are available, with *G. pallida* being partially controlled by *Gpa5* and *G. rostochiensis* being controlled by *H1*. Currently, both of these genes have markers available, though they do not have 100% concordance with resistance phenotypes. These are termed HC for the haplotype-specific PCR marker for *Gpa5* (Sattarzadeh et al., 2006) and 57R for a marker for *H1* (Finkers-Tomczak et al., 2011).

Previous studies have suggested that both genes are members of the nucleotide binding site leucine rich repeat (NLR) class of plant resistance gene (*R* gene). This allowed for the use of enrichment sequencing techniques, termed RenSeq, to reduce genome complexity and aid in the identification of these genes. Through the use of a combined, novel approach (termed SMRT-AgRenSeq-d) of PacBio HiFi based RenSeq (termed SMRT-RenSeq), association genetics RenSeq (AgRenSeq) and diagnostic RenSeq (dRenSeq), we were able to assemble complete NLRomes of known resistant varieties and identify resistance gene candidates. We have identified nine candidates for *Gpa5* and five candidates for *H1*. Phylogenetic analyses of these candidates have allowed for the development of novel KASP markers that are undergoing validation. Additional in planta work is ongoing to identify which candidate represents the *R* gene.

These markers will be deployed in breeding programs, leading to enhanced resistance in varieties, helping to secure the seed potato industry and improving food security.

177. Screening wild potato genotypes for a novel source of resistance to the root-knot nematode, *Meloidogyne chitwoodi*

Misghina Goitom Teklu[1], Casper Schaik[1], Richard Visser[1], Jack Vossen[1]
1 Wageningen University and Research, The Netherlands

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: breeding, in vitro screening, quarantine nematodes, relative susceptibility, *Solanum* tuberosum.

ABSTRACT:

Cultivar resistance was a major part of the management strategies of the quarantine root-knot nematode, *Meloidogyne chitwoodi* in The Netherlands. Successful introgression of resistance in the first generation of potato genotypes was demonstrated on a limited source of resistance (*S. bulbocastanum*, *S. hougasii*, and *S. fendler*i) The first resistant cultivar is now on the market in 2023. So far, the genotypes developed in The Netherlands were based on introgression of a single resistance gene. As a baseline for diversifying the sources of resistance, much more screening effort was needed for future breeding programs. In the project Holland Innovative Potato (HIP), research was undertaken to find a new novel source of resistance to *M. chitwoodi* (MC-31), a standard reference population in Europe. More than 500 genotypes from 319 accessions, belonging to 118 species from the collection of WUR-Plant Breeding, were used in a high-throughput in vitro resistance screening method followed by pot validation bioassays in the glasshouse. *Solanum* accessions from North and South America with different ploidy (2x, 3x, 4x, and 6x), and endosperm balance numbers (1- 4 EBN) were used to cover the whole spectrum of the genotypes. Overall results from both the in vitro and pot bioassay indicate promising wild potato genotypes with a high level of resistance ~ 99% compared to cv. Desiree was found. On those resistant genotypes quality of the tubers was improved and was acceptable for both industrial processing with tuber-knot index values of (< 10) and as seeds with a fraction of clean tubers of (> 98%). Further, details on the methodology, results, and strategies for the integration of the resistance found into cultivated potatoes will be discussed.

SHORT ORAL COMMUNICATIONS

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182. It's not that easy! How root colonization by endophytic Sebacinales fungi affects sedentary plant-parasitic nematodes *Krzysztof Wieczorek*

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185. Two decades of monitoring of potato cyst nematodes in Serbia. *Nikola Grujic, Branimir Njezic and Milan Radivojevic*

85. Responses of plant parasitic and free-living nematodes to cover cropping in *Narcissus* fields

Vongai Chekanai[1], Roy Neilson[1], David Roberts[1], Simon Edwards[2], Matthew Back[2]
1 Ecological Sciences, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland, United Kingdom.
2 Crop and Environment Research Centre, Harper Adams University, Newport TF10 8NB, England, United Kingdom.

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Oral
Keywords: *Pratylenchus* spp. *Aphelenchus*, *Aphelenchoides*, *Ditylenchus*, French marigold, Oilseed radish

ABSTRACT:

The UK produces 70% of global *Narcissus* cut flowers and bulbs worth over £100M annually. *Pratylenchus* penetrans and *Ditylenchus dipsaci* are serious plant parasites of *Narcissus*, causing a reduction in the viability, quality and marketability of bulbs and flowers. Management practices such as lifting after two, three and four years, limited rotations, and bulk handling predispose Narcissus to nematode attack. Growers rely on pre-planting methods such as hot water treatment, quarantine/field sanitation and nematode-free bulbs, as there are no available nematicides with label permission for use on *Narcissus*. Non/poor host cover crops reduce nematode numbers through starvation and can provide alternative sustainable nematode management strategies.

Field experiments were set up in Scotland and the Isles of Scilly to assess the effects of phacelia, oilseed radish, Indian mustard, Japanese oats, and French marigold on *Pratylenchus*, *Ditylenchus*, *Aphelenchus* and *Aphelenchoides* spp., and free-living nematodes. Disturbed and undisturbed fallow were controls, and the experiment was replicated in 5 randomised blocks. Nematode populations were monitored at planting, pre-incorporation, and six weeks post-incorporation. *Ditylenchus* spp. were present at low numbers (Based on the results, French marigold and oilseed radish are options for *Pratylenchus* management in Narcissus fields and other important crops. Indian mustard, Phacelia and Japanese oats are not advisable cover crops for *Pratylenchus* management in Narcissus rotations. Oilseed radish exhibited additional benefits of boosting beneficial free-living nematodes, probably due to the high biomass produced, which stimulates bacterial decomposition, potentially improving nutrient cycling.

86. Defining and characterizing the core effectorome of the most prevalent plant pathogen in the world: the root-knot nematodes

Junior Lusu Kika[1], Unnati Sonawala[1], Sebastian Eves-van den Akker[1], Lida Derevnina[1]
1 Crop Science Centre, Department of Plant Sciences, University of Cambridge, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: root-knot nematodes, effectors, core effectorome, gland cell sequencing.

ABSTRACT:

Root-knot nematodes (RKNs, *Meloidogyne* spp.) inflict substantial agricultural losses, surpassing billions of dollars annually. To successfully parasitize their host, RKNs produce and secrete effectors. Effectors are synthesized in the nematode oesophageal salivary glands and injected into plant cells via a syringe-like stylet. Once inside, effectors suppress the plant s immune system and manipulate its physiological functions to support the nematode’s growth and proliferation. Effectors therefore dictate the outcomes of parasitism and underpin RKN success. However, despite being major players in pathogenicity, the function of few RKN effectors have been characterised. Additionally, their identification is hindered by the lack of unique conserved protein motifs, necessitating innovative solutions. The establishment of gland cell sequencing technology has provided an opportunity to characterize a multitude of effectors across several RKN species, which will substantially advance our understanding of the processes involved during RKN-plant interactions. Leveraging the latest gland-cell sequencing technology, we will analyse transcriptomes of four *Meloidogyne* spp. at different life stages, chosen based on their genetic diversity, varying ploidy levels, and differing host ranges, and define their ‘core’ effectorome through comparative genomics approaches. Preliminary data from gland cells of pre-parasitic second-stage juveniles of *M. incognita* are currently being analysed and will be presented. We hypothesize that gland cell-specific transcriptome libraries will provide a mean to establish a more comprehensive list of candidate effectors in RKNs and that functional characterization of core effectors from root-knot nematodes will provide better understanding of RKN pathogenesis.

87. Nematology in the digital age: mastering communication

David Pires[1], Maria Joao Camacho[1], Ana Fundurulic[1], Maria L. Inácio[1]
1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, Portugal

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: education, outreach, public engagement with science and technology, science communication

ABSTRACT:

Traditionally, scientists used to make new contacts at conferences and symposia, crucial hubs for social interaction that laid the foundations for lifelong collaborative networks. However, these networking opportunities were somewhat limited in space and time. Nevertheless, this paradigm shifted with the increasing access to the internet, democratizing online communication and revolutionizing the way we interact with peers. Nowadays, virtual interaction can foster connections with potential employers, mentors, and collaborators. Furthermore, leveraging social media can significantly enhance research impact, by reaching a much wider audience than the scientific community. Indeed, funding bodies are increasingly prioritizing international collaborations and research addressing challenges, while actively involving the public in science and technology. Hence, producing outputs with tangible societal impact, whether through policymaking, engagement with stakeholders or otherwise, can increase chances of securing funds. Here, we review the benefits of communication tools after our first year coordinating the dissemination, communication, and exploitation of the EU-funded project ‘PurPest - Plant pest prevention through technology-guided monitoring and site-specific control’, which aims to speed up diagnostics of key plant pests, including the pinewood nematode.

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88. The elicitor COS-OGA enhances finger millet defense against parasitic nematodes

<u>Cecilia Wanjau</u>[1], Grace Kinyanjui[3], Beth Waweru[1], Njira Pili[4], Peter Masinde[2], Samson Longolol[2], Tina Kyndt[1], Godelieve Gheysen[1]
1 Department of Biotechnology, Ghent University, Proeftuinstraat 86, 9000 Gent, Belgium
2 Department of Agriculture, Meru University of Science and Technology, P.O Box 972-60200, Kenya
3 Department of Biological Sciences, University of Embu, P.O Box 6-60100, Kenya
4 Department of Biological Sciences, Moi University, Kenya

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: FytoSave, FytoSol, priming, *Pratylenchus vandenbergae*, *Meloidogyne javanica*

ABSTRACT:

Finger millet is an important grain crop for food security in Africa and Asia. It is naturally high in health-promoting nutrients and outperforms staple cereals and legumes in nutritional value. Plant-parasitic nematodes are an emerging threat to finger millet production and productivity. This study investigates the effectiveness of a mixture of chitosan oligomers (COS) and pectin-derived oligogalacturonides (OGA), (COS-OGA) in priming finger millet for defense against major plant-parasitic nematodes. The effect of two COS-OGA formulations, FytoSol and FytoSave, was evaluated in two finger millet varieties (OKHALE-1 and KNE1034), known to be susceptible to *Pratylenchus vandenbergae* and *Meloidogyne javanica*. Finger millet plants were treated, in a pot experiment, with foliar applications of COS-OGA and inoculated either with *P. vandenbergae* or *M. javanica*. Our results showed significant reduction in number of *P. vandenbergae* in the two varieties treated with FytoSave (80-87%) and FytoSol (80-89%) without trade-offs in growth and development of the plants. In a separate pot experiment, both FytoSol and FytoSave enhanced resistance to *M. javanica*, by reducing the number of galls with FytoSol (85%) and FytoSave (91%), as well as suppressing nematode development, 45 days after inoculation. Subsequently, the OKHALE-1 variety was grown in a field naturally infested with plant-parasitic nematodes, and similarly treated with FytoSol and FytoSave. The number of plant-parasitic nematodes in mature plants was reduced by 66% (Fytosol) and 58% (Fytosave) after 100 days. Furthermore, grain yield per plant increased by 91% (Fytosol) and 81% (Fytosave), and the 1000-seed weight increased by 28% (Fytosol) and 38% (Fytosave). Quantitative real-time polymerase chain reaction (q-RT-PCR) analysis indicated that COS-OGA elicitors induce defense in finger millet. Key genes such as WRKY45, terpene synthase, polyketide synthase and glutathione-S-transferase were strongly upregulated by FytoSave in primed finger millet plants, suggesting the activation of defense-hormone pathways, secondary metabolite biosynthesis and detoxification pathways. In conclusion, this study showcases that COS-OGA elicitors effectively activate defense pathways, leading to the suppression of nematode reproduction and development without compromising finger millet growth and development. The high efficacy of COS-OGA both under controlled and field conditions, highlights FytoSave and FytoSol as promising priming agents for enhancing the immunity of finger millet plants and potentially other cereal crops.

89. Deciphering the cuticular microbiota of *Meloidogyne graminicola* exposed to the soils of Cambodian rice fields cultivated under conservation agriculture or conventional farming

Michel Barbier[1], Cécile Perrollaz[1], Kimsrong Uon[2], Jamel Aribi[1], Pierre Czernic[1], Florent Tivet[3], Sreymom Sieng[4], Vang Seng[4], Malyna Suong[2], Lionel Moulin[1], Stéphane Bellafiore[1]
1 PHIM Plant Health Institute, Univ Montpellier, IRD, CIRAD, INRAe, Institut Agro, Montpellier, France
2 ITC Institute of Technology of Cambodia, Phnom Penh, Cambodia
3 AIDA Agroécologie et Intensification Durable des cultures Annuelles, Univ Montpellier, CIRAD, Montpellier, France
4 CARDEC Cambodian Conservation Agriculture Research for Development Center, Department of Agricultural Land Resources Management, General Directorate of Agriculture, Ministry of Agriculture, Cambodia

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: *Meloidogyne* graminicola, agricultural practice, cuticular microbiota, soils microorganisms

ABSTRACT:

Root-knot nematodes (genus *Meloidogyne*) are among the plant-parasitic nematodes with the greatest impact on agriculture. During their parasitic cycle, the stage 2 juvenile larvae (J2s) spend part of their life cycle in the soil where they come into contact with a variety of microorganisms. Some of these microorganisms can attach to their cuticle, forming a cuticular microbiota that is transported into plant roots. In the last ten years, few studies have been interested in this particular microbiota. It has been shown that it is a very specific microbiota, different from the one of the soil where evolved the nematode (Elhady *et al*., 2017) and that it can play an important role during the parasitic cycle of the nematode (Topalovic *et al*., 2019).

Meloidogyne graminicola (*Mg*) is a major pest of rice (*Oryza sativa*), mainly found in Southeast Asian rice fields, and especially under flooded growing conditions. The aim of my thesis is to describe the microbial diversity associated with the *Mg* s cuticle evolving in these particular agrosystems subjected to drought or anoxia (flooded condition) depending on the water regime. For this study, we first collected soil samples from conventional and conservation-farmed rice fields in Cambodia before sequencing their microbiota, and then studied by the cuticular microbiota of pre-disinfected larvae incubated in these soils. Analysis of the sequencing data will tell us which microorganisms are recruited by the nematode cuticle and whether there is a core-microbiota in the *Mg* species. These data should enable us to study the role played by this microbiota during the plant infestation by the nematode and consider exploiting microorganisms beneficial to rice health.

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90. NemaWater of a plant-parasitic nematode contains diverse proteins involved in parasitism and immunity

Maximilian Euler[1], Badou Mendy[1], Florian M.W. Grundler[1]
1 Molecular Phytomedicine, INRES, University of Bonn, Bonn, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: PTI, NemaWater, PAMPs, plant-nematode interaction

ABSTRACT:

Plant-parasitic nematodes (PPN), especially cyst nematodes and root-knot nematodes (*Heterodera* spp., *Meloidogyne* spp.) cause significant yield losses in many important agricultural crops worldwide. Plants recognize pathogens through pathogen-associated molecular patterns (PAMPs) using pattern recognition receptors located on the cell membrane. The recognition of PAMPs triggers defense reactions known as pattern-triggered immunity (PTI). Numerous PAMPs have been identified from various plant pathogens such as bacteria and fungi, but very little information is available on PAMPs from PPNs that are sensed in the early stages of plant-nematode interactions. In previous work, we have shown that the aqueous diffusate (NemaWater) of the plant-parasitic nematode *Heterodera schachtii* contains proteinaceous molecules and is capable of activating PTI. A total of 193 proteins were identified by liquid chromatography-mass spectrometry (LC-MS). Among them are 75 cytoplasmic, 18 membrane-bound and 13 extracellular proteins with a predicted signal peptide for secretion. Some of them are thought to be involved in the activation or suppression of plant immunity, e.g. heat shock proteins or venom allergen-like protein 1. Here we present a selection of these proteins and characterize them with regard to their potential role in nematode biology, parasitism and their importance for plant immune defense.

91. Host suitability and feeding habit of *Aphelenchoides pseudobesseyi* on strawberry (*Fragaria ananasa*), rice (*Oryza sativa*), soybean (*Glycine max*) and cotton (*Gossypium*).

Lynhe Demesyeux[1], William T. Crow[1]
1 University of Florida, Dept. of Entomology and Nematology, Gainesville FL USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: Aphelenchoides

ABSTRACT:

Aphelenchoides pseudobesseyi is a newly described foliar nematode species for which the host range is unspecified. Plant parasitic species in the genus *Aphelenchoides* can be very damaging to suitable hosts. Therefore, this research proposed to investigate the host status of strawberry, rice, soybean and cotton to *A. pseudobesseyi*, and to determine the distribution and feeding habit of this nematode on these crops under greenhouse conditions. The experiments consisted of a RCBD of 5 blocks with 5 inoculated and 5 non-inoculated plants per block for each crop. Approximately 300 active *A. pseudobesseyi* were added to each strawberry and rice plants of the inoculated treatment when their new growth reached ~7 cm. The soybean and cotton plants were inoculated with ~70 nematodes at seed emergence and with ~ 350 nematodes when the plants had 2 to 4 leaves. Yield parameters were recorded for each crop separately. At the end of the experiment, all plants for each crop were separated into leaves, stems, husk (soybean), seed and bulb (cotton). To assess the feeding habit, the number of *A. pseudobesseyi* extracted from the surface and internal of each plant-part were recorded. For the strawberry, the final nematode count from internal extraction (mean ± SE = 105 ± 3) was more than one hundred time higher than from the external extraction (mean ± SE = 1 ± 2). Non-inoculated plants produced ~ 4 times more flowers and fruits (mean ± SE = 4.64 ± 0.45) than inoculated plants (mean ± SE 1.32 ± 0.23). No nematodes were recorded from the external extraction of neither plant parts of the rice, soybean and cotton. From the internal extraction, the nematode count averaged ~ 8 nematodes from the leaves and 0 nematodes from the other plant parts of these three crops. Additionally, there was no significant difference between the means of yield parameters of inoculated and non-inoculated rice, soybean and cotton. These results indicate that *A. pseudobesseyi* is capable of feeding endoparasitically on strawberry and caused yield loss to this crop under greenhouse conditions. *A. pseudobesseyi* does not reproduce nor influence the growth of rice, soybean and cotton.

92. Investigating the function of the *Heterodera schachtii* effector *Hs2B11* in plant-nematode Interactions

Joffrey Mejias[1,2], Anil Kumar[1], Parijat Juvale[1,3], Melissa Bredow[1], Thomas R Maier[1], Ekkachai Khwanbua[1], Sebastian Eves-van den Akker[4], Steven Whitham[1], Thomas J Baum[1]
1 Department of Plant Pathology, Entomology and Microbiology, Iowa State University, USA
2 CIRAD, UMR PHIM, Montpellier, France
3 BASF, Research Triangle Park, North Carolina, USA
4 The Crop Science Centre, Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: beet cyst nematode, *Heterodera* schachtii, BCN effector, yeast two-hybrid assay, subcellular localisation

ABSTRACT:

The beet cyst nematode (BCN), *Heterodera schachtii*, is a significant threat to profitable production of beet crops like sugar beet. Like other cyst nematodes, *H. schachtii* relies on specialized feeding structures known as syncytia within host roots to extract nutrients for its survival and reproduction. The formation and maintenance of syncytia are facilitated by a set of effector proteins secreted from the nematode's esophageal glands. Understanding effector functions and their molecular interactions with host plants is crucial for developing novel strategies to manage *H. schachtii* infestations. In this study, we used the model plant *Arabidopsis* to investigate the functional role of a novel *H. schachtii* effector, Hs2B11, whose orthologue was originally identified in gland-specific cDNA libraries of the soybean cyst nematode (SCN) *H. glycines*. *Hs2B11* is expressed in the dorsal esophageal gland and exhibits highest up-regulation early during infection. Structural analysis unveiled a unique tower-like structure of the Hs2B11 carboxyl (C)-terminal domain, which aligned serine residues across one surface. Hs2B11 localizes to the endoplasmic reticulum and cytoplasm of *Nicotiana benthamiana* plant cells. Through a yeast-two-hybrid (Y2H) approach, we identified pathogenesis-related protein 6 (AtPR-6), a serine proteinase inhibitor, as an interacting *Arabidopsis* protein. This interaction was further confirmed through pairwise Y2H analyses and *in planta* bimolecular complementation and split-*luci*ferase assays. Investigation of *Arabidopsis* lines harboring an AtPR-6 promoter-driven GUS construct revealed upregulation of the AtPR-6 promoter in response to BCN infection in the syncytium at early infection stages. Moreover, AtPR-6 overexpression and knockout (KO) lines displayed higher and lower immunity when treated with flg22, respectively, resulting in AtPR-6 KO mutants showing higher susceptibility to *H. schachtii* infection and plants overexpressing AtPR-6 being more resistant. Furthermore, *Arabidopsis* plants with moderate *Hs2B11* expression exhibited enhanced growth but reduced immune response to flg22 treatment, while those plants highly expressing *Hs2B11* showed the opposite phenotypes. Analysis of domain mutants suggested that AtPR-6 specifically interacts with the *Hs2B11* serine-rich C-terminus. Furthermore, a Y2H screen identified potential plant partners of AtPR-6, including two plant serine proteases and a novel putative cyst nematode-specific serine protease effector. We hypothesize that *Hs2B11* functions as a serine proteinase inhibitor decoy trap and that its

interaction with AtPR-6 prevents this protein from inhibiting plant serine proteases that regulate plant immunity. Collectively, our findings suggest that the Hs2B11–AtPR6 interaction plays a pivotal role in sugar beet cyst nematode parasitism.

The sugar-beet cyst nematode effector 2B11 targets the serine protease inhibitor of AtPR-6 to favor parasitism

178. Insights into the local transmission of the *Litylenchus crenatae mccanni* (Nematoda: Anguinidae) in the U.S.

Mihail Kantor[1], Goraya Mankanwal[1], Camelia Kantor[2]
1 Plant Pathology & Environmental Microbiology Department, The Pennsylvania State University, University Park, PA 16802, U.S.A.
2 Huck Institutes of the Life Sciences, Pennsylvania State University, State College, PA 16802.

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: *Litylenchus crenatae mccanni*, transmission, environmental factors

ABSTRACT:

The North American beech leaf nematode, *Litylenchus crenatae* ssp. *mccannii* (LCM) is the causal agent of the beech leaf disease (BLD), an emerging significant threat to hardwood forests in the Northeastern U.S. First identified in Ohio in 2012, the disease has since spread to 15 additional states, including expanding to Ontario, Canada. Early BLD symptoms include dark-green interveinal banding, followed by leaf thickening, ultimately leading to tree death. Currently, there is a lack of comprehensive understanding regarding LCM dispersal mechanisms, which poses a substantial challenge in managing BLD. Our main research objective was to examine the environmental and biotic factors involved in the local transmission of LCM. In 2023, at Penn State Forest, we established four experimental stations at variable distances from the BLD-infected beech trees. Every station was equipped with three water-filled funnels to facilitate data collection, which spanned two months starting from early September. Every other day, we conducted counts of both active and inactive LCM nematodes in each funnel. Additionally, we collected DNA from various biotic agents, including beech blight aphids and spider webs, for sequencing and analysis. Our findings revealed that active LCM nematodes were present in all the funnels, confirming that factors such as wind, rain, and temperature play a crucial role in mediating the transmission of LCM. A significant correlation was observed between the number of LCM nematodes in the funnels and the environmental variables. We employed molecular identification techniques to confirm the nematode species. Moreover, the molecular analysis of the DNA samples from the biotic life forms under the beech leaf canopy successfully identified the presence of LCMs further confirming the role of environmental factors on nematode mobility and their potential role in aiding local LCM transmission. This research sheds light on the dynamics of LCM dispersal, a critical step toward developing effective quarantine strategies for BLD.

179. Fairy-tail: root-knot nematodes and their unusual telomeres

Ana Paula Zotta Mota[1], Etienne Danchin[1]
1 Institut Sophia Agrobiotech, INRAE, Université Côte d'Azur, CNRS, Sophia Antipolis, France

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: telomeres, *Meloidogyne*, genome assembly

ABSTRACT:

Telomeres are nucleoprotein structures present at the end of all linear chromosomes and are associated with their protection and stability. The great majority of living organisms share a canonical combination of G-rich short simple repeats, maintained by the telomerase. To determine the nucleotide sequence of these important repetitive regions high quality and contiguous genomes are needed. Thanks to recent long-read sequencing technologies, we are now able to precisely determine these regions. We sequenced and assembled the genomes of three of the most devastating plant parasites from the genus *Meloidogyne*: *M. incognita*, *M. javanica*, and *M. arenaria*. Thanks to these new assemblies produced from long-read technology we were able to investigate the telomere sequence yet unknown of these species.

Against all odds, we could find neither the telomerase genes nor the canonical telomeric repeat sequence of *C. elegans* and conserved in other nematodes. Instead, at the end of some contigs, we found a composite G-rich degenerate repeat of ~280bp, composed of three motifs. Although the three species share the G-rich characteristic of these composite repeats, only one of the three motifs was conserved between them. We compared this new repetitive sequence against all nematode species with a sequenced genome, and apart from partial matches in closely related *Meloidogyne* species (e.g. *M. luci*) the repeat was absent from the rest of the nematodes. Although these species present this unusual telomere sequence, we observed that these composite regions are enriched on sequences able to form G4-quadruplex structures. G4-quadruplex are characteristic of telomeres, also acting on their stabilization and function. In addition, we analyzed the transcripts of these three species and identified telomeric repeat-containing RNA (TERRA-like) . This kind of transcript has the potential to form R-loops, and together with the G4 structures, form G-loops, which were previously described to be important for alternative lengthening of telomeres (ALT pathway).

Finally, using fluorescent in-situ hybridization we confirmed the repeat had a telomeric localization but only at one single extremity of most chromosomes, with the exception of a few chromosomes of *M. javanica* and *M. arenaria*. We also confirmed that the number of repetitive regions found was consistent with the number of chromosomes for each species. The discovery of a new kind of telomeric repeat in these species highlights the evolutionary diversity of chromosome protection systems despite their central roles and opens new perspectives towards the development of more specific control methods against these pests.

180. Targeting serotonin signaling to control plant parasitic nematodes

Lindy Holden-Dye[1], Fernando Calahorro[1], Catherine Lilley[2], Mirela Coke[2], Peter E Urwi[2], Vin O Connor[1]
1 School of Biological Sciences, University of Southampton, UK
2 School of Biology, University of Leeds, UK

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Oral
Keywords: nematicide, natural product, stylet, seed

ABSTRACT:

The neurotransmitter serotonin regulates vital nematode behaviours and in plant parasitic nematodes serotonin is a key regulator of stylet thrusting (Masler, 2007). The stylet is a lance-like structure that protrudes from the mouth and rhythmically contracts and relaxes to pierce the root of the host plant. This is a pivotal moment in the life cycle of the parasite and therefore arguably a rational target for plant protection. We have shown that targeting serotonin signaling impairs stylet thrusting in *Globodera sp.* and disrupts invasion of plant roots in vitro (Crisford et al., 2020).

Two serotoninergic targets have been selected for further investigation. MOD-1 is a serotonin-gated chloride channel which is apparently constrained to the Nematoda. We have developed a platform for the characterization of the MOD-1 pharmacophore using *Caenorhabditis elegans* for expression. This has yielded lead chemicals for further study for bioactivity against plant parasitic nematodes (Calahorro et al., 2022). We are also targeting presynaptic serotonin signaling using the vesicular-monoamine transporter (VMAT) inhibitor reserpine (Erickson et al., 1992). Reserpine is the active constituent of a traditional herbal remedy from *Rauwolfia serpentina*. In *Globodera pallida*, reserpine inhibits stylet thrusting at sub-micromolar concentrations and is irreversible over a time-course of 24 hours (Crisford et al., 2020). Trials are planned to test the efficacy of reserpine in the field.

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182. It’s not that easy! How root colonization by endophytic Sebacinales fungi affects sedentary plant-parasitic nematodes?

Krzysztof Wieczorek[1]
1 University of Natural Resources and Life Sciences Vienna, Austria

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: *Serendipita* spp., plant-parasitic nematodes, cyst nematodes, root-knot nematodes, fungus-derived substances

ABSTRACT:

Plant-parasitic nematodes (PPNs), especially sedentary cyst (CN, *Heterodera* spp.) and root-knot nematodes (RKN, *Meloidogyne* spp.), are a serious threat to many crops causing tremendous economic losses worldwide. Due to the ban of the most chemical nematicides, there is a strong need for new environmentally friendly alternatives. One of such promising options is the utilization of antagonistic fungal and bacterial microorganisms as biological control agents. Particularly different endophytic fungi such as arbuscular mycorrhiza fungi, Fusarium spp., Trichoderma spp., Paecilomyces spp. and more recently different Sebacinales spp. were shown to induce plant tolerance to abiotic and biotic stresses and to act through different beneficial pathways promoting the growth of the host. Hence, in last two decades various studies reported their considerable positive impact on host tolerance/resistance against different PPNs. However, more recently the number of reports showing for some cases the lack of these bioprotective effects or even enhancement in nematode populations caused by endophytic fungi is increasing. Therefore, to shed more light on this matter we tested *Arabidopsis* and different important crops including tomato and sugar beet in greenhouse and in in vitro experiments. We used plants directly colonized by different Sebacinales spp., such as *Serendipita indica* and *S. williamsii*, or plants treated with fungi-derived substances (cell wall extracts, culture filtrate, VOCs etc.) that were subsequently inoculated with PPNs, the sugar beet CN *H. schachtii* and the RKN *M. incognita*. Finally, we investigated several aspects of this tripartite interaction such as plant growth parameters, expression of defense-related genes, sugar metabolism and development of nematodes in comparison with noncolonized or nontreated controls. Interestingly, the results strongly depend on host plant, PPN and fungus species and the kind of endophyte application (living fungus, fungus-derived substances, direct or systemic interaction). In all cases, however, the fungal root colonization could counteract the negative impact of nematode infestation on plant biomass and growth. In terms of the bioprotective effects against nematodes, our data show, that while the colonization with the living fungi resulted in rather diverse results including even enhancement of nematode population, the application of fungi-derived substances led to reduction in nematode numbers.

Our work demonstrates that the beneficial endophytic Sebacinales spp. or their products have a promising potential against PPNs. However, in a case-by-case examination each specific combination of host plant, species of PPNs and fungi as well as the method of fungal application has to be separately evaluated and optimized.

183. Identifying and characterizing the NLR immune receptor network against root-knot nematodes in sweet potato

Unnati Sonawala[1], Sam Bruty[2], Lida Derevnina[1]
1 Crop Science Centre, Cambridge, UK
2 The John Innes Centre, Norwich, United Kingdom

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Oral
Keywords: Root-knot nematodes, immune receptor, NLRs, sweet potato

ABSTRACT:

Plants use their immune system to defend themselves against invading pathogens, including plant-parasitic nematodes. Integral to this system are nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins, commonly referred to as resistance (R) genes. An emergent model in NLR biology is that NLRs function in complex networks to activate defence responses wherein 'sensor' NLRs directly or indirectly recognize pathogen effectors and 'helper' NLRs induce downstream immune signalling. Although extensively studied in Solanaceous plants, like tomato, this sensor-helper NLR network remains under-explored in the *Convolvulaceae* family, which includes the important food crop sweet potato (*Ipomea batatas*). Root-knot nematodes (RKNs), especially *Meloidogyne incognita* and in more recent years, *M. enterolobii*, are serious threats to sweet potato production, however, how the sweet potato NLR network functions to mediate resistance to RKNs is currently unknown. We have recently delineated the phylogenetic network of NLRs in sweet potato using genomic and transcriptomic data. To identify the activated sensor and helper NLRs during RKN infection, we performed a dual transcriptomic study of sweet potato roots infected with *M. incognita*. This will not only help identify NLRs of importance but will also enable us to discover pathogen virulence factors expressed during infection. Latest results from this analysis along with the characterization of NLRs via transient expression in the model plant *Nicotiana benthamiana* will be discussed.

184. Quality control of entomopathogenic nematodes through infrared spectroscopy (FTIR-ATR, 2D-COS): tracing, modelling and prediction

Ernesto San-Blas[1], Gabriel Paba[3], Nestor Cubillan[4], Edgar Portillo[5], Mayamarú Guerra[1]
1 Instituto de Ciencias Agroalimentarias, Animales y Ambientales ICA3, Universidad de OHiggins, Chile
2 Centro de Biologia de Sistemas para la Sanidad Vegetal BIOSAV-UOH, Universidad de OHiggins, Chile
3 Postgrado de Biotecnologia Vegetal, Pontificia Universidad Católica de Chile, Chile
4 Universidad del Atlántico, Colombia
5 Instituto Venezolano de Investigaciones Científicas, Venezuela

Area: Entomopathogenic Nematodes
Type: Oral
Keywords: machine learning, Heterorhabditis indica, *Steinernema riobrave*, virulence, genetic algorithms, infectivity

ABSTRACT:

The main objective of this research was to evaluate the energy reserves of the entomopathogenic nematodes (EPN) *Heterorhabditis indica* and *Steinernema riobrave*, by using ATR/FTIR characterization, principal component analysis (PCA), two-dimensional correlation spectroscopic (2DCoS), integration of absorption bands and modelling by artificial intelligence. The levels of such reserves were associated with survival and infectivity of infective juveniles (IJs) towards *Galleria mellonella* according to their storage temperature: T1 (10°C), T2 (20°C) and T3 (30°C). These results suggest that lipids (especially triglycerides) represent the main source of energy in both species, whereas glycogen and trehalose act as alternative sources. In the case of *H. indica*, the highest percentages of infectivity were obtained by IJs from T2, while those from T3 were unable to infect *G. mellonella* larvae after 8 weeks of storage. On the other hand, the IJs of S. *riobrave* were more virulent compared to those of *H. indica*, indeed, they were more tolerant to low (T1) and high temperatures (T3). Thus, it was evidenced that energy reserves and infectivity are influenced by storage. Results similar to those presented in this study have been reported and conclude that Steinernematids survive much longer at high temperatures compared to Heterorhabditids ones. However, this depends on the species and the regulatory mechanisms involved in the acquisition of thermo-tolerance, such as saturation and desaturation of fatty acids and accumulation of stabilizing compounds of cell membranes, such as trehalose. Also, the results showed that mortality of *G. mellonella* larva according to the time and temperature of storage of EPN can be assessed by modelling the relations of chemical data of FTIR.

185. Two decades of monitoring of potato cyst nematodes in Serbia

Nikola Grujic[1], Branimir Njezic[2], Milan Radivojevic[1]
1 University of Belgrade-Faculty of Agriculture, Nemanjina 6, 1080 Belgrade, Serbia
2 University of Banja Luka, Faculty of Agriculture, Bosnia and Hercegovina

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Oral
Keywords: *Globodera*, survey, management, phytosanitary control, quarantine

ABSTRACT:

Potato cyst nematodes (PCN) are one of the major pests of potato worldwide. They are regulated quarantine pests in Europe and in Serbia. First surveys of PCN in Serbia started in 1990’s with the first records of *Globodera* rostochiensis and *G. pallida* from 2000 and 2006, respectively. Since then, regular yearly phytosanitary control of seed and partly mercantile potato production yielded 56 PCN infested fields. Since 2013, Faculty of Agriculture in Belgrade was granted by the Plant Protection Directorate of the Ministry of Agriculture of Republic of Serbia to carry out program of special surveillance of the quarantined areas and we herein present the current program results. Over the years all known PCN infested fields were resampled excessively by authors with at least eight soil samples per field taken in a zig-zag pattern across the field. Collected soil samples were dried in laboratory and cyst extracted with Fenwick elutriator. Species identification of PCN for all sampled fields was made by morphological means and for majority of fields confirmed by molecular means. Viability of PCN populations was obtained by individual cyst dissection of 50 randomly taken cysts and counting of viable content. Further, PCN viability was checked by second stage infective juveniles (J2) hatching tests in 0.6mM solution of Na-metavanadate. For most of the localities where PCN has been found (23PCN populations tested) pathotype tests have been done using commercial potato varieties. All PCN records were from Western Serbia distributed in five broader localities in Mavanski, Zlatiborski and Moraviki district. From 56 fields where PCN had been found since 2000, in resampling from 2013, 19 fields were found PCN free and those were mostly the older findings and/or records with originally low infestation levels. 36 fields were infested with G. rostochiensis and only one with G. pallid. There were no records with mixed infestations of PCN species. Most of the fields are with low to medium PCN infestations with around 20% of them with high infestation levels. Some fields were in monitoring for over 17 years including the one with *G. pallida*. The latter is left in fallow since 2005 and 2023 sampling and cysts analysis showed still viable population with positive J2 hatching test. PCN pathotype testing is underway for recent findings with up to now only Ro1 and Pa2 pathotypes recorded.

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POSTER

186. Investigation of xenocoumacins, natural products produced by entomopathogenic bacteria, on root-knot nematode control. *Fatemeh Sayedain, Peter Grün and Helge B. Bode*

187. Activity of plant-derived compounds on the virus-vector nematode *Xiphinema index*. *Trifone Daddabbo, Alberto Troccoli, Pinarosa Avato and Maria Pia Argentieri*

188. Role of *Meloidogyne chitwoodi* infecting different potato genotypes and investigation of the resistance mechanism. *Jessica Lange, Stefanie Hartje and Johannes Hallmann*

190. The effects of some plant methanol extracts on the egg hatching of root-knot nematodes (*Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne luci)*. *Emre Yılmaz, Yeter Küçüktopçu, Gökhan Aydınlı and Sevilhan Mennan*

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295. *Bursaphelenchus arthuri* from *Pinus pinea* in Portugal. *Hugo Silva, Joana M. S. Cardoso, Ricardo da Costa, Isabel Abrantes and Luís Fonseca*

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297. Discovery of a newly identified plant-parasitic nematode (Tylenchida: Anguinidae) infesting western sword fern leaves in Washington State rainforests, USA. *Sergio Álvarez-Ortega and Sergei A. Subbotin*

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186. Investigation of xenocoumacins, natural products produced by entomopathogenic bacteria, on root-knot nematode control

Fatemeh Sayedain[1], Peter Grün[1], Helge B. Bode[1]
1 Max-Planck-Institute for Terrestrial Microbiology, Germany

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ABSTRACT:

Root-knot nematodes (RKNs, *Meloidogyne* spp.) are obligate parasites that cause significant global agricultural production losses each year. Control of this nematode is difficult due to its wide host range and the prohibition of many biocide chemical compounds. *Xenorhabdus* bacteria, symbiotically associated with entomopathogenic *Steinernema* nematodes, are known to produce a large number of natural products with antimicrobial, insecticidal, and nematicidal properties. One group of natural products produced by some species of *Xenorhabdus* with antimicrobial activity are the xenocoumacins (XCNs). In this study, first, the freeze-dried supernatants of bacterial mutants created according to the easyPACID approach (easy Promoter Activation for Compound Identification) were tested on second-stage juveniles (J2) and eggs of *Meloidogyne incognita*. The results showed that supernatant of bacterial mutant producing both XCNs can paralyze second-stage juveniles of root-knot nematode after 48h. However, bacterial mutant supernatant only producing XCN1 was not able to cause paralysis on J2s. In addition, the supernatant containing XCNs with and without heating treatment, and in three different concentrations was able to reduce the hatching of root-knot nematode eggs. Additional experiments were followed to prove the correctness of initial results by extracting and purifying three natural products pre-XCN, XCN1, and XCN2 from *Xenorhabdus nematophila* mutants. When the concentration of XCN2 exceeded 250 µg/ml the mobility of second-stage juveniles declined to 50% and at the highest concentration 500 µg/ml 88% of juveniles were paralyzed and egg hatch was decreased by 40%. However, per-XCN and XCN1 did not affect juveniles. On top of that, no concentration of XCN2 affected the mobility of two species of entomopathogenic nematodes *S. carpocapsae* and *S. diaprepesi*. Our results can be promising in the strategy of integrated pest management using the simultaneous application of entomopathogenic nematodes and XCN2 and will provide an eco-friendly way to control plant-parasitic nematodes and soil pests.

187. Activity of plant-derived compounds on the virus-vector nematode *Xiphinema index*

Trifone Daddabbo[1], Alberto Troccoli[1], Pinarosa Avato[2], Maria Pia Argentieri[2]
1 Institute for Sustainable Plant Protection, National Council of Research, Via Giovanni Amendola 122/d, 70126 Bari, Italy
2 Department of Pharmacy-Drug Sciences, University of Bari Aldo Moro, Via Edoardo Orabona, 4, 70125, Bari, Italy

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Keywords: *Xiphinema index*, control, saponins, essential oils, garlic extract

ABSTRACT:

The dagger nematode *Xiphinema index* Thorne et Allen is a major threat in vineyards as main vector of the Grapevine Fanleaf Virus (GFLV). Ban of agrochemicals previously applied to reduce *X. index* populations encouraged the search for alternative control strategies, also including the use of plant-derived nematicidal compounds. The in vitro activity on *X. index* of saponins from *Medicago* species, essential oils (EOs) and their active constituents and a garlic extract formulation was investigated by exposing mixed-age *X. index* specimens to a range of concentrations and exposure times and then microscopically checking nematode immobility and mortality. All saponins from *Medicago* species induced 100% *X. index* mortality at a 500 µg mL^{-1} concentration between 8- and 48-hour exposures, while quillay and soybean saponins were significantly less active (19% and 29% mortality, respectively). Among saponin aglycones, hederagenin was the most active, as resulting in a complete nematode mortality at all the tested concentrations, while medicagenic acid and bayogenin resulted less active than their whole saponins. An almost complete *X. index* mortality occurred after a 2-hour exposure 4 and 2 mL L^{-1} concentrations of a garlic extract formulation, though more than 80% and 60% mortalities resulted even at 1.0 and 0.5 mL L^{-1} concentrations, respectively. EOs from *Artemisia herba-alba*, *Rosmarinus officinalis* and *Thymus saturejoides* resulted highly active on *X. index*, as causing a complete mortality even after a 24-hour exposure to 2 µg mL-1 concentrations. A poor activity was recorded for the EOs from *Cedrus atlantica* and *Mentha viridis*, which resulted in 6.8 and 7.4% peak mortalities, respectively, at 15 µg mL-1 concentrations, while a complete inactivity on *X. index* was found for an EO from *Citrus sinensis*. Among tested EOs components, the strongest activity on *X. index* was recorded for 1,8 cineole (about 44% mortality at a 15 µg mL^{-1} concentration), followed by thymol, thujone and α-pinene which ranged 35 - 41% mortalities at the same concentration. Activity of single components was always lower than the whole derivative EOs as indicating, in the absence of a predominant compound, synergistic effect of present components. Results indicate that all the tested plant compounds possess a nematicidal activity against *X. index*, though EOs and their constituents and garlic extract seems to be more reasonable candidates for the development of new nematicidal products.

188. Role of *Meloidogyne chitwoodi* infecting different potato genotypes and investigation of the resistance mechanism

Jessica Lange[1], Stefanie Hartje[2], Johannes Hallmann[1]
1 Julius Kuehn Institute, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany
2 Böhm-Nordkartoffel Agrarproduktion GmbH & Co. OHG, Ebstorf, Germany

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: medicinal plants, plant resistance, *Meloidogyne chitwoodi*

ABSTRACT:

The root-knot nematode *Meloidogyne chitwoodi* is a major pathogen on potato. Qualitative resistance to *M. chitwoodi* originating from *Solanum bulbocastanum* was investigated in splitting populations. Furthermore, wild species were screened for new sources of resistance including already known sources of resistance like *S. fendleri* and *S. hougasii*. Furthermore, the resistance mechanisms was studied against different *M. chitwoodi* populations. All experiments were carried out under greenhouse conditions. In general, tissue culture plants were transplanted in 400 ml plastic pots and after three days inoculated with 400 J2 of *M. chitwoodi*. Nine weeks later, plants were uprooted, eggs extracted in 1% chlorine solution and counted. In experiments on the resistance mechanism, plants were inoculated with the following populations of *M. chitwoodi*: Borken, Smakt and P3, a population that is virulent towards the resistance gene RMC1-blb derived from *S. bulbocastanum*. For studying nematode development in resistant genotypes, plants were harvested 4, 7 and 11 days after inoculation with *M. chitwoodi*. Roots were stained with acid fuchsine and juveniles inside the root counted according to their developmental stage (J2, J3, J4). Overall, phenotyping splitting potato populations resulted in plants being highly resistant to highly susceptible. Resistant candidates will be used in the future for backcrossing with S. tuberosum to obtain resistant cultivars. Looking at different *M. chitwoodi* populations, all three populations reproduced very well on Desirée, but only the virulent population P3 was able to reproduce on *S. bulbocastanum*. Furthermore, the virulent *M. chitwoodi* population P3 did not reproduce on *S. fendleri* and *S. hougasii*, which indicates a different resistance background of the latter two genotypes.

The project was funded with support from Federal Ministry of Food and Agriculture by decision of the German Bundestag (grant 28 a8701B19).

190. The effects of some plant methanol extracts on the egg hatching of root-knot nematodes (*Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne luci*)

Emre Yılmaz[1], Yeter Küçüktopçu[1] , Gökhan Aydınlı[2] , Sevilhan Mennan[1]
[1] *Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, 55139 Samsun, Turkey*
[2] *Ondokuz Mayıs University, Bafra Vocational School, Department of Crop and Animal Production, 55400 Samsun, Turkey*

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: plant methanol extracts, egg hatching, root-knot nematodes

ABSTRACT:

The root-knot nematodes are among the most important pests in agricultural areas and causes important crop losses all over the World. Due to the adverse environmental and human health effects of chemical nematicides, widely used in the control of plan-parasitic nematodes, the development of alternatives to chemical control strategies in recent years has been of great importance. These alternative strategies play an important role in promoting sustainable agricultural practices, preserving environmental health and reducing economic losses in agricultural production. The study was aimed to search the effects of methanol extracts from six different species of plants known to have effects against various pests, namely, *Nigella sativa*, *Laurus nobilis*, *Lavandula angustifolia*, *Foeniculum vulgare*, *Hypericum perforatum* and *Nicotiana tabacum* from various regions of Turkey, on the inhibition of egg hatchings of *Meloidogyne incognita*, *M. javanica* and *M. luci*. In order to obtain the required concentration (%20 w/v) for biological tests, pure solid extracts were prepared using a 10% solution of acetone /water (v/v). For each extract, 5 eggs masses were put into egg-hatching plates and 2 ml of the extract was applied. Same amount of sterilized water was use for the control plates and all plates were put into 25 °C incubators. The experiment continued for one month, by counting second stage juveniles under microscope at seven-day intervals. Experiments were 4 replicates and repeated one more times with same treatments and conditions. The inhibitions of ethanol extracts on the egg hatching were determined by comparing with the controls.

As a result, the inhibition of hatching was between 58% and 99% for three different species of root-knot nematodes. Among the species, *M. luci* is the most affected, while *M. incognita* is the least affected. *Lavandula angustifolia*, was the most effective, followed by *F. vulgare* and *N. sativa*. The most ineffective extract is *N. tabacum*, followed by *L. nobilis*. These findings highlight the availability of plant extracts as a potential alternative to effectively managing root-knot nematodes. However, wider trials are needed to ascertain the practical feasibility of these applications and to assess their feasibility on an industrial scale.

191. Testing chitosan-based Fosetyl-Al nanocrystals in *Meloidogyne javanica* control

Chiara Sciandra[1], Giulia Torrini[1], Francesco Barbieri[1], Sara Amoriello[1], Francesca Baldassarre[2], Pio Federico Roversi[1], Francesco Paoli[1]

1 CREA Research Centre for Plant Protection and Certification, via di Lanciola 12/a, Florence, Italy
2 University of Salento - Department of Biological and Environmental Sciences and Technologies, via Monteroni, Lecce, Italy

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: Fosetyl-Al, nanotechnology, nematicides, plant-parasitic nematodes, root-knot nematodes

ABSTRACT:

Plant-parasitic nematodes, which are ubiquitous and have high reproductive rates, are undoubtedly a serious threat to food security and plant health, causing an annual loss of approximately USD 100 billion worldwide. Root-knot nematodes *Meloidogyne* spp. are responsible for extensive yield loss of horticultural and field crops. The difficult management of plant parasitic nematodes often relies on chemicals. However, due to their negative impact on human health and the environment, many major nematicides have been withdrawn or banned in recent decades. The need for control measures with less environmental impact has led to the renewed search for alternative strategies, such as nanotechnology.

The aim of this study is to investigate the potential efficacy of a new formulation of Fosetyl-Al, a well-known systemic fungicide also used as bactericide, in the control of *Meloidogyne javanica*. In-vitro experiments were performed using chitosan-based Fosetyl-Al nanocrystals (CH-nanoFos), prepared by a sonication-assisted method, applying chitosan as a coating agent.

In order to determine the optimal concentrations of CH-nanoFos to be tested against *M. javanica*, a series of preliminary experiments were carried out. The potential nematicidal, nematostatic and hatching inhibition activities of three different concentrations of CH-nanoFos (1 mg/ml, 2 mg/ml and 4 mg/ml) at different exposure times were then compared.

Nematostatic and nematicidal activities were evaluated on second-stage juveniles after 24 and 48 hours of exposure to CH-nanoFos. There was a statistically significant interaction between the effects of the tested nanoproduct concentration and exposure time for both parameters evaluated. In general, the nematicidal effect increased as the tested concentration and exposure time increased. In contrast, the nematostatic effect decreased with increasing tested concentration and exposure time.

To investigate the potential hatching inhibitory activity, *M. javanica* eggs were incubated with CH-nanoFos and the number of juveniles was estimated after 3, 7 and 14 days. There was a statistically significant interaction between the effects of the tested nanoproduct concentration and incubation time, in fact, the inhibitory activity of CH-nanoFos on *M. javanica* egg hatching increased with increasing concentration and incubation time.

Regarding future perspectives, the efficacy of CH-nanoFos in controlling this root-knot nematode will also be evaluated *in vivo* through pot experiments. Its potential phytotoxicity is currently under investigation on tomato plants.

In addition, any changes in *M. javanica* morphology and the possible uptake of nanoparticles after exposure to CH-nanoFos will be investigated using electron and confocal microscopy.

192. Mechanism of the nematicidal effect of essential oil from *Cinnamomum cassia* on *Meloidogyne incognita* juveniles

Elena Fanelli[1], Trifone DAddabbo[1], Francesca De Luca[1], Pasqua Veronico[1]
1 Institute for Sustainable Plant Protection-CNR, Bari

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: *Cinnamomum cassia*, essential oil, *Meloidogyne incognita*, nematicidal effect

ABSTRACT:

Essential oils (EOs) have been widely acknowledged as potential candidates for the formulation of environmental safe crop pesticides, also including nematicidal products. In this study, an EO from *Cinnamomum cassia* was investigated for its toxicity to infective juveniles (J2) of the root-knot nematode *Meloidogyne incognita* by soaking or fumigation *in vitro* assays.

Nematode J2 were exposed for 4, 8 or 24 h to 12.5-100 µg mL^{-1} concentrations of the EO. The highest concentration of *C. cassia* EO induced above 93% mortality already after 8h incubation. At the 50 µg mL^{-1} concentration, J2 mortality was above 60 and 94% after 8 and 24 h contact with the EO solutions. A 24-hour exposure by fumigation to 12.5-100 µg mL^{-1} EO concentrations was also effective, although mortality rates were lower than in contact experiments. A 24-hour immersion or exposure to vapors of the 25 µg mL^{-1} EO solution resulted in 20 and 37% mortality rates, respectively, and most of the alive nematodes appeared immobile. This sublethal concentration was used for further experiments aimed to establish if J2 were still able to penetrate roots and develop. Attraction of nematodes to tomato roots was observed microscopically, and number of J2 touching the root tip was counted at 2, 4, 6, 8 and 16 h. At 24, 48 and 72 h roots were stained with acid fuchsin to record the number of nematodes that had penetrated the root. J2 soaked for 24 h in *C. cassia* EO were attracted to tomato root tip in lesser number than controls.

Development and reproduction of RKN were evaluated in an infection bioassay on potted tomato. At 7, 14 and 30 dpi, plants infected with nematodes soaked for 8 h and 24 h with the sublethal concentration did not display inhibition in parasitism in terms of reduced number of galls, egg masses, eggs per egg mass, as suggesting a nematostatic effect rather than a biocide activity of the EO at this concentration.

Since mechanisms and target sites of *C. cassia* EO action on *M. incognita* J2 are not yet known, studies on expression changes of target genes were addressed. Effect of 2- and 4-hour incubation of *M. incognita* J2 at the *C. cassia* OE sublethal concentration was investigated by qPCR measuring the expression levels of different genes involved in the motility and protection mechanisms.

These studies were funded by PNRR Agritech, task 3.2.5 ‘Nature-based solutions for natural resources and environment protection’.

193. Mitigating Grapevine Fanleaf Virus transmission (GFLV): exploring sunflowers as potential agents for virus clearance from the dagger nematode *Xiphinema index*

Julija Polanšek[1, 2], Myriam Claeys[3], Saša Širca[1]
1 Plant Protection Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia
2 Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
3 Nematology Research Unit, Department of Biology, Ghent University, Campus Ledeganck, Ghent, Belgium

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: *Xiphinema index*, GFLV, management, *Helianthus annuus*, transmission electron microscopy

ABSTRACT:

The dagger nematode species *Xiphinema index*, known for transmitting viruses, primarily target grapevines (*Vitis vinifera* L.) as their host plant. These nematodes feed on the root system in the soil, causing direct damage with a strong stylet and facilitating easier infection of grapevines by other pathogens. Besides direct damage, their importance lies in the transmission of Grapevine fanleaf virus (GFLV), resulting in infected vineyards with lower annual yield production and deterioration of yield quality. Due to the impossibility of completely eradicating the virus from infected plants, the challenge is to limit the presence of the virus and/or its vector *X. index* in the soil or to shorten the several years (7-10 years) period needed for a replanting stage of vineyards. In light of a prior experiment revealing the sunflowers (*Helianthus annuus* L.) as potential host plants for *X. index* populations [1], our research explores the potential of sunflowers to cleanse nematodes of GFLV. In a 6-month greenhouse experiment, vineyard soil with a high population density of *X. index* was used and subjected to three different pot treatments, with planted sunflower, with planted vine and pot without any crop (bare soil), to compare nematode numbers and the presence of GFLV in *X. index*. The nematode density was highest in pots with grapevine while, surprisingly, the number of individuals *X. index* was lowest in sunflowers, although we expected that this population would also increase based on previous research in comparison to bare soil (Villate et al., 2012). Transmission electron microscopy (TEM) was used to investigate the presence of viral components in nematodes from all treatments, revealing their visibility only in nematodes from pots with grapevine. Virulent particles were not observed under 12,000 x magnifications around the odontostyle as expected but were visible lower, around the odontophore [1, 2, 3].

The following season, the experiment will be scaled to determine whether sunflowers can have an effective impact on virus-free nematode population. The overarching goal of our research is to decrease *X. index* populations or their virulence in vineyards using a natural approach involving different crops or biological preparations.

The research activities are conducted within the program group Agrobiodiversity (P4-0072), financed by Slovenian Research and Innovation Agency (ARIS).

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194. Mode of action of two plant-derived compounds on the root-lesion nematode *Pratylenchus penetrans*

Pedro Barbosa[1], Marina Costa[1], Madalena Mendonca[1], Margarida Espada[1], Cláudia S. L. Vicente[1]
1 Mediterranean Institute for Agriculture, Environment and Development, University of Évora, Évora

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: 3-octanol, benzaldehyde, xenobiotics, nematicide

ABSTRACT:

The root-lesion nematode (RLN), *Pratylenchus penetrans,* is one of the most severe plant parasitic nematodes (PPN), responsible for productivity losses in a significant number of plant hosts. The chemical control of these PPNs relies on fumigants and non-fumigants compounds, which offer a systemic protection. Such control methods are costly and hazardous to the environment and to humans. Compounds naturally produced by plants may play an important role in nematode control. The aim of this study is to analyze the molecular response of *P. penetrans* to the nematicidal agent 3-octanol ($C8H18O$) and benzaldehyde ($C6H5CHO$) using a de novo transcriptome assembly approach. Previous work showed that both active compounds are strong nematicides of *P. penetrans* causing 100% mortality in standard direct contact for 24h (Barbosa et al., 2022). The mode of action was studied at a sub-lethal exposure for 30 min. The molecular response of *P. penetrans* to both compounds was similar regardless the different chemical composition. Overall, both compounds induced the activation of detoxification mechanisms of *P. penetrans* with significant up-regulation of metabolic pathways related with metabolism of xenobiotics by CP450, steroid hormone synthesis, retinol metabolism and drug metabolism, while affecting ascorbate and aldarate metabolism, starch/sucrose metabolism and several signaling pathways related with cellular immune response. Understanding the defense mechanisms of *P. penetrans* against natural nematicides can lead to development of tailor-made control solutions.

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Barbosa P, Faria JMS, Figueiredo AC, Mota M, Vicente CSL (2022) Control of the root lesion *Pratylencus penetrans*: the effect of nematicidal of plant-derived compounds. Revista das Ciências Agrárias 45(4): 3-13.

195. Reaction of strawberry cultivars to *Meloidogyne incognita* and *M. luci*

Gokhan Aydınlı[1], Hatice Beyza Eren[2], Sevilhan Mennan[2]
1 Ondokuz Mayıs University, Bafra Vocational School, Department of Crop and Animal Production, 55400 Samsun, Türkiye
2 Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, 55139 Samsun, Türkiye

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: *Fragaria x ananassa*, resistance, root-knot nematodes

ABSTRACT:

Strawberry (*Fragaria x ananassa* Duch.), produced for industrial and fresh consumption, is one of the most profitable fruit species in Türkiye. With increasing yearly production, Türkiye ranks among the top five strawberry-producing countries in the world. Root-knot nematodes, *Meloidogyne* spp., have the potential to reduce strawberry production worldwide significantly. Host plant resistance would be a cost-effective strategy for managing nematode damage in strawberry. Therefore, it is essential to determine the host suitability of currently cropped strawberry cultivars against *Meloidogyne* species. This study aimed to evaluate the resistance of nine commercial strawberry cultivars (Albion, Festival, Fortuna, Morty, Rubygen, Sabrina, San Andress, Sweet Charlie, 406) to *Meloidogyne incognita* and *M. luci* in the pot experiments. The strawberry seedlings were inoculated with 5,000 eggs of *M. incognita* or *M. luci*. The experiment was carried out under non-controlled greenhouse conditions in a completely randomized design with five replications and was conducted twice. Sixty days after nematode inoculation, nematode eggs were extracted from the roots and counted. The susceptibility of the cultivars was determined by calculating a reproductive factor (Rf = final egg count / initial number of eggs inoculated) and evaluated as resistant (Rf<1) or susceptible (Rf>1).

There were differences ($p<0.05$) in the Rf values among the nine strawberry cultivars for each *Meloidogyne* species. On the cultivars inoculated with *M. incognita*, the Rf values ranged from 0.49 (Rubygen) to 2.09 (Festival). Four cultivars (Rubygen, San Andres, Morty and Albion) exhibited resistance response to *M. incognita* (Rf<1). The Rf values of *M. luci* on strawberry cultivars ranged from 0.55 (Rubygen) to 2.37 (Festival). In addition to the cultivars that responded as resistant to *M. incognita*, Fortuna was resistant to *M. luci* (Rf<1). These strawberry cultivars, which were resistant to the selected *M. incognita* and *M. luci* isolates in this study, might be used in crop rotation to reduce these *Meloidogyne* populations in the soil.

196. Pea breeding for resistance to plant-parasitic nematodes

María Córdoba-Sánchez[1], Diego Rubiales[1], Pablo Castillo[1], Juan Emilio Palomares-Rius[1]
1 Sustainable Agriculture Institute-CSIC, Avda. Menéndez Pidal, 14004, Córdoba, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: nematode, pea, *Meloidogyne*, *Heterodera*, *Pratylenchus*, resistance, plant breeding

ABSTRACT:

Pea (*Pisum sativum*) is a grain legume widely cultivated in temperate climates, which has known agricultural and environmental benefits. The dry pea is the most widely cultivated legume in both Europe and Spain and, although its cultivation has declined in recent years, it is recovering rapidly. Specifically, in Spain, it has doubled in the last decade. This legume crop can be affected by several diseases. For this reason, genetic resistance is a very important tool in a context of extensive, low-input cultivation. One of the diseases that can affect the pea crop are plant-parasitic nematodes. In this case, we will study two major groups of plant-parasitic nematodes that can cause significant losses in pea: sedentary endoparasitic nematodes (root-knot nematodes, *Meloidogyne* spp., and the pea cyst nematode, *Heterodera goettingiana*) and root-lesion nematodes (*Pratylenchus* spp.). Some levels of resistance have been found mainly in wild species (*Pisum* spp.) to *H. goettingiana*, and in some pea cultivars to root-knot nematodes, but no resistance sources have been identified for root-lesion nematodes. The main objectives of this work are 1) to perform molecular identification of these nematodes damaging pea, 2) to develop and apply protocols for identification and characterization of resistance in large collections of pea germplasm, 3) to identify sources of resistance to these plant-parasitic nematodes, and 4) to identify molecular markers by genome-wide association mapping. To carry out the molecular identification, a series of primers will be designed using ribosomal (ITS region) and mitochondrial (COI) markers in order to quantify and identify the presence of a given species in different soil and root samples. On the other hand, for the identification and characterization of resistance of Pisum to different species of nematodes. This collection has several species from many different parts of the world. One characterized and analysed, a series of GWAS analyses will be performed to identify genes that are potentially important in the resistance or tolerance of some pea species to the different nematodes used in the bioassays.

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197. Unraveling the role of carbon metabolism on the pathogenicity of pinewood nematode fungal antagonists, *Esteya* spp.

David Pires[1], Cláudia Vicente[2], Manuel Mota[2], Maria L. Inácio[1]
1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, Portugal
2 Mediterranean Institute for Agriculture, Environment and Development (MED) & Global Change and Sustainability Institute (CHANGE), University of Évora, Évora, Portugal

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: biological control, carbon sources, nematophagous fungi, pathogenicity, pinewood nematode

ABSTRACT:

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a quarantine organism in Europe and the causal agent of pine wilt disease (PWD), a serious threat to pine forests worldwide. PWD results from complex interactions between the nematode, its insect vector, *Monochamus* spp., and host plants (mostly conifers). In Europe, this pest was first detected in Portugal, and has since spread to Northwestern Spain and Madeira Island. The non-specificity of synthetic chemicals and lack of environmental-friendly options to control this parasite have driven a surge in research focusing on natural enemies capable of suppressing the PWN. *Esteya* spp. are nematophagous fungi and promising biocontrol agents against the PWN. Carbon sources and carbon-to-nitrogen ratio were shown to influence the production of pathogenic conidia in *Esteya*. In this sense, we investigate the utilization of 49 carbohydrates by five *Esteya* isolates, from two species and different geographical origins. This should provide new insights on the pathogenic mechanisms of these nematophagous fungi against the PWN, and open the door for future research on the role of carbon catabolite repression, a crucial strategy utilized by microorganisms in nutrient-limiting conditions in the infective cycle of *Esteya*.

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198. Host suitability of *Vitis* spp. to *Meloidogyne ethiopica* and *M. luci*

Ivania Esteves[1], Beatriz Cantarinho[1], Leidy Rusinque[1,2,3], Maria de Lurdes Inácio[2], Isabel Abrantes[1], Carla Maleita[1,3]
1 Centre for Functional Ecology - Science for People and the Planet, TERRA Associate Laboratory, Department of Life Sciences, Faculty of Science and Technology, University of Coimbra (UC), Coimbra, Portugal
2 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, Portugal
3 Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering, UC, Coimbra, Portugal

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords:

ABSTRACT:

The host suitability of *Vitis vinifera*, cultivar Cabernet Sauvignon, and five *Vitis* rootstocks (Rupestris du Lot, SO4, 99R, 1103P and 197.16), commonly used in Portugal, to the root-knot nematodes (RKN), *Meloidogyne ethiopica* and *M. luci*, was evaluated in a pot assay under controlled conditions. Ten plants from each cultivar/rootstock were inoculated with 500 *M. ethiopica*/*M. luci* eggs (Pi=initial population density). The viability of the nematode inoculum was confirmed on tomato cultivar Coração-de-Boi, at 60 days after inoculation with 500 *M. ethiopica*/*M. luci* eggs. Non-inoculated grapevine plants were also included as controls. The pots were maintained in a growth chamber with environmental controlled conditions (23±2°C, 12 h photoperiod, ±60% relative humidity). Eight months after RKN inoculation, the number of galls/plant, number of egg masses, eggs/egg mass, final population density (Pf) and the reproduction factor (Rf=Pf/Pi) were determined. Host suitability was assessed based on root gall index (GI) and Rf. The results obtained showed that rootstock 197.16 was more susceptible to *M. ethiopica* (GI= 5; Rf= 12.5 ± 4.4) than to *M. luci* (GI= 3.1; Rf= 1.1 ± 0.6), whereas Rupestris du Lot was resistant/hypersensitive to *M. luci* (GI= 1.7; Rf= 0.6 ± 0.3) and susceptible to *M. ethiopica* (GI= 2.3; Rf= 1.4 ± 0.97). Cabernet Sauvignon and rootstocks 99R, 1103P and SO4 were resistant (GI= 0; Rf= 0) to both species. Substantial variation of Rf values occurred among replicates of the same host. Since differences in the reproduction of *M. ethiopica* and *M. luci* were observed only in two rootstocks among the five and, conversely to previous reports, the results suggest that these grapevines are not suitable differential hosts to distinguish these two RKN species.

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199. Nematicidal potential of *Mentha x rotundifolia* hydrolate by-product

María Fe Andres[1], Carmen Elisa Diaz[2], Juliana Navarro-Rocha[3], Azucena Gonzalez-Coloma[1]
1 Instituto de Ciencias Agrarias. CSIC. Madrid, Spain
2 Instituto de Productos Naturales. CSIC. Santa Cruz De Tenerife, Spain
3 Centro de Investigación y Tecnología Agroalimentaria de Aragón. Zaragoza, Spain

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: *Mentha*, hydrolate, essential oil, *Meloidogyne javanica*, nematicidal effects

ABSTRACT:

Because of the environmental, food safety, and health-related issues associated with the application of chemical nematicides, there is growing emphasis on botanical-based control agents of plant parasitic nematodes. Essential oils (EO) are obtained from the distillation process of aromatic plants, generating hydrolates as byproducts. In agriculture, the pesticide effects of hydrolates have been extensively described and, specifically, the nematicidal potential of some hydrolates has been reported. *Mentha x rotundifolia*, also known as apple mint, is an aromatic and medicinal plant of ethnopharmacological interest and several studies have demonstrated the antioxidant, antimicrobial, antifungal, insecticide, and nematicidal activity of its essential oil. Following a process of crop domestication, a chemically stable population of *M. x rotundifolia* has been developed for further valorization. The in vitro nematicidal effects of Spanish *M. x rotundifolia* EO, with piperitone oxide as the main component, have been previously demonstrated. In this work, we have studied the in vitro and in vivo nematicidal activity of M. x rotundifolia hydrolate by-product, obtained from the semi-industrial vapor-pressure essential oil extraction, against the root-knot nematode, *Meloidogyne javanica*. The essential oil, the hydrolate, and its organic fraction caused high mortality of juveniles, suppressed egg hatching, and reduced nematode infection and reproduction in tomato plants. The nematicidal compounds of the hydrolate have been identified by the bio-guided fractionation of the organic extract. These findings have important implications for the development of new nematode control products based on hydrolate compounds and highlight the recovery of waste from essential oils extraction.

200. Potential of *Paenibacillus polymyxa* as biocontrol agent for *Meloidogyne* spp. in tomato

Wim M.L. Wesemael[1], Jackeline A. Bakengesa[2], Lieven Waeyenberge[3], Dieter Slos[3], Lirette M. Taning[3]

1 Flander Research Institute for Agriculture, Fisheries and Food ILVO, Merelbeke, Belgium and Ghent University, Department of Plants and Crops, Gent, Belgium
2 Ghent University, Department of Biology, Nematology unit, Ghent, Belgium
3 Flanders Research Institute for Agriculture, Fisheries and Food ILVO

Area: Chemical, Biological, Botanical and Cultural Control of Nematodes
Type: Poster
Keywords: *Meloidogyne incognita*, *Solanum lycopersicum*, organic production, endophytic bacteria

ABSTRACT:

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint for organic tomato production in Flanders, Belgium. *Meloidogyne incognita*, *M. hapla* and *M. javanica* occur on a regular basis in soils of organic greenhouses. The number of root-knot nematodes can amount to several thousand per 100 ml of soil. This is due to the rapid reproduction of these nematodes. Low initial numbers (1-10/100 ml of soil) in combination with a good host plant and optimal temperature results in very high final densities after a few months. The currently applied control measures such as the use of known resistant varieties, soil steaming or non-chemical soil disinfection are inadequate. In certain crops (including tomatoes), resistance is present in some cultivars (rootstocks), but this is often incomplete, i.e. not for all types of root-knot nematodes. With soil steaming, the entire soil life is killed, including the beneficial soil life, while it is precisely that useful soil life that is of great importance in organic cultivation. Biological control agents can be a valuable alternative and endophytic bacteria may be excellent candidates. These microorganisms colonize root tissues inter- and/or intracellularly and have a mutualistic interaction with host plants. The endophytes may improve plant growth by supplying the host with nutrients, conveying stress tolerance, or inducing systemic resistance. Research is often restricted to in vitro or pot experiments on effectivity and their suppressive mechanism has not been fully clarified. Potential of *Paenibacillus polymyxa* as a bio-control agent for root-knot nematodes (*M. fallax*, *M. chitwoodi*, *M. javanica*, *M. enterolobii*, *M. incognita* and *M. hapla*) was examined *in vitro* and *in vivo*. A 100% bacterial suspension (BS) caused J2 mortality and reduced hatching 97%-99% for all tested species. More than 90% J2 mortality occurred after 24 hours of exposure in 100% BS. In a naturally infested organic glasshouse a one-time application of *P. polymyxa* showed an effect on *M. incognita*, especially at lower initial populations. Through DNA barcoding changes in nematode communities were also monitored. With research on the role of endobacteria in the cropping system and the functional interactions among endobacteria, crops, and the environment combined with knowledge on the mode of action, future selection and use of endophytic bacteria will be enhanced.

201. Analysis of the plant secondary metabolites produced in response to the infection of *Pratylenchus penetrans*

Marina Costa[1], Joao Trindade[2], Jorge M.S. Faria[1], Maria L. Inácio[1], Dora Teixeira[2], Cláudia S. L. Vicente[3]

1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal.

2 Hercules Laboratory & Chemistry and Biochemistry Department of Science and Technology School, University of Évora, Palácio do Vimioso, Largo Marquês de Marialva, 7000-809 Évora, Portugal.

3 MED- Mediterranean Institute for Agriculture, Environment and Development & CHANGE- Global Change and Sustainability Institute, University of Évora, Pólo da Mitra, 7006-554 Évora, Portugal

Area: AREA 1
Type: Poster
Keywords: nematicide, *Pratylenchus* penetrans, potato cultivars, secondary metabolites, UPLC-QTOF-MS

ABSTRACT:

The root lesion nematode (RLN) *Pratylenchus penetrans* is a migratory endoparasite that affects high-value crops, resulting on the reduction of crop yield, and consequent economic impact. The application of pesticides increases crop production costs, causes environmental hazards, and health risks to humans. The study of plant metabolism in response to nematode infection can help to understand the mechanisms of plant defences and, consequently, in the identification of plant secondary metabolites (SM) with potential nematicidal proprieties. The aim of this study is to analyse the production of SM by tolerant and susceptible potato cultivars (*Solanum tuberosum*) infected with *P. penetrans*, using two methods of SM extraction, namely, the ultrasound method and a double methanol extraction. Roots of susceptible (cv. Agria and cv. Kennebec) and tolerant (cv. Laura) potato cultivars infected and non-infected (control) with *P. penetrans* were extracted using two different procedures. For each extraction method, three biological replicates, with four technical replicates each, were analysed using UPLC-QTOF-MS. To evaluate the extraction efficiency of the methods, the plant extracts were doped with known concentrations of the metabolites quercetin and azelaic acid, since these compounds were described has being potentially involved in plants defence mechanisms against pathogens. The optimization of the extraction method was performed using a QTOF-MS targeted approach for these compounds, and the chosen method will be used in the untargeted metabolomic analysis of the plant samples. Considering the current restrictions on the use of chemical nematicides, there is a need to search for more sustainable alternatives for RLN control.

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202. Compatibility of EPNs and essential oils: a novel approach for the integrated pest management of the truffle beetle

Ivan Julià Brunat[1], Marina Seco de Herrera[1], Ana Morton[1], Daniel Tapia[2], Juliana Navarro-Rocha[3], Fernando Garcia del Pino[1]
1 Universitat Autonoma de Barcelona, Bellaterra 08193, Spain
2 Instituto de Ciencias Agrarias, CSIC, Madrid 28006, Spain
3 Centro de Investigación and Tecnologia Agroalimentaria de Aragón, Zaragoza 50059, Spain

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: *Allium sativum*, *Satureja montana*, *Mentha suaveolens*, Steinernema feltiae, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*

ABSTRACT:

The European truffle beetle, *Leiodes cinnamomeus*, is the most important pest in black truffle (*Tuber melanosporum*) plantations. Entomopathogenic nematodes (EPNs) are promising biological control agents against *L. cinnamomeus*. Essential oils (EOs) are also recently being investigated for the control of the adults of this pest. Therefore, both control methods could be combined in Integrated Pest Management (IPM) programs to enhance their efficacy. However, limited information exists regarding the effects of the EOs on EPNs and so their compatibility. The aims of our work were to study the effects of three previously described insecticidal and nematicidal essential oils, *Allium sativum*, *Mentha suaveolens*, and *Satureja montana*, on the survival, infectivity, reproduction, and attraction behaviour of three EPN species: *Steinernema feltiae*, *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. Therefore, we conducted four experiments under laboratory conditions: lethal effect assay, sublethal effect assay, fumigant effect assay and chemotaxis assay. *Allium* EO caused the highest mortality rates in all three EPN species at 24 and 72 hours after application whether through direct contact in the lethal assay (97-99%) or fumigation (40-42%), and it also reduced their infective capacity on *Galleria mellonella*. *S. montana* EO caused significantly higher mortality rates (6-8%) than control (0-3%) at 72 hours in the lethal assay. It also displayed repellent properties against *S. feltiae* and *H. bacteriophora* in the chemotaxis assay. In contrast, *M. suaveolens* EO exhibited minimal impact on the survival, infectivity and reproduction of all three EPN species. Our results suggest *M. suaveolens* oil may be the most compatible EO for use integrated with EPNs. However, further validation under field conditions and in the presence of *L. cinnamomeus* is necessary to confirm the practical applicability of these findings.

204. Virulence of *Heterorhabditis bacteriophora* Azorean strains against *Popillia japonica* under laboratory and field conditions

Hugo R. Monteiro[1], Rubén Beltrí[1], Ángel Ros[1], Mário B. Teixeira[1], Jorge Frias[1], Nelson Simões[1], Anna Garriga[2]

1 Centro de Biotecnologia dos Açores, Faculdade de Ciências e Tecnologia, Universidade dos Açores, Portugal

2 Dept. Biologia Animal, Vegetal i Ecologia, Universitat Autonoma Barcelona, Spain

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: biological control, native strains, field application

ABSTRACT:

Popillia japonica was introduced on Azorean Islands in the early 1970s, and the great interest in controlling the pest using entomopathogenic nematodes (EPNs) has driven several investigations over the years. The EPN *Heterorhabditis bacteriophora* is the species with better performance against the larvae, although infectivity rates have proven inconsistent in actual field applications. With the recent invasion of *P. japonica* in Europe, there has been a renewed effort to identify efficient strains, thus leading to the isolation of native *H. bacteriophora* strains from the Azorean archipelago. The expectation is that these native strains will be better adapted to local climatic and soil conditions, resulting in improved performance in field applications. This study aimed to assess the virulence of six new Azorean isolates of *H. bacteriophora* and two established strains to third instar larvae of *P. japonica*. Both laboratory and field experiments were conducted to compare their effectiveness. In laboratory conditions, strains FA16 and Az29 were determined to be the most virulent, whereas strains such as FAPF were less effective. Conversely, in a field experiment involving constrained PVC plots, strain Az148 demonstrated to be the most virulent strain, whereas FA16 exhibited limited infection rates. These findings highlight the diverging virulence observed between laboratory and field experiments, emphasizing the importance of testing multiple native strains under environmental conditions, and recognizing that high EPN efficacy observed in laboratory conditions may not necessarily translate into the complexity of natural environments, as evidenced by the FA16 infection rates.

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205. Isolation and characterization of new *Heterorhabditis bacteriophora* strains from the Azores Archipelago

Rubén Beltrí[1], Anna Garriga[1,2], Hugo R. Monteiro[1], Duarte Toubarro[1], Nelson Simões[1]
1 Centro de Biotecnologia dos Açores - Faculdade de Ciências e Tecnologia, Universidade dos Açores, Portugal
2 Dept. Biologia Animal, Vegetal i Ecologia - Universitat Autonoma Barcelona, Spain

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: entomopathogenic nematodes, biological control, *Heterorhabditis bacteriophora*

ABSTRACT:

Entomopathogenic nematodes (EPNs) play a crucial role in biological pest control, and despite the availability of commercial strains, the ongoing quest for native nematodes adapted to diverse environments persists.

In the present study, six new strains of *Heterorhabditis bacteriophora* isolated from the Azores Archipelago were characterized, involving the establishment of taxonomic relationships and the evaluation of biological traits. This comprehensive analysis included phylogenetic assessment, mortality rates, lethal times, and measurements of reproductive potential. This approach allowed to identify native strains adapted to the local environment and compare their efficacy with a previously characterized highly virulent Azorean strain, Az29, using the model organism *Galleria mellonella*.

The results revealed varying infection rates among strains, with Az29 exhibiting higher efficacy due to a shorter median lethal time at a fixed dose (26.7h). In comparison, FAPF demonstrated lower efficacy at the same dose, with a median lethal time of 49.2 hours and only reaching 65% mortality after 72 hours of exposure. In lethal dose experiments, strains such as SMLV and SMTU showed comparable or slightly better performance than Az29 on *G. mellonella*. Alongside phylogenetic analysis and progeny measurements, diverse virulence profiles were identified. This characterization aids in evaluating the potential of isolates for screening against different insect pests, contributing to the ongoing development of sustainable pest management strategies.

The primary objective of this study was to gather crucial information for the practical application of locally isolated EPNs as biological control agents. The nematodes isolated in this study could potentially be employed in biological control programs against invasive pests, considering that native EPN isolates possess physiological traits adapted to local ecological conditions. Currently, the newly isolated EPNs are undergoing testing as biological control agents against pest insects, with a particular focus on targeting the Japanese beetle *Popillia japonica*.

Acknowledgements: This study had the support of the Fundação para a Ciência e a Tecnologia (FCT) through the Pluriannual Program for the Funding of R&D Units (UIDB/05292/2020 and UIDP/05292/2020) granted to CBA, and the project AGWAS-EPNs (2022.06153.PTDC). This work was supported by the project IPM-Popillia (grant Nr. H2020-EU.3.2.1.1. / ID: 861852), funded by the European Union s Horizon 2020 Research and Innovation Programme. The author's work is supported by the postdoctoral grant Margarita Salas from the Ministerio de Universidades (Spain).

206. The first detailed determination of entomopathogenic nematodes in Samsun

Nida Doluel[1], Gokhan Aydinli[2], Sevilhan Mennan[1]
1 Ondokuz Mayis University, Agricultural Faculty, Turkey
2 Ondokuz Mayis University, Bafra vocational School, Turkey

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: EPN, Samsun

ABSTRACT:

The aim of this study was to determine the entomopathogenic nematodes (EPN) in mostly natural parks and forests (60) and rarely agricultural areas (40) which are using to grow hazelnut and corn in Samsun Province, Middle black see Region of Turkey during 2023. A total of 100 soil samples were collected and EPNs were obtained by using the insect bait method, the last instar larvae of *Galleria mellonella* L. (Lep.: Pyralidae) were used as bait.

Of the 100 soil samples collected, 11 % were positive for entomopathogenic nematodes. Although studies on the detection of EPN have been carried out in many areas in Turkey, it is the first comprehensive survey conducted to determine EPN in Samsun, and the percentage of EPN detection is generally above the Turkey average. The samples obtained generally belong to the Heterorhabditidae and Steinernematidae families, and species identification continues using morphological and molecular methods.

207. Occurrence of *Oscheius insectivorus* in Iran, a new record, and its potential as a biocontrolling species

Mohammad Amiri Bonab[1], Mohammad Reza tighi[1], Didar Azizi[1], Majid Pedram[1]
1 Tarbiat Modares University, Tehran, Iran
2 Tarbiat Modares University, Tehran, Iran
3 Tarbiat Modares University, Tehran, Iran
4 Tarbiat Modares University, Tehran, Iran

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: Bioassay, identification, molecular, Rhabditidae

ABSTRACT:

In a survey on entomopathogenic/insect related nematodes occurring in northern Iran, an isolate of *Oscheius* sp. was isolated form a nymph of a scarabaeid beetle, collected from Golestan province. A number around 500 individuals of females and juveniles of the abovementioned species were recovered from the haemocoel of the dissected specimen. About 100 specimens of the recovered population were retained on the cadaver of the dissected beetle on a water agar plate. One female specimen was separately put on another water agar plate. The plates were incubated at 28°C. During a period of 3-20 days, both plates were inspected. A high number of specimens were recovered from the plate including the cadaver of insect in 3-5 days. The increasing of the population in second plate occurred in 20 days. This species is mostly characterized by 2030-2850 µm long females, six separated lips and E-pore located in basal bulb region. The D2-D3 expansion segment of this species was sequenced. A BLAST search using this locus revealed it has maximal identity with two sequences EU195968 (belonging to an European isolate of *O. insectivorus*) and MN381940 (recorded in GenBank as *O. shamimi*). The morphological characterization of this species is in progress.

The infection ability of this isolate on *Galleria mellonella* larvae was evaluated in both soil and water agar plates. A high rate of mortality was observed in both trials after 3-5 days.

208. Pathogenicity tests with entomopathogenic nematodes isolated from chestnut groves on *Cydia* and *Curculio* larvae

Sara Amoriello[1], Agostino Strangi[1], Immacolata Iovinella[1], Chiara Sciandra[1], Leonardo Marianelli[1], Pio Federico Roversi[1], Giulia Torrini[1]
1 CREA Research Centre for Plant Protection and Certification, Florence, Italy

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: Biological control agents, chestnut phytophagous insects, *Steinernema carpocapsae*, *Steinernema feltiae*

ABSTRACT:

The Italian chestnut-growing, particularly in rural areas, continues to represent an ecological niche and a reservoir of cultural and historical significance, as well as an important economic source for farmers.

Chestnut cultivation is threatened by phytophagous insects that cause early drops of fruits and considerable losses of harvested product. Tortrix moths [*Cydia fagiglandana* (Zeller), *C. splendana* (Hübner), *Pammene fasciana* (L.)] and chestnut weevils (*Curculio elephas* Gyllenhaal, *C. propinquus* Desbrochers des Loges) are considered among the most economically damaging native species.

The study of entomopathogenic nematode (EPN) communities in these cultivated areas aims to enhance the knowledge useful for the development of low-impact control strategies, in order to promote the proliferation and conservation of these natural biological control agents for the control of harmful insects.

Monitorings were carried out in autumn and spring period, in two different types of chestnut groves in Northern Italy: pure and mixed with other tree species.

A clear predominance of EPNs was recorded in mixed chestnut groves, where the genus *Steinernema* was the most frequent. In particular *S. feltiae* was the most abundant species (31.3% of positive samples), however it was exclusively isolated in mixed chestnut groves. Moreover, two new *Steinernema* species were isolated in the spring period sampling, one only in mixed and the other in both types of chestnut grove. These species are under description.

Laboratory tests were carried out to assess the pathogenicity of *S. feltiae* and *S. carpocapsae* strains isolated from chestnut groves against *Cydia* and *Curculio* larvae. The results obtained indicate that both EPN species are excellent biocontrol agents. In particular, *S. carpocapsae* gave the best results against both pests, killing 100% of *Cydia* and *Curculio* larvae.

209. Virulence of native populations of *Steinernema feltiae* (Filipjev) against *Lycoriella ingenua* (Dufour)

Nikola Grujic[1], Tanja Drobnjakovic[2], Nikola Andjelkovic[1], Jelena Lukovic[2], Svetlana Milijasevic-Marcic[2], Ljiljana Santric[2], Ivana Potocnik[2], Dejan Marcic[2]
1 University of Belgrade-Faculty of Agriculture, Belgrade, Serbia
2 Institute of Pesticides and Environmental Protection, Belgrade, Serbia

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: *Agaricus bisporus*, entomopathogenic nematodes, mushroom fly

ABSTRACT:

Pest and disease control in cultivated mushroom production in Serbia is mainly based on chemical pesticides that are toxic to non-target organisms, humans and the environment. The European and national policies, as well as the United Nations Sustainable Development Goals, strongly encourage the use of low-risk active ingredients and sustainable alternatives to chemical pesticides in mushroom disease and pest control. The mushroom fly, *Lycoriella ingenua* (Dufour) (Sciaridae: Diptera) is the most damaging mushroom pest in Serbia and globally. The aim of the study was to evaluate the virulence of two locally isolated populations of *Steinernema feltiae* (Filipjev) (strains P9 and K2) against the mushroom fly.

Nematodes were reared on grater wax moth last instar larvae, and infective juveniles (IJ) not older than 15 days were used in the experiments. Experiments were conducted in plastic Petri dishes (35 mm in diameter) with the bottom surface covered with filter paper. Volume of 0.5 ml of nematode suspension containing 0, 50, 100, 200, 500, and 1000 IJ were applied to filter paper and 10 fourth larval instar of *L. ingenua* were added subsequently in each Petri dish. Every concentration of both nematode populations were replicated five times and whole experiment was repeated twice. The mortality of mushroom fly larvae was recorded three and seven days after exposure to nematodes. Log-probit regression analyses of the concentration-mortality data showed that both local populations of *S. feltiae* tested caused significant mortality of the fourth instar larvae of *L. ingenua*. In the dose-response bioassays, the following LC50s (median Lethal Concentrations) were estimated: 15.7718 and 18.2742 nematodes/larva (i.e. 16 and 18 IJ/cm2), for populations P9 and K2, respectively. Values LC90 for both populations corresponded with the highest IJ concentration used, around 100 IJ/cm^2. A lethal dose ratio test showed that the local population P9 was significantly more virulent toward the mushroom fly larvae. Experiments regarding *in vivo* testing of efficacy of local populations of *S. feltiae* against *L. ingenua* are ongoing.

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210. Potential of different strains of *Steinernema feltiae* (Filipjev) to control *Lycoriella ingenua* (Dufour) and impact on mushroom yield

Nikola Grujic[1], Tanja Drobnjakovic[2], Nikola Andjelkovic[1], Jelena Lukovic[2], Svetlana Milijasevic-Marcic[2], Ljiljana Santric[2], Ivana Potocnik[2], Dejan Marcic[2]
1 University of Belgrade-Faculty of Agriculture, Belgrade, Serbia
2 Institute of Pesticides and Environmental Protection, Belgrade, Serbia

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: *Agaricus bisporus*, entomopathogenic nematodes, mushroom fly

ABSTRACT:

The study was focused on improvement of integrated management strategy against the mushroom fly *Lycoriella ingenua* (Dufour) on the white button mushroom *Agaricus bisporus* (Lange) Imbach. Impact of different strains of entomopathogenic nematodes (EPN) *Steinernema feltiae* (Filipjev), commercial and two indigenous from Serbia (K2, P9), on regulation of mushroom sciarid fly and the influence on mushroom yield was evaluated. Experiment was conducted in air-conditioned mushroom growing chamber. Commercially produced phase III mushroom substrate were placed into 24 plastic containers (w 31 × d 21.5 × h 13 cm). Each plastic container was inoculated with 20 last instar larvae of *L. ingenua*. EPN were produced on last larval instars of *Galleria mellonella* L. and less than a week old infective juveniles (IJ) were used in the experiment. Nematode suspension of each of the three EPN strains containing 50000 IJ were added on the substrate surface in each plastic container on the day before casing, at the casing time and seven days after casing. Containers were held in the insect rearing cages and density of adults of *L. ingenua* was observed weekly using yellow sticky traps inside each cage. Preliminary results of ongoing experiment showed the highest efficacy in pest control of commercial EPN strain, followed by two native strains. No statistically significant differences were found in efficacy between two Serbian strains. Also, no statistically significant differences in mushroom yield were observed among control and all treatments using different strains of EPNs.

This research was supported by the Science Fund of the Republic of Serbia: Green program of cooperation between science and industry #GRANT No 3/4848 (2023-25) Microbial recipe for edible mushroom production - MICRO-MUSH, and Ministry of Science, Technological Development and Innovations of the Republic of Serbia: project No 451-03-47/2023-01/ 200214.

211. *In vivo* vs *in vitro* activation of *Heterorhabditis bacteriophora* from the transcriptomics perspective.

Jana Ilgová[1], Sara Sreibr[2], Pavel Dobes[2], Jiri Vorel[3], Jacek Marciniak[2], Jana Hurychová[2], Martin Kasny[1], Pavel Hyrsl[2]

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
2 Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
3 CESNET, 160 00 Prague 6, Czech Republic

Area: Entomopathogenic Nematodes
Type: Poster
Keywords:

ABSTRACT:

Entomopathogenic nematodes (EPNs), such as *Heterorhabditis bacteriophora*, have gained recognition as effective biocontrol agents against insect pests, offering an eco-friendly alternative to chemical insecticides. This preliminary study delves into the molecular dynamics of *H. bacteriophora* infection, shedding light on the responses to host molecules.

Invasion strategy of *H. bacteriophora* involves the active penetration of the host by third-stage larvae, also called infective juveniles (IJs). Upon contact with the host or its molecules, IJs start the process called activation or recovery, which involves the transition from the non-active stage to the infective one. Within a few hours after the host colonisation, IJs release symbiotic bacteria of the genus *Photorhabdus*, which cause host septicaemia and death.

Under experimental conditions, the infection process and activation of IJs are often simulated by challenging IJs with various host-derived materials (such as tissue homogenate or haemolymph). This study aims to bridge the gap in understanding how *in vitro* activation of *H. bacteriophora* corresponds to the *in vivo* infection.

We are conducting a comparative RNA-seq analysis at various time points throughout the *in vitro activation* and *in vivo* infection of the greater wax moth, *Galleria mellonella*. Our analysis focuses on five critical time points (3, 6, 9, 12, and 15 hours post-infection) to capture the dynamic changes in *H. bacteriophora* gene expression during IJs infection in vivo. *In vitro* activation involves exposure of IJs to *G. mellonella*-derived homogenates for three periods (3, 6 and 9 hours), simulating the interaction with host tissues.

Recognizing the low recovery of IJs after *in vivo* infection, we employ a single-cell RNA NGS library preparation strategy, followed by sequencing at the Illumina NovaSeq 6000 platform. Differential expression analysis will identify key transcripts by mapping reads to a reference transcriptome. Our objective will be to evaluate the extent to which *H. bacteriophora* gene expression during *in vitro* activation mirrors the *in vivo* infection dynamics.

This pilot data will contribute to the understanding of *H. bacteriophora in vivo* molecular strategies and address the relevance of *in vitro* activation models in studying the infection process.

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212.

Daniela Vidaurre [1], Edna Bode[1], Li Su[1], Helge B. Bode[1]
1 Department of Natural Products in Organismic Interactions, Max-Planck Institute for Terrestrial Microbiology, 35043 Marburg, Germany
2 Molecular Biotechnology, Department of Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany
3 Department of Chemistry, Philipps-Universität, Marburg 35043, Germany

Código: 208
Area: AREA 1
Type: Poster

Keywords:
ABSTRACT:

213. Monitoring the *Photorhabdus* spp. bacterial load in *Heterorhabditis bacteriophora* dauer juveniles over different storage times and temperatures: a molecular approach.

Christopher Ogaya[1]
1 e-nema, Germany

Area: Entomopathogenic Nematodes
Type: Poster

Keywords: *Photorhabdus laumondii*, single copy gene, *Heterorhabditis bacteriophora*, qPCR, WT strains

ABSTRACT:

The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* and *Photorhabdus* spp. bacteria are obligate symbiotic partners. The EPN dauer juveniles (DJs) search for new insect hosts, penetrate them, and release a few bacterial cells in the haemocoel. Thereafter, the bacteria grow exponentially, overrun the insect's immune system, and kill the host. To date it is well known that the interaction between environmental stresses and storage time can affect survival of *H. bacteriophora* DJs. However, it is not well known to which extent stress conditions can affect the symbiotic bacterial load inside the DJ. Therefore, for EPN as industrially produced biological control agents, tracking the symbiotic bacterial cells in the DJ can be a very useful tool. We have used the available genetic information from both bacteria and nematode partners, to develop a low-skill-demanding fast assay to monitor the bacteria in the DJs of *H. bacteriophora* DJs. By screening the genome of the *P. laumondii* DE2 strain, single copy genes with no homology to any other bacterial accessions were identified and used for absolute quantification of DJ bacterial cells through qPCR assays. Subsequently, we evaluated the bacteria in different *H. bacteriophora* materials of varying ages (storage times). A 750-fold reduction in bacteria was detected between freshly produced *H. bacteriophora* DJs and 300-days stored DJs carrying the *P. laumondii* strain DE2. In parallel the bacteria were monitored in long time stored *H. bacteriophora* WT strains. A higher bacterial density was detected in *H. bacteriophora* WT strains after a long storage period (> 300 days). Additionally, we monitored the bacteria in formulated long time-stored nematodes at different temperatures. A rapid decrease in the bacterial density over time for nematodes stored below the optimal storage temperature

214. Heterologous expression of a Cysteine-rich secretory protein (CAP) from entomopathogenic nematode with Sterol-binding activity

Jorge Frias[1], Duarte Toubarro[1], Hugo Monteiro[1], Nelson Simões[1]
1 Biotechnology Centre of Azores (CBA), Faculty of Sciences and Technology, University of the Azores, 9500-321 Ponta Delgada, Açores, Portugal

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: entomopathogenic nematodes, cysteine-rich secretory protein, sterol-binding activity

ABSTRACT:

The control of insect pests in agriculture presents significant challenges, including the development of pest resistance, and the adverse effects of chemical insecticides on beneficial species, human health, and the environment. In response to this problem, there is a need to explore safe and effective alternatives for pest control. This brings the attention of the scientific community to bioinsecticides, which can be natural organisms, but also their corresponding metabolic products or natural molecules, which can be used against insect pests. The *Steinernema carpocapsae* nematode is known to release several excretory/secretory products (ESPs) upon contact and during the parasitic infection process of their hosts. Different proteins have been identified in the nematode ESPs, some of which are immunomodulators, and others cause tissue damage and insect lethality. One family of proteins that were found in the ESPs of this nematode is the CAP (cysteine-rich secretory protein/antigen 5/pathogenesis related-1) protein., but the functional role of these proteins in their respective hosts, remains unknown. To e*luci*date the biological function of the Sc-CAP protein, this study focused on the cloning, heterologous expression, and characterization of this secreted protein, which was first identified in the ESPs from the nematode s parasitic stage and sequence retrieved from transcriptomic analysis. The structural comparisons of the Sc-CAP protein model determined by AlphaFold2 were conducted, revealing related structures from other parasitic nematodes of vertebrates, such as *Brugia malayi*, *Necator americanus*, *Ancylostoma caninum*, and *Heligmosomoides polygyrus*. Several studies reported that this family of proteins released by parasitic nematodes has sterol binding ability. The expression conditions and optimization of the recombinant protein were investigated, and both in silico docking analysis and in vitro cholesterol-binding assays were conducted to assess the protein s sterol binding capacity. The results showed that the Sc-CAP protein has a strong binding affinity to cholesterol and docking analyses of sterols on the CAP protein revealed potential molecular interactions. Thus, suggesting that this venom protein being able to bind to these types of compounds may have immunomodulatory or lipidic sequestration functions against insects. This study provides valuable insights into the potential role of CAP proteins in bioinsecticide development and highlights the importance of exploring natural, biological alternatives for pest control in agriculture.

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215. Characterization of *Heterorhabditis indica* isolate and investigation of its efficacy against the European corn borer

Tevfik Ozalp[1], Esmeray Ayhan[1], Erkan Yilmaz[1]
1 Bornova Plant Protection Research Institute. Turkey

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: EPN, *Ostrinia nubilalis*, *Heterorhabditis indica*

ABSTRACT:

Entomopathogenic nematodes (EPNs) are biological control agents used to control of different insect pests. It is important to investigate the efficacy of EPNs in different insects in order to match the appropriate EPN species with the targeted pest for effective integrated pest management programs. Though primarily known as a corn pest, the European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae) exhibits a broad host range among herbaceous plants, potentially causing significant economic damage beyond corn production. This study aims to molecularly identify this entomopathogenic nematode found in a soil sample taken from a greenhouse in Antalya, Türkiye, and investigate its infection ability to *O. nubilalis*. For this purpose, DNA was isolated from nematodes cultured on *Galleria mellonella* and PCR was performed using the D2A/D3B primers that amplify to the D2-D3 region of the 28s rDNA. The PCR product was then sequenced. The nematode was identified as Heterorhabditis indica according to analysis performed with NCBI BLASTn. The phylogenetic relationship of the *H. indica* isolate with other *Heterorhabditis* spp. was investigated. In vitro studies, 250 ij and 500 ij were inoculated into the late-stage *O. nubilalis* larvae. It was observed that deaths began in the O. nubilalis larvae 36 hours after both inoculation densities. The mortality rate was found to be 87.2% and 100% after 48 hours at 250 ij and 500 ij, respectively. To our knowledge, this is the first study to investigate the efficacy of *H. indica* on *O. nubilalis* larvae.

216. Characterization of a new species of entomopathogenic nematode *Oscheius* sp. (Nematoda: Rhabditidae) from Georgia (Caucasus)

Oleg Gorgadze[1], Alessio Vovlas[2], Alberto Troccoli[2], Elena Fanelli[2], Eustachio Tarasco[3], Ebunoluwa Ajobiewe [2], Francesca De Luca[2]
1 Institute of Zoology of Ilia State University, Tbilisi 0162, Georgia
2 Institute for Sustainable Plant Protection-CNR, Via Amendola 122/D, 70126 Bari, Italy
3 Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari – 'A. Moro', via G. Amendola, 165/A, 70126 Bari, Italy

Area: Entomopathogenic Nematodes
Type: Poster
Keywords: insectivora-group, molecular, *Oscheius*, morphometrics, Phylogeny, Taxonomy

ABSTRACT:

Surveys in the village of Shamgona in Samegrelo Region of West Georgia, a new nematode species belonging to *Oscheius* genus was isolated from soil of hazelnut orchard. Leptoderan bursa and spicules with hook-like endings are the main characters for assigning the new species to Insectivora group. The new *Oscheius* sp. is closely related to *O. colombiana*, *O. carolinensis*, *O. myriophila*, and *O. rugaensis* at morphological level. The most prominent distinctive morphological feature is the presence of eight lateral lines. The female has the largest body and widest body size, respectively, 1816 (1591-2329) µm and 136 (106-143) µm compared with other *Oscheius* spp. Spicule sizes of males and values of coefficients a and c are also different. Entomopathogenecity tests were carried out on *Galleria mellonella* and *Tenebrio molitor*. Molecular analyses were carried out by amplifying ITS, D2-D3 expansion domains and mitochondrial COI as markers. BLAST Search at NCBI revealed no corresponding sequences.

217. Partial biofumigation with brassica cover crops for potato cyst nematode suppression

Francis Kawalya[1], Matthew Back[1], Simon Edwards[1], Joe Roberts[1], Roy Neilson[2], Jonathan Snape[3]
1 Harper Adams University, Newport, TF10 8NB, UK
2 James Hutton Institute, Invergowrie Dundee DD2 5DA, Scotland, UK
3 James Hutton Limited, Invergowrie Dundee DD2 5DA, Scotland, UK

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: partial biofumigation, brassica cover crops, potato cyst nematodes

ABSTRACT:

Potato cyst nematodes (PCN), *Globodera* rostochiensis and *G. pallida*, are economically important pests of potatoes that account for an annual yield loss in the UK of about £31 million. Most effective nematicides have been withdrawn due to human health and environmental concerns, creating a need for sustainable management strategies. Such strategies include partial biofumigation using cover crops that produce glucosinolates from their roots to generate biocidal volatile organic compounds. A glasshouse study investigated the suppression of PCN eggs by partial biofumigation with brassica cover crops in relation to the microbial activity of the soil. Potato cyst nematodes, *G. pallida* of size 300-500 µm were placed in nylon sachets and seeded in 20 cm diameter pots containing either autoclaved or non-autoclaved soil. Five cover crop treatments including *Brassica carinata* var. Cappuccino, *B. juncea* var. Caliente 199, *Eruca sativa* var. Trio, *E. sativus* var. Nemat and *Raphanus sativus* var. Terranova were tested with fallow (unplanted) pots used as a negative control. Each cover crop treatment was planted in autoclaved or non-autoclaved soil and maintained in the glasshouse set at 15/5° C Day/night temperatures, respectively for 12 weeks. Dry root biomass, viability of PCN eggs, and total microbial activity were assessed after the termination of the experiment. The root biomass between brassica cover crops varied with R. sativus showing the greatest biomass. Furthermore, *B. juncea*, *R. sativus* and *E. sativus* var. Nemat reduced the viability of PCN eggs when compared to the fallow treatment. Similarly, the soil type (autoclaved and non-autoclaved soil) affected the total viability of PCN and the total microbial activity during the study with a significant reduction in PCN viability within non-autoclaved soil whereas the total microbial activity was significantly higher in the non-autoclaved soil when compared to the autoclaved soil. This study suggests that brassica cover crops such as *B. juncea* and *R. sativus* can reduce PCN viability and are candidates for PCN management by partial biofumigation. Further work is needed to validate the observations made from this study at the field scale.

218. The response of resistant pepper and wild eggplant cultivars to different inoculation levels of *Mi-1.2* virulent and avirulent isolates of *Meloidogyne incognita*

Gulsum Uysal[1], Zübeyir Devran[3]
1 Bati Akdeniz Agricultural Research Institute, Akdeniz University, Antalya, Türkiye
2 Akdeniz University, Antalya, Türkiye
3 Plant Protection Department, Faculty of Agriculture, Akdeniz University, Antalya, Türkiye

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: eggplant, Me1 gene, pepper, resistance, root-knot nematode

ABSTRACT:

Root-knot nematodes (*Meloidogyne* spp.) are one of the major risks to vegetables in agricultural production. Infection of vegetables by *Meloidogyne* spp. can cause a severe decrease in the yield. In recent years, with increasing environmental awareness and the effective use of developing technology in agricultural production, there is a tendency towards alternative control methods to chemicals. Successful management of root-knot nematodes in vegetable production is mainly done with the use of resistant cultivars. Densities of root-knot nematodes can affect the performance of resistance genes. The aim of the study was to investigate the reaction of different inoculation densities of *Mi-1.2* virulent (V6) and virulent (S6) *Meloidogyne incognita* isolates on resistant pepper bearing *Me1* gene cv. MT01 and wild eggplant *Solanum torvum* cv. Hawk. The study was conducted under the controlled conditions (25±1°C, 60±5% humidity and 16:8 photoperiod). Experiments were designed based on a randomized block design with 5 replications and repeated two times. Second stage juveniles (J2s) of *M. incognita* isolates were inoculated the plants when they had four true leaves seedlings. 5000 J2s and 10000 J2s were inoculated into each plant s two cm depth of root zone. Plants were uprooted eight weeks after inoculation and then softly washed the roots under the tap water. The numbers of galls, egg masses on roots were evaluated and indexed according to 0-5 scale. As a result of the study, *Mi-1.2* virulent and avirulent *M. incognita isolates* showed different reactions in two inoculum densities on resistant pepper cv. MT01 and *S. torvum* cv. Hawk. Gall indices were found 3 and 3.7 on resistant pepper and 2.8 and 3.6 on *S. torvum*, respectively, at 5000 and 10000 J2s inoculum levels. However, *Mi-1.2* virulent isolate caused 5 and 2 gall indices on pepper and eggplant in both inoculum levels.

219. Successful eradication the root knot nematode *Meloidogyne luci* in Slovenia

Melita Theuerschuh[1], Saša Širca[1], Nik Sisic[1], Barbara Geric Stare[1]
1 AGRICULTURAL INSTITUTE OF SLOVENIA

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: eradication, *M. luci*, root knot nematode

ABSTRACT:

The root knot nematode (RKN) *Meloidogyne luci* was found in Slovenia in a greenhouse in the village of Šmartno near Ljubljana during a RKN field survey in 2015. The infested tomato plants showed symptoms of decline, leaf chlorosis and large galls on the roots. The nematode presence was high as more than 80 % of plants were heavily infested. RKN were spread over approximately 1,000 m2 in two greenhouses. Tomato roots were examined and thirty RKN females were isolated for species identification using isozyme patterns. The nematodes were identified as *M. luci* based on L3 esterase phenotype and N1 malate dehydrogenase phenotypes. Identity of *M. luci* was confirmed with sequence analysis of mtDNA, stretching from the gene for cytochrome oxidase subunit II through to a portion of the 16S rRNA gene (acc. nos. LT837518 and LT837519). This pest species was already found in Slovenia in 2003 in Dornberk (Primorska region), at that time reported as *M. ethiopica*. However, finding of *M. luci* near Ljubljana in 2015 represented a new finding at different region approximately 100 km apart from the 2003 location. What is more, *M. luci* presents a serious threat to agricultural production if it spreads to other growing areas since it has a very broad host range of monocotyledonous and dicotyledonous plants, including important agricultural crops such as tomatoes, peppers and potatoes. In 2015, strict phytosanitary measures were therefore ordered for the infested area, including the application of Dazomet, a soil sterilant. All infested plant material from the affected greenhouse was destroyed. The strict phytosanitary measures were in place for several years. The infested area was monitored every year. The following eradication measures were implemented: use of the plant protection product Velum Prime (Bayer) with the active ingredient fluopyram and cultivation of: (i) tomato varieties that are resistant to *M. luci* (*Solanum lycopersicum*), (ii) poor hosts or non-host plants for *M. luci* (i.e. peppers *Capsicum annuum*, chillies *Capsicum annuum*) and (iii) medium-good hosts: (i.e. aubergines *Solanum* melongena). Strict plant protection measures and close cooperation with the owner of the infested greenhouses led to a successful eradication of *M. luci* at this location. In 2022 and 2023, the presence of *M. luci* was not detected anymore at this location which constitutes as successful eradication of RKN *M. luci* in Slovenia. As the climate conditions are favourable for survival of *M. luci* in many regions of EPPO countries, the authors suspect that this nematode species could already be or become more widespread in Slovenia and possibly in other European and Mediterranean countries. Further, the climate change with increased temperatures may result in even significantly greater spread and damage in several agricultural crops in the open field by *M. luci* in the future.

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220. Activity of medium-carbon chain fatty acids against the root lesion nematode

Jorge M S Faria[1], Pedro Barbosa[3], Maria L Inácio[1], Cláudia S L Vicente[3]

1 INIAV, I.P., National Institute for Agrarian and Veterinary Research, Quinta do Marquês, 2780-159 Oeiras, Portugal ,

2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

3 MED, Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Évora University, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: direct contact bioassays, fatty acids, nematicides, root lesion nematodes, sustainable pest management

ABSTRACT:

The root lesion nematodes (RLN), *Pratylenchus* sp., can pose a significant threat to important crop species, e.g., potato, onion, or carrot, with the development of large populations, which can heavily reduce crop productivity. The pest management strategies imposed are many times not entirely successful given the migratory endoparasitic nature of these pests allied to a commonly wide host range. Currently, research is focusing on sustainable cultural practices and the development of biopesticides or biocontrol agents. Medium-chain length fatty acids extracted from aromatic plants have shown a high nematicidal activity against other plant parasitic nematodes, namely, the root-knot nematodes or the pinewood nematode. In the present work, decanoic, undecanoic and dodecanoic acids, C10, C11, and C12 fatty acids, respectively, were tested against the RLN *P. penetrans*, in direct contact bioassays, for 24 h. These compounds were added to aqueous suspensions of the RLN, at 1 mg/mL. Dead and live nematodes were counted and compared to control assays. Decanoic acid (C10) showed the strongest activity, inducing 100% mortality, while the activities of undecanoic (C11) and dodecanoic (C12) acids were lower, 94 and 40 %, respectively. Activity appears to be related to carbon chain length which suggests that these compounds might act on specific groups of proteins.

221. Management of *Meloidogyne incognita* through the use of resistant pepper varieties and rootstocks under the greenhouse

Caridad Ros Ibáñez[1], Celia Martínez Mora[1], Adriana Fuensanta Esteban López[1], Victoriano Martínez Alarcón[1], María Ángeles Hernández Colucho[1], Ana María Fita Fernández[2]

1 Instituto Murciano de Investigación y Desarrollo Agrario y Mediambiental, Murcia (IMIDA), Spain
2 Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: root knot nematodes, resistance genes, nematode management, *Capsicum annuum*, undisinfected soil

ABSTRACT:

The cultivation of pepper is one of the emblematic crops of Campo de Cartagena (Southeast of Spain), the strong restrictions on the use of soil fumigants and the limitation of the use of sheep manure for biosolarization in the management of soil pathogens, for Being an area vulnerable to nitrates, paints a difficult scenario that threatens the sustainability of the crop. Last season, many farmers grew varieties and grafted plants on nematode-resistant rootstocks on undisinfected soil. With the aim of studying the viability of this practice, 5 commercial varieties carrying nematode resistance genes (Cabanas, Kation, Katmandu, Nelto, Pretoria) and 4 resistant rootstocks (Alcos, Beldrock, Robusto and Oscos) were evaluated. cultivated the susceptible variety Maestral (Fito seeds) without grafting in a greenhouse homogeneously infested with a population of *M. incognita* in the process of virulence selection for the Me3 gene for resistance to the nematode. Nematode population density and nematode incidence and severity were measured during cultivation every 6-8 weeks and at the end of the trial and production. The varieties carrying the Me1 resistance gene were less affected by the nematode than those carrying the Me3 gene, which did not show significant differences with respect to the susceptible ungrafted variety. The population density in the soil at the end of the crop decreased between 57-99%, compared to the susceptible variety, where genotypes carrying the Me1 gene were grown. The differences in production are due to the intrinsic characteristics of each genotype, however the rootstocks improved the performance of the susceptible variety without grafting. Preliminary results show that the cultivation of varieties carrying the Me3 gene in soils infested with populations virulent to the gene could affect yield. On the other hand, it remains to be verified whether the reiteration of genotypes carrying the Me1 gene in undisinfected soil could lead to the appearance of nematode population to this gene.

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222. *Meloidogyne chitwoodi* management using *Solanum* extracts

Laura Soraia Perpétuo[1], Fátima Nunes[2], Isabel *Luci* Conceição[1], Maria José Cunha[3], Maria Teresa Batista[4]

1 University of Coimbra, Centre for Functional Ecology - Science for People and the Planet (CFE), Associate Laboratory TERRA, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra
2 University of Coimbra, Faculty of Pharmacy, Azinhaga de Santa Comba, 3000-548 Coimbra
3 Polytechnic University of Coimbra, Research Centre for Natural Resources Environment and Society (CERNAS) and Coimbra Agriculture School, Bencanta, 3045-601 Coimbra
4 University of Coimbra, CERES, Department of Chemical Engineering, 3030-790 Coimbra

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: aqueous extracts, integrated nematode management, plant-derived nematicides, root-knot nematodes

ABSTRACT:

Plant-parasitic nematodes (PPN) like the root-knot nematode (RKN) *Meloidogyne chitwoodi* are key factors in reducing crop quality and yield, thereby impacting global trade and market prices. This study explores the potential of *Solanum linnaeanum* and *S. sisymbriifolium* aqueous extracts and their fractions in managing this PPN quarantine species. Using a combination of phytochemical analysis and in-vitro bioassays, the research evaluates the nematicidal potential of these *Solanum* species, which are known for their resistance to *M. chitwoodi*. The study focuses on the efficacy of root extracts of S. linnaeanum (Sl R) and immature fruit extracts of S. sisymbriifolium cv. Sis 6001 (Ss F) on the nematode hatching, infectivity, and reproduction. Phytochemical composition was screened by High Performance Liquid Chromatograph coupled to a Photodiode detector and a Linear Ion Trap Mass Spectrometer (HPLC-PDA-LIT/MS). Ions at m/z 414 were identified, suggesting the presence of different phytochemicals with the spirostane structure derived of the solasodine, a steroidal alkaloid. In addition, ions at m/z 271 and 253 detected in Ms2 and Ms3 support this identification. The most active fraction obtained from the fractionation of Sl R and Ss F extracts showed significant reduction in *M. chitwoodi* infectivity compared to controls and registered the highest concentration of steroid structure phytochemicals. The results indicate the potential of *S. linnaeanum* roots and *S. sisymbriifolium* immature fruits as a natural alternative to commercial synthetic nematicides. This aligns with the growing emphasis on sustainable and environmentally friendly pest management strategies in agriculture. The study underscores the need for continued exploration of plant-derived nematicides, particularly those from the Solanaceae family, to combat PPN effectively. These findings contribute to the broader understanding of plant-nematode interactions and highlight the potential of leveraging botanical resources in integrated nematode management.

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223. Morphological and molecular characterization of nematodes affecting potato in Uganda.

Rose Mwesige[1], Joseph Kisitu[2], Marjolein Couvreur[2], Joseph Maosa[2], Laura Cortada[2], Danny Coyne[4], Frank Kagoda[1], John Jones[3], Wim Bert[2]
1 National Agricultural Research Organization, Uganda
2 Nematology Research Unit, Department of Biology, Ghent University, Belgium
3 Cell & Molecular Sciences Department, The James Hutton Institute, Dundee, UK
4 International Institute of Tropical Agriculture, Kenya

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: PCN, PPNs, prevalence, distribution

ABSTRACT:

Potato cyst nematodes (PCN) are quarantine pests with the potential to cause significant yield losses in Uganda. The agronomic factors that influence their establishment and proliferation are crucial for effective management. However, the distribution and importance of PCN and other plant-parasitic nematodes (PPNs) in potatoes remain relatively understudied. Soil and root samples were collected from potato fields across South-Western, North-Eastern, and Central Uganda, in the districts of Kabale, Kisoro, Rubanda, Kapchorwa, Mbale, Kween, Masaka, Mubende, Kassanda, Arua, Kamuli, Kabarole, Lira, Wakiso, and Zombo, to investigate the occurrence, distribution, and abundance of PPNs. PCN was recovered in 22% of the 224 fields sampled. Notably, Kisoro district exhibited the highest PCN prevalence with 62% of 29 fields sampled in the district. Additionally, a higher prevalence of PPN species was observed in the North-Eastern and South-Western regions compared to Central Uganda. Soil samples provided a greater PPN diversity than root samples. The most prevalent non-cyst nematode genera detected were spiral nematodes (*Helicotylenchus* spp.), followed by root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.). *Xiphinema* spp. and criconematids were also present, though with lower prevalence and densities. Further morphological and molecular analyses (using D2-D3 of 28S rDNA, COI, COII, and Nad5 of mtDNA sequences) will determine the exact species' identity of these PPN. For the PCN, high-throughput DNA sequencing methods will be employed to identify genetic variations between populations, linking them to individual fields. Our study shall provide critical insights into PPN occurrence in key potato-growing regions of Uganda, raising awareness among farmers, agricultural officers, and researchers. Moreover, it will provide policymakers with valuable guidance on addressing nematode pest issues, ultimately enhancing potato yields and growers incomes across Uganda.

224. Importance of volatile compounds on the infection cycle of Pine Wilt Disease (PWD)

Ana Fundurulic[1], Jorge M. S. Faria[1], Maria L. Inácio[1]
1 INIAV, I.P., National Institute for Agrarian and Veterinary Research, Oeiras, Portugal
2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: plant parasitic nematodes, volatile organic compounds, early detection, plant health

ABSTRACT:

The plant parasitic pinewood nematode (PWN), *Bursaphelenchus xylophilus*, causes pine wilt disease (PWD) in susceptible pine trees. In Europe, PWD greatly impacts the Iberian pine forests, leading to economic losses in the wood industry. The early detection and faster monitoring in the field, in plant nurseries, or at border transport entrance points is of the utmost importance if this disease is to be contained. The utilization of volatile organic compounds (VOCs) for the detection of plant diseases relies on the fact that when plants are subjected to pathogenic agents, they elicit distinct VOC emissions in response. The analysis of these VOC signatures can provide a non-invasive approach for the early detection of PWD. However, in the PWD disease complex, several organisms contribute to VOC emissions, making disease detection more intricate. Furthermore, specific VOC profiles associated with early infection, symptom development, and advanced stages of PWD may differ. Addressing these challenges is necessary for the development of reliable and practical VOC-based diagnostic tools, enhancing the overall capacity for early PWD detection and monitoring. In this work the main VOCs reported to have an influence in several stages of the PWD infection cycle were reviewed and analyzed according to their chemical composition. Examining contributing VOC emissions during the PWD infection cycle allows the identification of reliable biomarkers for disease detection. These biomarkers can be employed not only in greenhouse conditions but also in the field to assess plant health.

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225. Smart technologies for plant parasitic nematodes detection

Maria João Camacho[1], Jorge M. S. Faria[1], Ana Fundurulic[1], Maria L. Inácio[1]
1 Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Av. da República, Quinta do Marquês - Edifício Florestal, 2780-159 Oeiras, Portugal
2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: in-field detections, lab-on-chip, GC system, *Bursaphelenchus xylophilus*, *Globodera pallida*

ABSTRACT:

In the agricultural sector, different technologies can be used as part of crop management strategies, aiming to promote sustainability and increase overall crop productivity. A growing number of devices is emerging for the detection of crop pests and diseases. Thus, advances in sensing technologies are opening new opportunities for precision agriculture. Here, we present a Lab-on-chip approach, based on a magneto resistive biosensor, developed for detection of the pale potato cyst nematode - *Globodera pallida*, and a portable in-field sensor-system, based on gas chromatography (GC), developed for detection of specific Volatile Organic Compounds (VOCs) emitted by plants under stress induced by the pinewood nematode *Bursaphelenchus xylophilus*. This work provides insights for new strategies to develop smart devices for nematode in-field and at the entry ports detection.

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226. Exploring *Xenorhabdus bovienii* secondary metabolites as alternative strategy for controlling *Meloidogyne incognita*

<u>Maria Del Mar González-Trujilo</u>[1], Juan A. Artal[2], Ignacio Vicente-Díez[1], Ruben Blanco-Pérez [3], Miguel Talavera[4], Sergio Álvarez-Ortega[5], Jorge Dueñas-Hernani[1], Raquel Campos-Herrera[1]

1 Instituto de Ciencias de la vid y del Vino (ICVV, Gobierno de La Rioja, CSIC and Universidad de La Rioja) Logroño, La Rioja, Spain
2 ARTAL Smart Agriculture, Paterna, Valencia, Spain
3 Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Spain
4 IFAPA Alameda del Obispo, Córdoba, Spain
5 Universidad Rey Juan Carlos, Móstoles, Madrid. Spain

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: plant-parasitic nematodes, reproduction indicators, pest control, tomato plants, sustainable production.

ABSTRACT:

Plant-parasitic nematodes (PPNs) considerably hinder global agricultural production, causing losses estimated at US$ 157 billion annually. Among the different PPNs, *Meloidogyne incognita* poses a critical threat to agriculture due to its direct detrimental effects on plants and its ability to facilitate second infections by opportunistic pathogens. This dual threat significantly complicates the development of effective control and management strategies. Secondary metabolites (SMs) produced by bacteria in the genus *Xenorhabdus*, a symbiont of entomopathogenic nematodes (EPNs) of the genus *Steinernema*, have generated increasing interest due to their ability to control various pests and diseases. We hypothesized that exposure of *M. incognita* to the SMs would cause dormancy or even mortality, reducing plant damage. This study explored the efficacy of four SMs (SM1, SM2, SM3, and SM4) produced by different strains of *X. bovienii* in the control of *M. incognita*. SMs were obtained after the bacterial fermentation in tryptone-soybean broth (TSB), centrifugation, and filtration. The investigation comprised both *in vitro* and *in vivo* experiments. Initially, we assessed the impact of SMs at different concentrations (10%, 40%, and 90%) on the motility of two *M. incognita* populations (Chipiona and Alos). Our observations revealed pronounced immobility, indicative of significant efficacy, reaching proportions over 60% mortality after 4 hours for the highest concentration and 24 hours for the medium concentration in all SMs evaluated. In subsequent *in vivo* trials, we evaluated the capability of *M. incognita* to reproduce on tomato plants (number of galls and egg masses, Gall index scale: 1-10) and its impact on plant growth (dry root and above plant weight), exposure to SMs at two different volumes (10 and 20 mL) and times (30 and 60 days). The overall results showed that SM applications reduced the Gall index and, in some cases, enhanced plant growth. Gall and egg mass counting varied among SM types, with SM3 showing the highest reductions for both nematode populations. Our findings suggest that SMs from *X. bovienii* constitute a promising, novel, sustainable approach to managing *M. incognita* infestations in agriculture. However, further research is necessary to ascertain the broader ecological impacts, particularly on non-target

organisms, to fully validate the use of these metabolites as an effective and environmentally responsible pest control strategy.

227. Strategies for the management of *Meloidogyne incognita* in pepper greenhouses with Nemasol® (metam sodium 510) in the Spanish southeast

Caridad Ros Ibañez[1], Celia Martínez Mora[1], Adriana Fuensanta Esteban López[1], M Ángeles Hernández Colucho[1], Victoriano Martínez Alarcón[1], Jean-Michael Rabasse[1], Enmanuel Medico[1]
1 Instituto Murciano de Investigación y Desarrollo Agrario y Mediambiental, Murcia (IMIDA), C/ Mayor s/n 30150 La Alberca Murcia, Spain
2 Eastman Chemical Company Pantserschipstraat 207 9000, Gent, Belgium

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: nematode, disinfectation soil, *Capsicum annuum*, control

ABSTRACT:

Meloidogyne incognita is one of the main problems of greenhouse pepper cultivation in the southeast of Spain. The limitations of use of soil fumigants and post-planting nematicides necessitate the establishment of control strategies which rely on the different tools available. Currently, the only soil fumigant that can be used in Spain is Metam Sodium (MS) and the reference product in post planting is Velum . With the objective of evaluating their efficacy alone and in strategy, a trial was established in a pepper greenhouse homogeneously infested with a population of *M. incognita*. The strategies evaluated were: i) soil disinfection (SD) with MS at 300l/ha, ii) SD with MS at 300l/ha + Velum during cultivation (V), iii) SD with MS at 600l/ha, iv) SD with MS at 600 l/ha + V, v) untreated, vi) V. Each treatment was applied through drip-irrigation under TIF (Totally Impermeable Film) left in place during six weeks and planted with 3 rows of the variety Maestral in randomized blocks. The nematode population in the soil, the incidence and severity of nematode damage, the number of egg masses per plant and the yield were measured. No juveniles were found in the plot treated with MS while still present in the untreated. The incidence of infestation by nematode was significantly lower in MS treated plots, at both rates 300-600 l/ha, than in Velum and non-treated (55, 80, 100 and 100% respectively). The severity on root damages (Gall Index 0-10) was also significantly lower in all treatments in comparison to non-treated (1,7, 1,8, 2,9 and 3,7). MS at all rates has permitted a significant decrease of incidence and severity in comparison to control. In this trial, Velum used alone did not differ significantly from the untreated, but its application after metam translated to a numerical decrease of incidence and severity. No significant differences in yield were observed between treatments mainly due to the medium level of disease pressure. In the conditions of this trial, Metam sodium at 300 or 600l/ha has permitted a significant control of nematodes in Peppers, and the addition of the post-planting nematicide Velum in a strategy based on MS achieved a better control than with each product applied alone. NEMASOL® (Metam sodium 510) can be considered as an efficient tool for building integrated strategies with other products used in post-planting.

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228. Tobacco rattle virus and Trichodoridae: building blocks of a systemic and sustainable approach to disease control

Pella Brinkman[1], Doretta Boomsma[2], Roberto Miglino[2], Michaela Schlathoelter[3], Marcel von Reth[3], Thea van Beers[4], Leendert PG Molendijk[1]
1 WUR Field crops, Lelystad, The Netherlands
2 HZPC Research B.V., Metslawier, The Netherlands
3 P.H. Petersen Saatzucht Lundsgaard GmbH, Grundhof, Germany
4 Agrifirm NWE B.V., Apeldoorn, The Netherlands

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: TRV, trichodorid, Integrated Crop Management

ABSTRACT:

Tobacco rattle virus (TRV) is widespread in many countries around the world and is transmitted by root-feeding trichodorid nematodes (*Trichodorus* spp. and *Paratrichodorus* spp.), and in some plant species is also spread by infected seeds. Both virus and vector have a broad host range. In potato TRV is causal agent of 'spraing' disease, also known as 'corky ring spot' (CRS), which is characterized by necrotic rings in the tuber flesh and affects the marketability and value of potato seed lots. This is a relevant problem for the potato processing industry that demands a minimum of damage in the tubers.

Restriction in the use of current synthetical nematicides and farming on sandy soils is increasing the spread of both virus and vector, which translates in increasing economic importance of this disease. Following the call for a sustainable and resilient agriculture (FAO Sustainable Development Goals) we have started a multidisciplinary non-chemical approach for this disease management. The approach is based on Integrated Crop Management (ICM), which includes the choice and order of crops in the rotation, the availability of resilient crops, soil management, monitoring and targeted control. We focus on four building blocks: 1) Breeding for new potato cultivars that are fully resistant to TRV, 2) Breeding for resistance to TRV in green manure crops, 3) Knowledge of the host plant status for trichodorid nematodes and TRV and 4) Implementation into the farmer's practice. This will decrease dependency on and emission of synthetic crop protection agents.

229. Integrated strategies for the control of Nacobbus aberrans s.l. in tomato

Ana Laura Sosa[1], Natalia Soledad Girardi[1], Laura Cristina Rosso[2], Maria Alejandra Passone[1]

1 CONICET-UNRC Laboratorio de Ecología Microbiana, Universidad Nacional de Río Cuarto (UNRC), Ruta Nac. 36, Km 601, Río Cuarto, Argentina.
2 IPSP-CNR Istituto Per La Protezione Sostenibile Delle Piante, Sede Di Bari, Via G. Amendola, 122/D, 70126, Bari, Italy.

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: biocontrol, *Nacobbus aberrans*, tomato, *Purpureocillium lilacinum*, broccoli extract

ABSTRACT:

Microorganisms in the tomato rhizosphere ecosystem develop associations, many of which can be beneficial or pathogenic for this crop. The plant parasitic nematode (PPN) *Nacobbus* sp. cause significant economic losses to horticultural farmers in Argentina. The distribution of this genus is limited to the American continent and is consider a quarantine pest. Biological control strategies of PPN are environmentally friendly alternatives that ensure the sustainability of the horticultural system. The aim of this study was to evaluate the potential of the combined application of broccoli aqueous extract (BAE) and *Purpureocillium lilacinum* SR14 fungus in tomato plants (*Solanum lycopersicum* var. Platense) for the management of *N. aberrans* s.l. Three-week-old seedlings were transplanted into pots containing the following treatments: T1: control (sterile horticultural soil), T2: naturally infested horticultural soil (NIHS), T3: NIHS + SR14(1x106 spores/g), T4: NIHS + BAE(12%), T5: SHNI + SR14(1x106 spores/g) + BAE(12%). Six replicates per treatment were carried out and plants were maintained in a greenhouse. Data, collected at 120 days post-transplant, revealed that tomato growth was affected in NIHS(T2). Plants showed a 47% and 30% reduction in weight and length, respectively, compared to uninfected soil (T1). The highest number (4 tomatoes/plant) and weight mean (80 g) of fruits were recorded in T4. The fungus alone or combined with BAE (T3, T4 and T5) effectively controlled N. aberrans s.l. population (reduction percentages: galls = 35, 90 and 93%, egg masses = 5, 94 and 90% and reproduction factor = 90, 93 and 98%). Physicochemical and biological analyzes were also performed to evaluate the effect of the treatment on the soil ecosystem. The data showed an increase in organic matter for all treatments compared to the initial condition. Nutrient values such as P, N and S were maintained throughout the trial. Regarding the soil mycobiota, the fungal count (cfu/g) was around 106 for all treatments, but a lower diversity was observed in T5 with a prevalence of *P. lilacinum* (3x104). As regards the nematofauna, treatments did not significantly influence the number of individuals (n°/g of soil) belonging to the different trophic groups (phytopathogens: 16, bacteriophages: 19, predators: 10 and fungivores: 15), with the exception of T4, where a higher count of omnivores was observed (30). Finally, studies on microbial activity revealed that treatments did not influence the counts (MPN/g) of ammonifying (mean: 1.9x102) and denitrifying agents (mean: 2.7x1013), while T4 and T5 reduced the density of 1 log unit of nitrate - reducing bacteria (mean: 3.4x106) and aerobic-heterotrophic bacteria (mean: 3.9x108). In

conclusion, the integrated strategy reveal a synergistic effect for the control of *N. aberrans* s.l., without affecting tomato plant development, nutrient content and soil microbiome.

230. NemDetect: early detection of quarantine nematodes in potatoes using remote sensing

Saša Širca[1], Nik Susič[1], Barbara Geric Stare[1], Uroš Žibrat[1], Nicole Viaene[2], Jonathan Van Beek[1]
1 Agricultural Institute of Slovenia (KIS), Ljubljana, Slovenia
2 Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium

Area: Integrated Management of Plant-Parasitic Nematodes
Type: Poster
Keywords: detection, nematode potato infestation, hyperspectral imaging, plant parasitic nematodes,

ABSTRACT:

Plant-parasitic nematodes cause significant economic losses in crop production, with root-knot nematodes (RKN) and potato cyst nematodes (PCN) being the top two in terms of agronomic impact. Effective pest management requires accurate detection and monitoring, especially methods for early (pre-symptomatic) detection, as nematode damage can take years to become visible and lead to full field infestations if unmanaged. Remote sensing using hyper- and multispectral sensors enables pre-symptomatic detection at field scale, but it needs to reliably distinguish between biotic stress from nematodes and abiotic stress such as drought, and account for variety heterogeneity. Nemdetect focused on four nematode species and included drought stress in experiments to ensure accuracy. The project was divided into four parts: literature review, identifying implementation opportunities, pilot case studies, and knowledge transfer. The literature review analysed the current state-of-the-art in remote sensing for plant health assessment and provided guidelines for future research. Identifying implementation opportunities assessed the potential of remote sensing for nematode detection and monitoring. Pilot case studies tested the effectiveness of remote sensing in detecting nematode infestations and distinguishing between biotic and abiotic stress, for above-ground parts of potato plants and tubers. Knowledge transfer disseminated the findings to stakeholders. The pilot case study used hyperspectral imaging to identify infested tubers and plants in a greenhouse setting. In addition to accurate detection of infected plants, including identification of nematode species, latent infections in tubers were accurately detected. Greenhouse-scale models were validated using UAV multispectral and airborne hyperspectral imaging at field scale in Belgium. Overall, remote sensing methods show promise for early detection and management, but additional data are needed for more robust predictive models.

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231. Monitoring of *Bursaphelenchus xylophilus* and focus on *B. abietinus* in the Italian territory

Sara Amoriello[1], Leonardo Marianelli[1], Giuseppe Mazza[1], Stefania Simoncini[1], Francesco Paoli[1], Pio Federico Roversi[1], Giulia Torrini[1]
1 CREA - Research Centre for Plant protection and Certification, Florence, Italy

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: *Abies alba*, pinewood nematode, plant parasitic nematodes, priority pest

ABSTRACT:

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is one of the most important phytosanitary problems in conifers worldwide, as it can infest most *Pinus* species and other conifers, causing rapid wilting of the trees. It has been included in the EPPO A2 list as a quarantine organism and classified as a priority pest in the EU due to its serious potential economic, environmental and/or social impact (Regulation 2019/1702/EU).

The risk of introduction and spread of PWN in Italy is high due to the intensive trade between the countries where the organism occurs and the availability of a wide range of susceptible hosts, as well as the presence of its vector on the Italian territory. According to the European legislation, annual phytosanitary surveillance programs are carried out to confirm the presence or absence of the pest in national coniferous forest stands.

On the Italian territory, annual monitoring includes the sampling of standing declining and dead trees, pallets and bark mulch, and vector insects through baited traps.

To date, no samples from the forest have tested positive for *B. xylophilus*, but a progressive and increasing decline in silver fir (*Abies alba*) stands has been observed for several years. More detailed investigations in these environments have made it possible to isolate numerous specimens of *Bursaphelenchus abietinus* from almost all the silver fir samples collected and analyzed. It is possible that this nematode contributes to the observed declines which, in combination with abiotic and biotic factors (e.g. temperature, drought and high density of bark beetles), can also lead to tree mortality. Further investigations on the pathogenicity of *B. abietinus* will be investigated in future studies.

232. First investigation of potato cyst nematodes (*Globodera pallida* and *Globodera rostochiensis*) distribution in the Sila National Park

Giulia Torrini[1], Sara Amoriello[1], Agostino Strangi[1], Stefania Simoncini[1], Laura Tomassoli[2], Michele Santaniello[3], Vincenzina Scalzi[4], Silvia Landi[1]
1 CREA Research Centre for Plant Protection and Certification, Florence, Italy
2 CREA Research Centre for Plant Protection and Certification, Rome, Italy
3 Consorzio P.P.A.S., Camigliatello Silano (CS), Italy
4 Servizio Fitosanitario Regione Calabria, Catanzaro, Italy

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: Calabria region, quarantine nematodes, infestation level

ABSTRACT:

Cyst nematodes (*Globodera* spp.) represent a serious threat to potato crops. The *Globodera rostochiensis* species was found for the first time in Italy in 1960, while the *Globodera pallida* in 1977. Due to their relevant damage, they have been included in Annex II - Part B of EU Regulation 2072/2019 as quarantine nematodes relevant to the Union and known to be present in the EU territory. The presence of both species has recently been reported in Calabria and their spread is feared. The key element of the potato sector in this region is the high quality of the production: the Sila National Park, which extends into the provinces of Cosenza and Catanzaro, is designed for certified production such as Protected Geographical Indication (PGI) and represents one of the most suitable areas for the potato production in Italy.

In the framework of a triennial project, a task is aimed to assess the distribution and infestation level of cyst nematodes in this economically important district. During the potato growing season in 2023, cyst investigation was carried out. Twenty-five plots (1 ha each) distributed in the whole district were selected, 8 in the Sila Piccola and 17 in the Sila Grande, to evaluate the distribution of these two species. Soil samples were taken from each plot according to the standard methodology reported in EU Regulation 1192/2022. Cysts were extracted from the soil samples using Fenwick Can and identified by morphological and molecular methods.

Approximately 50% of the analyzed samples were infested with *Globodera* spp. with a different distribution in the area: higher in the Sila Piccola (63% of the analyzed samples were infested) than in the Sila Grande (48%). The prominent species was *G. pallida*, while *G. rostochiensis* was found in only three sites in the Sila Grande. Only one sample showed the presence of both species. Only four sites, mainly located in the Sila Grande, showed high infestation levels.

This investigation provides an initial picture of the distribution of these pests in this area and might represent an important support for phytosanitary management. Further analyses are necessary to monitor how these species may increase their spreading.

This work was supported by SILAPEST ‘Research and sustainable development of control actions against potato pests in the Sila area’

233. Leaf biochemistry and hyperspectral fingerprints of biological control measures against *Meloidogyne hapla* in a susceptible white-fruited strawberry

Nik Susič[1], Uroš Žibrat[1], Nika Cvelbar Weber[2], Jaka Razinger[1], Andrej Voncina[1], Matej Knapic[1], Saša Širca[1], Barbara Geric Stare[1]

1 Agricultural Institute of Slovenia, Plant Protection Department, Ljubljana, Slovenia

2 Agricultural Institute of Slovenia, Department of Fruit Growing, Viticulture and Oenology, Ljubljana, Slovenia

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: white strawberry, *Meloidogyne hapla*, root-knot nematode, biological control, Bacillus cf. firmus

ABSTRACT:

Plant-parasitic nematodes are a common threat in agricultural production systems associated with yield losses. Several species of plant-parasitic nematodes have been reported to cause damage to strawberries, and among them, the root-knot nematodes such as *Meloidogyne hapla* are the most common. *M. hapla* was reported to have damaging effects on strawberries even at low levels of soil infestation, so measures to control it are necessary. Due to the toxicity of many classical chemical nematicides and soil fumigants, alternative biological control strategies for root-knot nematodes are being investigated, such as the use of nematicidal bacteria. These bacteria can interact closely with plant roots, leading to potentially different outcomes that are poorly understood. We conducted pot experiments in the greenhouse and in plastic tunnel to test the nematicidal and potential plant growth-promoting effects of bacterium *Bacillus* cf. *firmus* on white-fruited strawberry plants (*Fragaria chiloensis* (L.) Mill.) infected with *M. hapla*. The effects of *M. hapla* and *Bacillus* cf. *firmus* on strawberry plants were tested either in isolation or in combination. Analysis of secondary metabolites in strawberry leaves and hyperspectral imaging were used to assess the plants in different treatments. The effects were different in the two experiments. In the greenhouse pot experiment, the bacteria significantly reduced the *M. hapla* population, comparable to the control by the chemical nematicide Velum (Bayer). A significant increase in the chlorophyll index was measured in the *Bacillus*-treated plants, and they had a significantly lower tannin content compared to the nematode-infected, chemically treated plants (over 30 % difference). In the tunnel pot experiment, *Bacillus* showed no nematicidal activity compared to the untreated control. However, a significant increase in chlorophyll index, a higher content of various procyanidin oligomers (over 20 % difference) and hydroxycinnamic acids (over 35 % difference) were measured in *Bacillus*-treated plants compared to nematode-infected plants. In both experiments, we used hyperspectral imaging in the spectral range of 400-2500 nm and image analysis to distinguish the plants in the different treatments. Kernel PCA (kPCA) or Partial Least Squares (PLS) methods in combination with Support Vector Machines (SVM) were used for discriminations. Analysis of the hyperspectral data allowed discrimination of plants between different treatments with an overall classification success rate of over 91 % in both experiments. The overall analysis highlighted the inherent variability in the success of

biocontrol when conducted in a controlled or field-analogous environment. At the same time, it demonstrated the usefulness of the remote sensing approach for high-throughput plant phenotyping in terms of assessing plant growth or the presence and spread of pathogens.

This study was funded by Horizon 2020, Grant agreement No. 817946-EXCALIBUR and ARRS (P4-0072, P4-0431).

234. EUPHRESCO-Melosurvey: Detection and distribution of *Meloidogyne chitwoodi* and *M. fallax* within Europe

Evelyn van Heese[1], Ines Gabl[2], Mette Vestergård[3], Barbara Geric Stare[4], Saša Širca[4], Caridad Ros Ibáñez[5], Sofia Windstam[6], Ingrid Areskoug[6], Refik Bozbuga[7], Mustafa Imren[8], Solveig Haukeland[9], Anne Sophie van Bruggen[1]

1 Netherlands Institute for Vectors, Invasive Plants and Plant Health (NIVIP), National Plant Protection Organization (NPPO), NVWA, Wageningen, The Netherlands
2 Austrian Agency for Health and Food Safety, Vienna, Austria
3 Department of Agroecology, Aarhus University, Denmark
4 Agricultural Institute of Slovenia, Ljubljana, Slovenia
5 Murcian Institute of Agricultural and Environmental Research and Development (IMIDA), Spain
6 Swedish Board of Agriculture, Sweden
7 Eskisehir Osmangazi University, Odunpazari, Eskisehir, Türkiye
8 Bolu Abant Izzet Baysal University, Türkiye
9 Norwegian Institute for Bioeconomy Research, Norway

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: sampling strategy, survey, diagnostics, quarantine nematodes

ABSTRACT:

Meloidogyne chitwoodi and *M. fallax* have been detected in various countries in Europe (Belgium, France, Germany, The Netherlands, Portugal, Spain, Sweden, Switzerland, United Kingdom and Türkiye) with different frequencies of occurrence reported, varying from ‘few occurrences but eradicated or under eradication’ to ‘present in several fields’. These nematodes have a wide host range that includes important agricultural crops. It is challenging to detect these nematodes because of the rapid population decline in the absence of host plants. At the same time, low numbers of remaining nematodes can rapidly increase once suitable host plants are grown.

Therefore, to assess the current distribution in Europe and to prevent further spread, it is important to conduct reliable and sensitive surveys. Recent first findings in Lithuania and Denmark confirm this significance.

The main goal of this EUPHRESCO project is to develop better and harmonized sampling strategies for the detection of *M. chitwoodi* and *M. fallax*. EU legislation prescribes that tubers should be checked for absence of these nematodes in case of seed potatoes. EPPO protocols describe how this can be done (PM 3/69 and PM 9/17). Improvement and harmonization can be achieved by knowledge sharing. During workshops we will compare and discuss sampling strategies and diagnostic methods, identify relevant risk-factors, identify knowledge gaps and develop a network. Prior to the workshops questionnaires on sampling strategy and diagnostic methods will be prepared and sent to all EU and EPPO Member States. Furthermore, the occurrence and distribution of *M. chitwoodi* and *M. fallax* will be assessed in the partnering countries. The intention is to agree on a validated and harmonized approach for *M. chitwoodi* and *M. fallax* surveys. To design risk-based and statistically sound surveys it is essential to prioritize host plants for the survey area, to identify relevant risk-factors to be able to target those areas or

fields that are most at risk of being infested, and to have a proper understanding of the sensitivity of the applied sampling and diagnostic methods.

235. Crop production in spatial conditions, risks associated with plant-parasitic nematodes

Hoël Hotte[1], Pauline Le Devendec[1], Sylvain Fournet[2], Didier Fouville[2], Nathan Garcia[1], Aude Letort[1], Nicolas Mariette[1], Josselin Montarry[2], Fabrice Ollivier[1], Maureen Scipion[1], Eric Grenier[2], Laurent Folcher[1]

1 French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Plant Health Laboratory, Nematology Unit, Le Rheu, France
2 IGEPP, INRAE, Institut Agro, Univ Rennes, Le Rheu, France

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: space farming, risk, plant heath, plant-parasitic nematode, *Meloidogyne enterolobii*, *Solanum lycopersicum*

ABSTRACT:

Agricultural production in space is a key issue for future spatial challenges. Travelling to Mars or setting up a base on the Moon need the production of food. Crop production is considered as one of the main future sources of nutrition by biogenerative systems. Despite the fact that this production is monitored, plant-parasitic organisms could potentially become pests as in agriculture on Earth. Crop parasitism could even compromise a mission in this confined and production-dependent environment. In this context, the IMOTEP project (Infection in Microgravity of Plant-parasitic TElluric Organisms Project) initiated by ANSES and INRAE has been funded by the French National Center for Space Studies (CNES). This project focuses on the risks associated with plant-parasitic nematodes following two main approaches. The first one focuses on plant-nematode interactions considering a specific environment of microgravity coupled with radiations. In these extreme conditions, knowledge about of the plant infection, completion of a full life cycle and reproduction rate is lacking in the scientific literature dedicated to plant-parasitic nematodes. Furthermore, after several generations, we plan to consider both some behavioral and physiological traits that could be affected such as the ability to infect and multiply or genomic alterations. The second approach involves studies regarding the extreme resistance capabilities of some plant-parasitic nematode species. Various parameters such as extreme temperatures at low Earth orbit, UVs and the vacuum of space, will be tested. Preliminary results in microgravity conditions without radiation were obtained with *Meloidogyne enterolobii* and *Solanum lycopersicum*. Microgravity was simulated using 2D agitation and 50 M. *enterolobii* larvae were inoculated in each replica (12 replicates for the microgravity modality and 12 controls in terrestrial gravity). Results showed that the nematodes have the ability to infect the host plant and to start their development cycle. The experiment was stopped after 17 days, before the end of the complete cycle of M. *enterolobii* and individuals in the 3rd and 4th stages were counted. No significant difference (F1,23 = 2.23, P = 0.15) in the infection rate was observed between the microgravity modality (5.17%, SE = 0.87) and the terrestrial gravity modality (9.0%, SE = 2.42). These results demonstrate a potential risk on crop production in space. Experiments are still under way and should soon validate the capability of individual nematodes to complete a full cycle.

236. Re-examination of identity and prevalence of plant parasitic nematodes in Cyprus

Michalakis Christofofou[1], **Antria Tsalakou[1]**, **Christos Christodoulou[1]**, **Dimitris Tsaltas[1]**
1 Cyprus University of Technology, Limassol, Cyprus

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: plant parasitic nematodes, molecular barcoding Identification

ABSTRACT:

Several reports in from the 70s up to 90s revealed the presence of a significant number of plant parasitic nematodes in the Island of Cyprus. Philis and Siddiqi (1976), reported the morphological identification of 58 plant parasitic nematode (PPN) species and 22 genus, from soil and root samples from various locations on the island of Cyprus. Since then, the land use, crop type and agricultural practices has change over the year and inevitably PPN presence and density. In the presence study, we re-identify PPNs extracted from soil and root samples from several crop types and altitudes on the island using molecular barcoding techniques. Soil sample's locations are marked in an ArcGIS pro geodatabase and a map is generated for future reference. At present the following nematode species have been identified: *G. rostochiensis*, *G. pallida*, *M. incognita*, *H. latipons*, *M. incognita*, *M. javanica* and *M. arenaria*, *T. semipenetrans*, *P. penetrans* and *X. index* from 25 soil samples from potato, tomato, cucumber, citrus, banana, vine, cereals.

237. Real-time PCR as a tool for detection and identification of *Bursaphelenchus xylophilus* and *B. mucronatus* based on trace amounts of their DNA left in the vector - the pine sawyer beetle, *Monochamus galloprovincialis*

Anna Filipiak[1], Marek Tomalak[1]
1 Institute of Plant Protection - National Research Institute, Wladyslawa Wegorka 20, 60-318 Poznan, Poland

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: detection, diagnostics, insect-vector, pine wilt disease

ABSTRACT:

The quarantine nematode *Bursaphelenchus xylophilus* is a causal agent of the pine wilt disease. In Europe, the nematode is vectored by the pine sawyer beetle, *Monochamus galloprovincialis* which is also a natural vector of the native, nonpathogenic *B. mucronatus*. Based on the established regulations, long term detection surveys of pine wood and vector-insects for the presence of *B. xylophilus* are obligatory in all EU member countries. The lack of precision in early detection of the pine wood nematode in the vector may have significant consequences for the future in the newly infested forest stands.

The main objective of the presented research was to use the real-time PCR technique for the fast and precise detection, and identification of Bursaphelenchus nematodes associated with *M. galloprovincialis*.

The research conducted on beetles collected in forest stands in Poland revealed exclusive presence of the native, nonpathogenic nematode *B. mucronatus*. At the beginning of the beetle flight season (June-July) the real-time PCR technique always detected this nematode species in the case of its physical presence in the body (i.e. tracheas) of dissected insects. At that time, the physical absence of nematodes in the beetles always resulted in negative reaction results with no DNA amplification. However, at the end of the flight season (September-October) the real-time PCR of material extracted from beetles with no nematodes generated a negative or positive result. It was also found that for the tested beetles in which the nematodes were present, the fluorescence signal was higher than that in individuals with no physical presence of nematodes. The positive reaction results could indicate the earlier presence of nematodes which left their remains in the beetle after departing from its body during maturation feeding and/or egg laying of the vector. This trend has been also confirmed for *B. xylophilus* in strictly laboratory conditions where naturally dead, squeezed and partially macerated juveniles were mixed with the beetle body homogenate and subjected to the similar detection procedure.

The obtained results confirm extremely high sensitivity of the real-time PCR method used in detection and identification of both nematode species which may simultaneously occur in *M. galloprovincialis* beetles. It allows for a precise detection of the nematodes based on trace amounts of their DNA left in the vector insects.

238. Nematology reference lab of Portugal: 15 years of research and services on the pinewood nematode

M.L. Inácio[1,2], Margarida Fontes[1], Marina Cardoso[1], Nídia Laureano[1], Jorge Faria[1,3], David Pires[1,3], Rita Varela[1,3], Luis Bonifácio[1,2], Pedro Naves[1,2] & Edmundo Sousa[1,2]
1 National Institute for Agrarian and Veterinarian Research (INIAV, I.P.), Oeiras, Portugal
2 GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQN NOVA), Oeiras, Portugal.
3 Mediterranean Institute for Agriculture, Environment and Development (MED), University of Évora, Pólo da Mitra, Apartado 94, 7006-554 Évora, Portugal

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: Pinewood nematode, laboratory services, diagnostic, research

ABSTRACT:

The Nematology Lab of INIAV (National Institute for Agrarian and Veterinary Research) in Oeiras integrates the National Reference Laboratory (NRL) for Plant Health in Portugal. NemaINIAV plays a crucial role in the national survey for the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease (PWD).

Over the past 15 years, NemaINIAV has processed more than 50,000 wood samples (from trees and wood packaging materials) for nematode extraction, morphological identification of specimens, and molecular confirmation when necessary. Similarly, over 15,000 longhorned beetles (*Monochamus galloprovincialis*), the insect vector of PWN, caught in the national trapping system, underwent processing for nematode extraction, identification, and molecular confirmation.

NemaINIAV offers services for PWN detection with the goal of preventing the spread and introduction of this pest from wood material and ensuring compliance with local and EU legislation. Also, the Nematology team has played a leading role in various ongoing research projects, including the projects PurPest (2023-2026) - Plant pest prevention through technology-guided monitoring and site-specific control, Forest Research_UK (2022-2026) - Pinewood nematode, PineProtect (2022-2025) - Exploring the biocontrol potential of *Esteya* spp. against the PWN in *Pinus pinaster*, PineEnemy (2018-2022) - Exploring the Nematode Mycobiota interactions, to ascertain the role of fungi in the PWD complex, NemaWAARS (2022-2024) - A motif to unveil mechanisms of parasitism gene regulation in the PWN as a target for disease control and plant resistance, PinasterPWN (2018-2022) - Development of molecular markers for resistance to PWD in *Pinus pinaster*, EU project PWN (2016-2022) - Support to scientific activities to enhance the efficiency and reliability of surveillance for PWN, in particular in the buffer zone in Portugal, EcoVETOR (2016-2020) - New eco-friendly approaches for the biomanagement of the PWN vector at Eurasia, NemaTransfer (2016-2019) - Can the PWD cycle be broken? Decoding transfer mechanisms of the PWN between its insect-vector Monochamus galloprovincialis and the host tree, PLURIFOR (2016-2019) - Risk management plans targeting rural forestry areas sensitive to biotic and abiotic risks.

As an NRL, NemaINIAV is a part of the European Reference Lab for Plant Parasitic Nematodes and the Panel on Diagnostics in Nematology of EPPO. This involvement provides the national

perspective on the diagnostic challenges of PWN. Numerous training sessions for national and foreign students, technicians, and inspectors have been conducted over the last decade. In this overview, we present the diverse activities of NemaINIAV over the last decade on the PWN, focusing on research and development (projects, supervisions, training, and publications), community service extension and consultation, and interactions with other Plant Health labs.

Funding: This work was partly funded by the Portuguese Foundation for Science and Technology (*Fundação para a Ciência e a Tecnologia*, FCT) through the project NemaWAARS 421036 FCT-ASP-PLA-1108

239. Updates on EUPHRESCO Melorisk: preventing *Meloidogyne graminicola* spread in European rice paddies

Leidy Rusinque[1,2], Carla Maleita[2], Maria L. Inácio[1]
1 National Institute for Agricultural and Veterinarian Research (INIAV), Oeiras, Portugal
2 University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Coimbra, Portugal

Area: Quarantine nematodes: Diagnostics, Legal and Regulatory Aspects
Type: Poster
Keywords: Detection, quarantine, identification

ABSTRACT:

Rice (*Oryza sativa*) stands as a vital staple food, sustaining over half the global population. In Europe, rice cultivation is restricted to a few southern European countries, with a total area of about 425,000 ha and an annual production of 2.8 million tonnes. Despite its significance, rice production faces a critical challenge from plant-parasitic nematodes, with root-knot nematodes (RKN, *Meloidogyne* spp.) being of major concern. Among them, the rice RKN *M. graminicola* (*Mg*) is particularly damaging, accounting for up to 70% of yield losses. *Meloidogyne graminicola* has been identified as a serious pest of rice in Europe and reported in Italy's Piedmont and Lombardy regions. Consequently, it has been added to the European and Mediterranean Plant Protection Organization (EPPO) A2 List of pests recommended for quarantine regulation. Moreover, urgent measures have been taken to prevent its further introduction and spread within the European Union, and so, it has entered in force the COMMISSION IMPLEMENTING REGULATION (EU) 2022/1372 of 5 August 2022 as regards temporary measures to prevent the entry into, the movement and spread within, the multiplication and release in the Union of *Meloidogyne graminicola*. Additionally, the changing patterns in annual mean temperature and rainfall are expected to impact *Mg*'s infection rate, development, and reproduction causing alterations in nematode's abundance and geographical distribution and, ultimately, posing a significant threat to cereal production in temperate regions. This project started in 2022 and aims to: 1) assess the occurrence and distribution of *Mg* in the partnering countries, 2) validate the *Mg* diagnostic methods (morphology, biochemical and molecular), 3) sequence (NGS) the genome of Italian and other *Mg* isolates, and 4) evaluate host suitability of several rice cultivars to *Mg*. During the last two years, surveys have been carried out by the partners and will continue for one more year to have enough information to build distribution maps. An assay on host suitability has been set up to evaluate the susceptibility to *Mg* of the main rice cultivars, cultivated by Portuguese farmers. Moreover, the project has succeeded to establish a network of researchers not only in the EPPO zone but also in Australasia and Latin American countries, exchanging knowledge, protocols, and biological material. In that frame, EUPHRESCO-MeloRisk has a partnering project MeloRisk Australasia: Reducing the risk of exotic root-knot nematodes in Australasia to support early detection and identification of exotic RKN in the Australasian zone. The following outcomes will be expected: 1) develop knowledge to support risk analysis of spread with trade, especially in Mediterranean countries, 2) enable the availability of isolates for morphological, biochemical and molecular studies, 3) increase the availability of robust sequence data through

Q-bank and Genbank, 4) improve the understanding of *Mg* epidemiology through accurate characterisation, and 5) develop sustainable management strategies.

This a Research Consortium with more than ten partners and more than twelve authors. The research consortium is composed by the following partners/institutions:

Maria L. Inácio[1], Leidy Rusinque[1], Rita Varela[1], Ines Gabl[2], Nicole Viaene[3], Ani Becheva[4], Mirela Borisova[4], Giulia Torrini[5], Agostino Strangi[5], Evelyn van Heese[6], Gerrit Karssen[6], Tim Warbroek[6], Daniel Apolônio[6], Barbara Geric Stare[7], Nik Susič[7], Cristiano Bellé[8], Giovanna Curto[9], Francesca De Luca[10], Alberto Troccoli[10], Elena Fanelli[10], Antonio Evidente[11], Alessio Cimmino[11], Marco Masi[11], Marieke Beltman[12], Salvador Noges[13], Xavier Serrat[13], Sandra Mace[13], Refik Bozbuga[14], Dilek Dinçer[15], Hulya Demirbas Pehlivan[15], Tony Pattison[16], Vanessa S. Mattos[17], Juan Palomares-Rius[18], *Luci* Conceição[19], Isabel Abrantes[19], Carla Maleita[19]

[1]National Institute for Agricultural and Veterinarian Research, Portugal
[2]Austrian Agency for Health and Food Safety, Austria
[3]Flander Research Institute for Agriculture, Fisheries and Food Research, Belgium
[4]Bulgarian Food Safety Agency, Bulgaria
[5]CREA Research Centre for Plant Protection and Certification, Florence, Italy
[6]Netherlands Food and Consumer Products Safety Authority, the Netherlands
[7]Agricultural Institute of Slovenia
[8]Phytus Institute, Brazil
[9]Emilia Romagna Plant Protection Service, Italy
[10]National Research Council, Italy
[11]University of Naples Federico II, Italy
[12]ClearDetections, The Netherlands
[13]University of Barcelona, Spain
[14]Eskisehir Osmangazi University, Türkiye
[15]Biological Control Research Institute, Türkiye
[16]Queensland Department of Agriculture & Fisheries
[17]Scientia Terrae, Belgium
[18]Spanish Research Council CSIC, Spain
[19]University of Coimbra, Portugal

240. Novel Strategies for durable resistance against potato cyst nematodes

Daan Langendoen[1], Johan Hopman[1], Mark Sterken[2], Aska Goverse[2], Geert Smant[2]
1 Averis Seeds, The Netherlands
2 Wageningen University and Research, The Netherlands

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Poster
Keywords: potato cyst nematode, resistance, management, hatching, rotation, allele dosage

ABSTRACT:

Potato cyst nematodes (PCN) are plant parasitic nematodes parasitizing on potato roots. PCN include two species: *Globodera pallida* and *G. rostochiensis* and cause major yield losses globally. With the introduction of PCN resistant potato varieties, the amount of PCN infested arable land steadily declined in the Netherlands. However, since 2013 infestation rates are increasing again, probably due to a resistance breakthrough by *G. pallida*. Since resistance breakthroughs are occurring through all currently used single and double stacks of PCN resistance genes, research on alternative durable strategies is necessary. The research for my PhD thesis will focus on novel strategies to combat PCN in the Netherlands, including (I) decreased host-induced hatching of nematodes from cysts, (II) differentially expressed genes due to altered resistance gene allele dosage, (III) discovery of novel PCN resistance genes from wild *Solanum* species and (IV) the effect of resistance genes rotation on virulence in PCN.

241. Pin nematodes (*Paratylenchus* spp.) parasitizing olive groves in the Mediterranean Basin

Rosa Ana Salazar-Garcia[1], Juan E. Palomares-Rius[1], Ilenia Clavero-Camacho[1], Carolina Cantalapiedra-Navarrete[1], Alba N. Ruiz-Cuenca[2], Pablo Castillo[1], Antonio Archidona-Yuste[1]

1 IAS-CSIC - Instituto de Agricultura Sostenible-CSIC, Avda. Menendez Pidal sn, 14004, Córdoba, Spain
2 Departamento de Biologia Animal, Biología Vegetal and Ecología, Universidad de Jaén, Campus Las Lagunillas, Jaén, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: pin nematodes, diagnosis, ecology, olive groves

ABSTRACT:

The main objective of this research was to investigate the biodiversity of pin nematodes of the genus *Paratylenchus* (obligate plant-ectoparasitic nematodes) in Mediterranean olive groves with different management strategies (traditional, organic and high-density intensive) in Greece, Morocco, Portugal and Spain.

A total of 44 olive groves were surveyed (with 6 replicates per grove) during olive flowering stage (spring 2023) giving 264 sampling sites. The survey comprised the most common olive varieties cultivated in the Mediterranean Basin, including Adramitini, Arbequina, Cobrancosa, Hojiblanca, Kolovi, Koroneiki, Picholine, Picual, Picudo and Sikitita. Soil samples were collected within 5-30 cm soil depth, and nematodes were extracted from a 500 cm3 subsample of soil by centrifugal flotation. Pin nematodes were identified based on integrative taxonomic approaches, including morphological and molecular characterization focused on D2-D3 expansion segments of 28S rRNA. Eighteen *Paratylenchus* species were identified including *Paratylenchus goodeyi*, *P. holdemani, P. idalimus, P. indalus, P. israelensis, P. neoamblycephalus, P. neoprojectus, P. pedrami, P. projectus, P. salubris, Paratylenchus* sp. 5 SAS, *P. sheri*, *Paratylenchus* sp. D, *Paratylenchus* sp. 1 FL, *Paratylenchus* sp. 94bald, *P. veruculatus*, *P. wuae, and P. zurgenerus*). The most common pin nematode was *P. holdemani* (frequency 13.26%, in all three management strategies in Greece, Morocco and Spain), followed by *P. neoprojectus* and *P. pedrami* (frequency 9.47% and 8.71%, respectively, in traditional and organic management in Greece, and traditional and high-density intensive management in Greece, Portugal and Spain, respectively). *Paratylenchus idalimus* was detected for the first time in Spain in traditional olive groves (Picual and Hojiblanca) in low densities. Four species were not specifically identified and need additional studies (*Paratylenchus* sp. 5 SAS, *Paratylenchus* sp. D, *Paratylenchus* sp. 1 FL, and *Paratylenchus* sp. 94bald). Nematode population densities ranged from 0.01 to 6.84 nematodes/cm3 of soil, but no plant damage was detected, although additional studies are needed to clarify the influence of these nematode population densities on plant olive growth and yield.

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242. Functional analysis of core SPRYSEC proteins from the potato cyst nematode *Globodera pallida*

James A. Price[1], Cheyenne Chan[2], David McLean[3], John T. Jones[1]
1 Cell & Molecular Sciences Department, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK
2 School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK
3 Division of Plant Sciences, School of Life Sciences, University of Dundee at The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Poster
Keywords: potato cyst nematode, *Globodera*, effector, SPRYSEC

ABSTRACT:

The potato cyst nematode *Globodera pallida* has complex interactions with its hosts. The nematode induces a large, multinucleate syncytium in the roots of host plants and ensures that this is protected from host immune responses for the duration of the nematode's life cycle. Effector proteins, secreted proteins that manipulate the host to the benefit of the pathogen, play critical roles in this process. Genomic analysis has revealed that *G. pallida* has very large, expanded gene family encoding SPRY domain proteins, some of which, the SPRYSECs, are deployed as effectors. Some SPRYSECs have been shown to suppress defence responses induced by activation of resistance proteins in Solanaceous plants. We have identified a core subset of SPRYSEC proteins that are present and expressed in a variety of *G. pallida* populations. We show that many of these are expressed specifically in the dorsal gland cell of *G. pallida*. These core SPRYSECs locate to a wide range of different host cell compartments including the cytoplasm, the nucleolus, chloroplasts and endoplasmic reticulum. Two of the core SPRYSECs suppress host defence responses. Future work will be aimed at identifying the host targets of these proteins.

243. Exploring genetic interactions in *Bursaphelenchus* species: implications for potential genetic pollution between *Bursaphelenchus xylophilus* and *B. mucronatus*

Yuzuki Ikeda[1], Kenichi Yanagisawa[2], Naoko Ishikawa[3], Yoshihisa Suyama[3], Ryoji Shinya[1]
1 Meiji University, Kanagawa, Japan
2 Nagano Prefecture Forestry Research Center, Nagano, Japan
3 Tohoku University, Miyagi, Japan

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: pine wilt disease, *Bursaphelenchus mucronatus*, Phylogeny and evolution, speciation, genotyping

ABSTRACT:

Bursaphelenchus xylophilus, the causal agent of pine wilt disease, is closely related genetically to *B. mucronatus*, a species of weaker pathogenicity. The latter comprises two subspecies: *B. m. mucronatus*, native to East Asia, and *B. m. kolymensis*, native to Europe. Previous laboratory experiments have demonstrated the ability of *B. xylophilus* and *B. mucronatus* to produce hybrids. Field studies in China using single gene markers have suggested the presence of hybrids between *B. xylophilus* and *B. m. mucronatus*. In this study, we used genome-wide genotyping analysis using MIG-seq to determine the presence of hybrids in natural populations in Japan. A total of 117 vector beetles, *Monochamus alternatus*, were examined and 32 were found to carry both *B. xylophilus* and *B. m. mucronatus*. DNA was extracted from 170 individual nematodes isolated from 17 of these beetles and then subjected to MIG-seq analysis. The results identified 1,401 single nucleotide polymorphisms (SNPs) showing the presence of four hybrids between *B. xylophilus* and *B. m. mucronatus*. These hybrids were detected in populations where both species were equally abundant or where *B. m. mucronatus* dominated. To understand the dynamics of hybrid individuals in the field, we investigated the number of eggs laid and hatchability in crosses between *B. xylophilus* and *B. m. mucronatus* as well as *B. m. kolymensis*. The results showed a significant decrease in both the number of eggs laid and hatchability in interspecific crosses. Although fecundity was reduced, reproductive isolation between these two species was apparently incomplete. The potential for genetic pollution from backcrossing remains, especially with *B. m. mucronatus*, which has higher populations than hybrids, although the proportion of hybrids detected in this study is low. This study also raises the possibility of hybrid formation and genetic pollution involving *B. xylophilus* and *B. m. kolymensis* in European populations. These findings highlight the importance of understanding the ecological implications of genetic interactions between closely related species and emphasize the need for further research on the potential consequences of genetic pollution in natural ecosystems.

244. Effect of Indian crested porcupine (*Hystrix indica*) activity on abundance and diversity of soil free-living nematodes

Stanislav Pen-Mouratov[1], David Pen[1]

1 The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University, Israel

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: herbivores, biodiversity, porcupine, feces, soil properties, bioindicator, soil biota, free-living nematodes.

ABSTRACT:

Herbivore species' activities, abundance, diversity and diets can exert various effects on the processes, functions and properties of ecosystems, and directly or indirectly effect changes in soil community structure. Herbivores' fecal depositions are an important source of essential chemicals, such as phosphorus and nitrogen, for soil organisms. Through the input of nutrients via their urine and feces, as well as trampling and selective browsing, herbivores can modify the structure and composition of soil ecosystem inhabitants. The impact of nutrient cycling through feces and urine on soil ecosystems has been well-studied for large and small herbivores, however, reports on the effects of medium-sized herbivores' excreta on the soil biota are scarce. To narrow the gap, we determined the impact of feces deposition of the Indian crested porcupine (Hystrix indica) on the abundance, structure and diversity of free-living nematode communities in Israel's Mediterranean region during the annual dry and wet seasons. Results confirmed our assumption that medium-sized herbivores can also have a significant impact on soil free-living nematodes and their habitats. Fecal deposits of the Indian crested porcupine were found to exert various effects on the observed soil properties, and mainly on the abundance of soil free-living nematodes. In accordance with earlier findings, we showed that soil properties and the observed external environmental factors have a seasonal effect on the abundance of soil free-living nematodes. Environmental indices showed that nutrient supply through the porcupine's urine and feces can have different (stimulatory or inhibitory) impacts on the abundance of the soil communities, affecting the abundance and structure of the soil free-living nematode population during the observed months and seasons of the year.

Herbivores' fecal depositions are an important source of essential chemicals, such as phosphorus and nitrogen, for soil organisms. The impact of nutrient cycling through feces and urine on soil ecosystems has been well-studied for large and small herbivores, however, reports on the effects of medium-sized herbivores' excreta on the soil biota are scarce. To narrow the gap, we determined the impact of feces deposition of the Indian crested porcupine (Hystrix indica) on the abundance, structure and diversity of free-living nematode communities in Israel's Mediterranean region during the annual dry and wet seasons.

245. Plant-parasitic nematodes in *Prunus* groves and ecological factors affecting their distribution in Spain.

Ilenia Clavero-Camacho[1], Antonio Archidona-Yuste[1], Carolina Cantalapiedra-Navarrete[1], Pablo Castillo[1], Juan Emilio Palomares-Rius[1]

1 Institute for Sustainable Agriculture-CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: nematodes, *Meloidogyne*, *Pratylenchus*, *Paratylenchus*, rootstock, distribution, soil, grove-use, almond, peach.

ABSTRACT:

This research aimed to determine the prevalence and distribution of plant-parasitic nematodes (PPNs) in *Prunus* groves, as well as the influence of explanatory variables describing soil, climate and agricultural management in structuring the variation of PPNs community composition. A total of 218 sampling sites were surveyed and 84 PPN species belonging to 32 genera were identified based of an integrative taxonomic approach. *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Pratylenchus penetrans* and *P. vulnus*, were identified as potential pathogenic nematodes in this survey. Other species could be potentially pathogenic because of the high levels found in some fields, such as *Paratylenchus hamatus*, and *P. tenuicaudatus*. Seven soil physico-chemical (C, Mg, N, Na, OM, P, pH and clay, loamy sand and sandy loam texture classes), four climate (Temperature seasonality-standard deviation ×100 (Bio04), max temperature of warmest month (Bio05), precipitation of wettest month (Bio13) and precipitation of driest month (Bio14)) and four agricultural management variables (grove-use history less than 10 years, irrigation, apricot seedling rootstock, and Montclar rootstock) were identified as the most influential variables driving spatial patterns of PPNs communities. In particular, younger plantations showed higher values for species richness and diversity indices than groves cultivated for more than 20 years with *Prunus* spp. This study has important implications for the successful design of sustainable management strategies (i.e. resistant rootstocks) in the future in this agricultural system.

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247. Environmental tolerance of *Bursaphelenchus tadamiensis* in tree sap flow

Kyoka Yoshitomi[1], Ryoji Shinya[1], Natsumi Kanzaki[2]
1 Meiji University, Kanagawa, Japan
2 Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto, Japan

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Poster
Keywords: acid tolerance, alcohol tolerance, high osmotic pressure tolerance, hypoxia tolerance, environmental adaptation

ABSTRACT:
Organisms develop their stress tolerance in order to thrive under hostile environments. One such habitat, sap flow, contains a wide variety of carbohydrates that increase the osmotic pressure of the environment. In addition, yeast fermentation within this habitat provides conditions characterized by low pH, high alcohol content, and low oxygen concentration - harsh environmental factors that challenge many organisms. A nematode, *Bursaphelenchus tadamiensis*, which was repeatedly isolated from the sap flow of *Quercus* trees, is suspected to adapt to the sap flow environment, but its adaptation (tolerance) has not been confirmed or examined experimentally. This study represents an initial exploration of the adaptive mechanisms of *B. tadamiensis* in the sap flow environment. The nematode was exposed to four stress conditions hypothesized to be present in the sap flow environment. Tolerance levels were quantified based on survival rates after exposure, and *Caenorhabditis elegans* and *B. okinawaensis* were employed as negative controls. *B. tadamiensis* showed a considerably high tolerance to low pH and alcohol, i.e., the survival rates under pH 2.0 and 15.1% alcohol conditions were significantly higher than the control species. *B. tadamiensis* also showed high tolerance to hyperosmotic and hypoxia conditions. The survival rate of *B. tadamiensis* after exposure to 15 and 20 w/w% glucose solution was significantly higher than that of *C. elegans*, and the rates after 48-288 hours of exposure to hypoxic conditions were comparable or higher than those of *B. xylophilus*, which was previously reported to be highly tolerant to hypoxia. These results suggest that *B. tadamiensis* is adapted to the sap flow environment. Notably, this tolerance was observed in the adult stage, indicating that *B. tadamiensis* does not necessarily require a specialized stage, such as dauer larvae, for survival in sap flow.

247, Identification of *Oscheius myriophilus* (Nematoda: Rhabditidae) on gastropods and its comparable virulence to *Phasmarhabditis papillosa* against *Arion vulgaris*, *Deroceras reticulatum*, and *Cernuella virgata*.

Ziga Laznik[1], Stanislav Trdan[1]
1 University of Ljubljana, Biotechnical faculty, Slovenia

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: slug parasitic nematodes, *Oscheius myriophilus*, *Phasmarhabditis papillosa*, *Arion vulgaris*, *Deroceras reticulatum*, *Cernuella virgata*.

ABSTRACT:

Between July and September 2021, researchers collected 100 specimens of the Spanish slug, *Arion vulgaris*, and dissected their cadavers to examine them for parasitic nematodes. Molecular techniques were used to identify the nematodes, which confirmed the presence of *Oscheius myriophilus*, marking the first recorded instance of this nematode in a gastropod host. To test the virulence of Slovenian strains of *O. myriophilus* and *Phasmarhabditis papillosa*, and their effects on the feeding behavior of the Spanish slug, grey field slug (*Deroceras reticulatum*), and vineyard snail (*Cernuella virgata*), laboratory bioassays were conducted using nematodes grown in vivo. Nematodes were applied at various doses ranging from 10 to 500 nematodes/gastropod. The results showed that *O. myriophilus* and *P. papillosa* caused significant mortality of the Spanish slug, while being less effective against the vineyard snail and grey field slug. Nematodes were more virulent at lower temperatures (15 °C) than at higher temperatures (20 °C) tested in the experiment. Additionally, both nematode species significantly reduced gastropod herbivory. The potential use of *O. myriophilus* and *P. papillosa* as biological control agents against gastropods was discussed.

248. Comparative study of nematode taxocenoses (Dorylaimida and Mononchida) associated with two typical Mediterranean habitats (holm oak forest and olive grove) in Andalusia, Spain

Miriam García-Ruiz[1], Reyes Peña-Santiago[1]

1 Departamento de Biología Animal, Biología Vegetal and Ecología. Universidad de Jaén. Campus Las Lagunillas s/n, 23071, Jaén, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords:

ABSTRACT:

The composition and structure of the nematode taxocenoses (dorylaims and mononchs) dwelling two habitats, a pristine (holm-oak) forest and an agro-ecosystem (olive grove) are analyzed to explore the effect of land use change on soil biodiversity. Twenty (ten per each habitat) soil samples were collected in two close enclaves of the province of Jaén, Andalusia, Spain. Nematodes were extracted by centrifugation, fixed in formaldehyde (4%), mounted in permanent slides, and identified to species level using light microscopy. A total of 1076 nematodes belonging to 36 species were examined. Their biogegraphical nature is diverse, including cosmopolitan (42%), Holarctic (12%), Paleartic (21%), Iberian (21%), and Andalusian (4%) elements. Species richness (α-diversity), abundance (number of specimens per sample) and frequency (number of soil samples where a species is present) were calculated and compared. Both species richness and abundance were higher in forest than in olive grove (10.3 ± 4.5 vs 6.7 ± 3.6, 73.7 ± 65.1 vs 34.0 ± 25.4, respectively), showing an appreciable diversity loss. Nevertheless, the observed differences were not statistically significant, probably due to the wide dispersion range of data. The composition and structure of the taxocenoses differ too as they share only two-fifths (39%) of their species, which displayed different values of abundance and frequency in both habitats. Two species, *Dorylaimellus* sp. and *Allodorylaimus paragranuliferus*, presented significant differences in their distribution, the former being associated with holm oak forest and the later with olive grove.

249. Host range characterization and crop rotation as useful tools to limit population densities of *Meloidogyne chitwoodi* and *M. fallax*

Nathan Garcia[1], Fabrice Ollivier[1], Nicolas Lenne[2], Sylvain Fournet[3], Laurent Folcher[1]
1 French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Plant Health Laboratory, Nematology Unit, Domaine de la Motte au Vicomte, 35653 Le Rheu, France
2 French Ministry for Agriculture and Food, General Directorate for Food (DGAL), Plant Health Department, 251 rue de Vaugirard, 75732 Paris, France
3 IGEPP, INRAE, Institut Agro, Rennes University, 35653 Le Rheu, France

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: quarantine nematode, pest management, greenhouse experiment, host range, population

ABSTRACT:

In EU member states, *Meloidogyne chitwoodi* and *M. fallax*, the root-knot nematodes, are regulated pests of major economic importance particularly in vegetable and potato crops. In these countries, phytosanitary legislation, enforce drastic and environmentally-friendly management practices. Alternative solutions to chemical nematicides are thus needed to deal with those organisms and to conciliate crop yield and environmental and human health. Among available innovative strategies such as for instance, inducing hatching of eggs in the absence of host plant, creating resistant plant cultivars thanks to resistance genes or stimulating antagonistic natural enemies for example, we studied and looked for plants and/or plant cultivar that does not multiply *M. chitwoodi* and *M. fallax*. At the French agriculture ministry's request, the tested plants and cultivars had to be economically interesting crops and could integrate crop rotations, in order to represent an alternative solution to black fallow that have been implemented according to the French legislation. Practically, the principle of these tests was to screen under confined greenhouse conditions the abilities of various plant species and cultivar to produce females and egg masses from an inoculum of nematode larvae. Plants were then classified in three categories defined as 'good'(> 1 female/g of roots), 'less good' (0 < female/g of roots < 1) or 'poor' (0 female/g of roots) host plants. The greenhouses experiments took place between 2009 and 2013 in order to test numerous plants and cultivars but also different populations of *M. chitwoodi* and *M. fallax*. Following the greenhouse tests, a field trial has also been set up to monitor the population dynamics of *M. chitwoodi* through different other modalities including crop rotations compared to a nematicide treatment and black fallow.

Due to the high number of tested cultivars, it appears that there were an important variability between cultivars of a same plant species in their ability to multiply *M. chitwoodi* and/or *M. fallax*. Overall, we however highlight that cultivars of carrots, beans and alfalfa were the most efficient 'less good' and 'poor' host plants for both species, but also radish or onion for *M. chitwoodi*. This also highlight the importance of such screening test regarding the differences between the cultivars.

Those results may now be used to set up the most efficient crop rotations, using the best cultivars in order to decrease the *M. chitwoodi* and *M. fallax* in the fields in addition with other management strategies.

250. Response of soil nematodes communities to cover crops in spinach production

Elyse Aubry[1], Jerry Akanwari[1], Tahera Sultana[2]
1 Brock University, St. Catharines, Ontario, Canada
2 Agriculture and AgriFood Canada, Vineland Station, Ontario, Canada

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: Nematodes, ecology, diversity,

ABSTRACT:

Recent studies have shown that Canadian growers are increasing relying on cover crops to improve the quality of the soil, suppress weeds, and manage diseases and pests. The use of cover crops in agricultural systems have since been identified as an environment friendly management practice in the province of Ontario. Although, cowpea and pearl millet are the most frequently used cover crops in southern region of Ontario their effect on nematodes communities are unknown. The main objectives of the study is to determine the impact of cowpea, pearl millet, and a mixture of cowpea and pearl millet on the abundance and diversity of soil nematodes communities. To explore these objectives, a comprehensive field study was conducted, focusing on two key stages: late-stage growth of cover crops (CC) and subsequent tillage with spinach planted and harvested (SP). The results show that, the average number of bacterivores was significantly higher in the Cow Pea treatment, having more than 16% than the treatment with no cover crops (Conventional Practice). However, no significant difference was observed in the number of fungivores and omnivores between ‘CC’ and ‘SP’ phases. The mixture of cover crops significantly reduce the number of herbivores (plant parasitic nematodes) over 18% compared to conventional practices Nevertheless, when spinach was cultivated after cover crops use, there was no significant difference, suggesting a potential recovery and reproduction of few plant parasitic nematodes on spinach. Pearl Millet had significantly lower species richness compared to the other treatments during the ‘CC’ phase but no significant differences was observed at the ‘SP’ phase. Also, no significant difference in Shannon-Wiener Diversity index between ‘CC’ and ‘SP’ phases. Our study shows the potential of cover crops to enhance overall soil health for sustainable agricultural production. Also, the study shows the significance of cover crop selection and implementation strategies in nematode management.

251. Free-living nematodes associated with olive groves in Mediterranean Basin - Preliminary results of *Soil O-live* EU Project

Miriam García-Ruiz[1], Joaquín Abolafia[1], Reyes Peña-Santiago[1]

1 Departamento de Biología Animal, Biología Vegetal and Ecología. Universidad de Jaén. Campus Las Lagunillas s/n, 23071, Jaén, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords:

ABSTRACT:

The study of free-living nematode fauna dwelling olive groves has received little attention in spite of being the most prominent and the most socioeconomically important fruit tree cultivated in Europe, especially across the Mediterranean Basin. At present, only 61 species and 35 genera belonging to 21 families and eight orders were previously recorded. In the course of a nematological survey conducted within the framework of the *Soil O-live project* (EU Horizon Program grant No 101091255), a total of 264 soil samples were collected in 44 locations of four countries (Greece, Morocco, Portugal and Spain), including traditional, intensive and organic tillage. Provisional results obtained from the analysis of 48 soil samples of eight Spanish groves reveal the presence of 64 species, 43 genera, 23 families and 7 orders. Diversity (species richness) reaches a value of 13.1 ± 4.6 (4-25) species per 50 cm3 soil. Abundance (number of specimens) is 70.1 ± 38.9 (9-157) nematodes per 50 cm3 soil. The most abundant species are *Acrobeloides nanus* (24.1% of the total), *Chiloplacus trilineatus* (7.7%), *Microdorylaimus* sp. (6.5%), *Acrobeles singulus* (5.1%), *Cervidellus vexilliger* (4.4%), *Dorylaimellus egmonti* (4.3%), *Aporcelaimellus obtusicaudatus* (3.8%) and *Dorylaimellus parvulus* (3.1%). Distributional data of diversity and abundance are analyzed to detect differences between groves, locations and tillage methods.

252. Preliminary Nematode data from high-altitude, extremely arid desert soil in Chile

Renáta Petrikovszki[1], Gergely Boros[1], Balázs Nagy[2]
1 Hungarian University of Agriculture and Life Sciences, Godollo, Hungary
2 The Globe Foundation, PermaChile Network, Budapest, Hungary
3 ELTE Eötvös Loránd University, Budapest, Hungary

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: Chile, atacama, biodiversity, free-living nematodes, PermaChile

ABSTRACT:

PermaChile is an international long-term research program, focusing on the effect of climate change in the Chilean Andes Mountains. The aim of the multidisciplinary data collection is the monitoring of changes in permafrost and their effect on soil properties.

In February 2020, soil samples were collected in the arid Puna Plateau (Dry Andes), the high mountain part of the Atacama Desert from the following areas: 3-3 samples from Laguna Verde (4350 m a.s.l.), Atacama Camp (5260 m a.s.l.), Tejos Camp (5830 m a.s.l.), Tejos Upper Lake (5900 m a.s.l.), and 5 additional samples from a puna-vegetated plateau-wetland, close to a mountain stream (3900 m a.s.l.). Active, free-living nematodes were extracted by Baerman funnel method from 25 g of soil, then their density was measured by counting in Gödöll (Hungary). After fixation with 8% formalin, their trophic group was determined as well.

No nematodes were found in the soil samples of Laguna Verde, Atacama Camp, and Tejos Camp area collected in 2020. However, at the bank of Río Lamas, a diverse density of nematodes appeared depending on habitat. A total of 8 genera with 99 individuals from sample 1. without plantation, 20 genera with 784 individuals from sample 1. under willow (Salix sp.), 18 genera with 2178 individuals from sample 1. under chervil (Anthriscus sp.), 12 genera with 152 individuals from sample 2., 17 genera with 262 individuals from sample 3. at 750 m level. Considering all the above, 38 nematode genera were identified including *Acrobeles*, *Acrobelophis*, *Aphelenchoides*, *Aporcelaimus*, *Cervidellus*, *Clarkus*, *Criconema*, *Dorylaimidae*, *Paratylenchus*, *Plectus*, *Tylencholaimus*, *Wilsonema*. Although the highest numbers of individuals were in the samples under chervil and willow plants, according to the food web analysis, the sample taken from the willow tree belongs to quadrant D, and the other 3 samples belong to the resource-limited and structured quadrant C. Sample 3 has a high abundance of c-p 4, which shows that this environment is ideal for omnivorous groups thanks to less disturbed conditions.

As part of the PermaChile project, we are conducting new samplings and including additional, new areas in the investigation of free-living nematode biodiversity.

The work was funded by the Hungarian National Research Fund (NKFIH 147424).

253. Changes in soil nematode populations caused by intensive farming practice in monoculture cucumber in Hungary: a case study

Dóra Sára Selmeczi[1], Renáta Petrikovszki[1], Anita Gódor[1], Péter István Nagy[1], Barbara Simon[3], Ferenc Tóth[2]

1 Department of Zoology and Ecology, Hungarian University of Agriculture and Life Sciences, Godollo, Hungary
2 Research Institute of Organic Agriculture, Budapest, Hungary
3 Department of Soil Science, Hungarian University of Agriculture and Life Sciences, Godollo, Hungary

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: artificial fertilizers, irrigation, salt stress, free-living nematodes

ABSTRACT:

The long-term effect of drip irrigation, continuous fertilization and soil disinfection against root-knot nematodes (*Meloidogyne incognita*) were monitored on free-living nematodes in a 25-year-old monoculture of cucumber (*Cucumis sativus* Monolit F1).

Soil properties (pH, salinity, plant available N, P and K, organic matter content) and nematological characteristics (trophic groups, c-p classes, abundance, indices) were determined on the chosen field.

All examined nematological indices proved a depleted, stressed and unstable environment. The application of fertilizers via dripping irrigation system led to high salinity which caused phytotoxicity for cucumber plants.

Highly stress tolerance nematode genera (*Acrobeloides* and *Cuticularia*) appeared in the area. The detection of male individuals of *M. incognita* means the alteration of the genetic composition of local *Meloidogyne* population which could result in adaptation of negative environmental changes.

All these changes in soil nematode communities can be attributed to intensive monoculture farming practice including drip irrigation and artificial fertilizers.

This work was supported by the New National Excellence Program (ÚNKP-22-4-II-MATE/11 and ÚNKP-23-4-II-MATE/3) of the Ministry for Innovation and Technology.

254. Distribution and prevalence nematode communities in three littoral areas of the Iberian Peninsula

Alba N. Ruiz-Cuenca[1], Joaquín Abolafia[1]
1 Departamento de Biología Animal, Biología Vegetal and Ecología, Universidad de Jaén, Campus Las Lagunillas, Jaén, Spain

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: coastal dunes, diversity, iberian peninsula, Rhabditida

ABSTRACT:

Nematodes are a large and diversified zoological group with a wide global distribution, being able even to be present in habitats with extreme conditions. Although coastal dunes can be considered as an adverse environment for these animals, numerous species are discovered there. In this study, a total of 222 sample sites of coastal sand dunes have been studied in order to know the nematofauna of these habitats. Thus, 42 coastal dunes from three littoral areas of the Iberian Peninsula coast (Atlantic coast, southern Mediterranean coast, and northern Mediterranean coast) were examined. The identification of these species were carried out based on integrative taxonomic approaches, including morphological and molecular characterization. Also, the prevalence was calculated and trophic structure of the nematode community was studied by subdividing into seven groups according to feeding type. A total of 120 species of nematodes were found, belonging to eight orders: Dorylaimida, Enoplida, Isolaimiida, Monhysterida, Mononchida, Plectida, Rhabditida and Triplonchida. The results showed the higher prevalence of the species belonging to the order Rhabditida, which were present in 84.2% of the sand dunes studied. On the other hand, the classification of nematofauna by trophic groups showed a total of seven groups as bacterial feeders, omnivores, hyphal feeders, and plant feeders shared a high prevalence (83.3%, 40.5%, 34.2%, and 32.8%, respectively), while predators, unicellular eukaryote feeders, and substrate ingesters appear with less than 11%. According to the geographical distribution of species per each littoral area, a total of 66 species were found in the Atlantic littoral, 75 species in the southern Mediterranean littoral, and 78 species in the northern Mediterranean littoral, showing the dunes located at the southern Mediterranean coast a higher percentage of rhabditid species (73.4%). The geographical distribution based on feeding habits shows that the bacteriophages are present in all littoral areas studied, having the southern Mediterranean coast a higher percentage of species of bacterial feeders (69.3%) as well as the highest prevalence of this trophic group (97.2%).

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255. The impact of eco-sustainable soil and phytosanitary management on nematode communities in vineyard

Silvia Landi[1], Giada dErrico[2], Francesco Binazzi[1], Stefania Simoncini[1], Andrea Rocchini[1], Sara Del Duca[3], Atruro Fabiani[3], Stefano Mocali[3], Paolo Storchi[4]

1 CREA Research Centre for Plant Protection and Certification, Florence, Italy
2 2 Dipartimento Scienze Agraria, Università di Napoli II, Via Università 100, 80055, Portici, Napoli, Italy
3 3 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca Agricoltura e Ambiente (CREA-AA), Via di Lanciola 12/A, Cascine del Riccio, 50125 Firenze, Italia
4 4 Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di Ricerca Viticoltura ed Enologia (CREA-VE), Viale S. Margherita 80, 52100 Arezzo, Italia

Area: Nematodes as Bioindicators, Diversity and Ecology
Type: Poster
Keywords: soil nematode indicators, free-living nematodes, plant-parasitic nematodes

ABSTRACT:

The objective of the LIFE GREEN GRAPES project was to decrease the use of plant protection chemical products on grapevine plants. The reduction was obtained by integrating cover crop management (permanent cover crop and green manure), use of defense inducers, and optimized timing of treatment applications by the Decision Support System. The present study aims to assess the potential effectiveness of Green Gapes management to prevent soil health degradation. Different indicators were used to evaluate the changes in biodiversity at different scales. Soil biodiversity was assessed on microbial, nematode, and microarthropod indicators. Specifically, the soil nematode community represents an efficient tool for understanding the efficacy of this management strategy, soil free-living nematodes and their predators are involved in the cycling of carbon, nitrogen, and phosphorous by grazing bacteria and fungi, instead, plant-parasitic nematodes may represent a serious concern in the vineyard, especially virus-vector nematodes.

The impact of soil management and control protocols was evaluated in a farm located in DOCG Chianti Classico (FI) district, characterized by 'Sangiovese' cultivar and usual tillage. Together with the two eco-sustainable soil managements, five different strategies for grapevine protection were also compared: Integrated Pest Management (IPM), IPM with fungicide reduction, organic control strategy, organic control strategy with 50% reduction of copper applications, and organic strategy with 100% reduction of copper applications. The soil nematode community was monitored by the nematode taxa abundance and soil nematode indicators. Moreover, trans-kingdom relations involving all studied taxa were investigated.

In general, the introduction of cover crops allowed a good separation between pre and post-treatment conditions for nematodes as evidenced by NMDS analysis. Moreover, a separation based on soil management was also evidenced. The indicators confirmed an increment of the Maturity Index (MI), which doubled at the end of the trial. The green cover showed the highest Structure index values due to the increase in predators, conversely, green manure increased the Channel index value because it benefited the fungal feeder populations. Plant-parasitic nematodes belonging to the Hoplolaimidae and Pratylenchidae families were dominant in green cover and green manure, respectively. After three years of eco-sustainable soil management application, the

connections of nematodes with the other taxonomic groups increased. The impact of phytosanitary management on the soil nematode community was almost nil.

256. Transcriptome analysis of *Meloidogyne incognita* during tomato parasitism in water stress conditions

Isabella Pentimone[1], Elena Fanelli[1], Pasqua Veronico[1], Laura Rosso[1], Aurelio Ciancio[1], Mariantonietta Colagiero[1], Maria Teresa Melillo[1], Francesca De Luca[1]
1 Istituto per la Protezione Sostenibile delle Piante-CNR, Via Amendola122D, 70126 Bari, Italy

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Poster
Keywords: water stress, *Meloidogyne incognita*, transcriptome

ABSTRACT:

Water stress and nematode parasitism are two stress sources with interacting effects in field conditions. However, few data are available on the effect of water stress on nematodes during plant parasitism. We collected tomato root segments containing galls induced by *Meloidogyne incognita* 7 and 14 days post inoculation (dpi) in parasitized plants, exposed or not to a water stress, to perform a comparative nematode transcriptome analysis. After filtering reads mapping on the tomato genome, the *M. incognita* ASM 18041v1 genome was used to identify genes up- and downregulated in M. *incognita* at 7 and 14 dpi, in both conditions. To assess time changes in *M. incognita* differentially expressed genes (DEG), RPKM cutoff values ≥ 10 were applied, between the two times course conditions. The DEGs, evaluated at 7 and 14 dpi, were considered significant when displayed at least a twofold change (Fc, $p < 0.05$) compared with unstressed condition. GO enrichment analyses included 93 and 703 genes up-regulated and 114 and 1093 down-regulated, at 7 and 14 dpi, respectively. Genes up-regulated at 7 dpi showed a significant enrichment of transcripts related to ion and phosphatase binding proteins along with pantothenate activity, and to several intracellular non-membrane organelle cellular components. The genes up-regulated at 14 dpi showed an enrichment of transcripts related to structural constituents of cuticle and peptidase activity. The genes down-regulated at 7 dpi showed an enrichment of transcripts for different binding proteins, with glycolytic and metabolic processes, mostly down-regulated at 14 dpi. Data contribute to the understanding of *M. incognita* gene expression patterns, useful for the management of this nematode in arid climates and water stress conditions.

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257. Exploring the complexity of sex determination in cyst nematodes

Muhammad Usman Ijaz[1], Santosh Bhandari[1], Florian M. W. Grundler[1], M. Shamim Hasan[1]
1 Molecular Phytomedicine, University Bonn, INRES, Bonn, Germany

Area: Nematodes Metabolism, Physiology and Parasitism Genes
Type: Poster
Keywords: Cyst nematodes, sex determination, plant-nematode interactions, dsRNA

ABSTRACT:

Cyst nematodes are plant parasites that threaten global food security. In the course of parasitizing the plant roots, they induce a nutrient cell system by changing the differentiation of the infested host cells in such a way that a syncytium is formed. This syncytium serves exclusively to feed the nematode. Although the nematodes are sexually dimorphic, the differentiation process into males and females only takes place when the food source is already established. However, the exact mechanism of sex determination and differentiation is still unknown. In this work, we show that several factors such as the hatching time of the beet cyst nematode (BCN, *Heterodera schachtii*), the time between hatching and feeding and the age of the host plant influence the sex differentiation of nematodes. This indicates that these and other non-genetically fixed factors have a decisive influence on sex determination. Recently published genomic and transcriptomic data for BCN finally allow us to investigate the genetic components of the mechanism of sex determination in more detail. The focus is on BCN genes that show sequential and functional homologies of sex determination traits in the extensively studied model nematode Caenorhabditis *elegans*. Using RNAi technology, we are currently investigating key components of the relevant signaling pathways to unravel the complexity of sex determination in cyst nematodes. Our work has the potential to provide multiple solutions to agricultural challenges contribute to sustainable agricultural practices and improve our understanding of fundamental biological processes.

258. RNA tomography reveals the spatial transcriptome of *Arabidopsis thaliana* roots during the early stages of *Heterodera schachtii* infection

Anna Pijnacker[1], Yuhao Wang[1], Jaap-Jan Willig[1], Rik Korswagen[2], Jonas Mars[2], Aska Goverse[1], Geert Smant[1], José Lozano Torres[1]

[1] Laboratory of Nematology, Wageningen University and Research, Wageningen, The Netherlands,
[2] Royal Netherlands Academy of Arts and Sciences and University, Hubrecht Institute, Utrecht, The Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: spatial transcriptomics, RNA tomography, nematode feeding site development, cell wall integrity

ABSTRACT:

Nematodes, a pervasive global threat to food security, cause billions of losses in crop production. Cyst nematodes, among the most damaging plant-parasitic worms, induce a permanent feeding site inside host roots. Current management strategies, such as resistance genes and pesticides, often lack durability or pose environmental risks. Therefore, the development of broad-spectrum nematode resistant plants is a necessity to secure food production. To address this critical need, we aim to contribute to this development by uncovering the cellular and molecular mechanisms underlying the initial cell wall modifications that enable nematode parasitism. We hypothesize that cell wall integrity receptors, essential for sensing cell wall damage and regulating developmental and immune responses, play a key role in nematode feeding site formation. We employed RNA tomography, a cutting-edge spatial transcriptomic technology, to study the initial stages of *Heterodera schachtii* infection in *Arabidopsis thaliana*. This approach enabled us to generate transcriptional maps of *H. schachtii*-infected *Arabidopsis* roots, revealing essential cell wall integrity genes involved in the initial stages of cyst nematode infection. Furthermore, we are generating spatial gene expression maps for *Meloidogyne incognita*-infected plant roots to compare cell wall integrity spatial transcriptomic profiles between root-knot and cyst nematode species. This comprehensive data will shed light on the onset of parasitism and pave the way for developing novel broad-spectrum nematode resistance in crop plants.

259. Transcriptome and functional analysis provide insights into tomato *Mi-1*-mediated temperature sensitivity responses to root-knot nematode

Zhijie Li[1], Erfeng Li[1], Jianlong Zhao[2], Zhenchuan Mao[1]
1 Tianjin Agricultural University, Tianjin, China
2 Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: Root-knot nematode, Mi-1 resistance gene, Temperature sensitivity, Plant immunity, Regulation pathway

ABSTRACT:

Root-knot nematodes are obligate endoparasites, which are infamous plant pathogens causing huge losses of horticultural plants globally every year. The tomato RKNs resistance gene Mi-1 is the sole commercially available resistant resource, which resistance will be lost when the soil temperature exceeds 28°C. Many studies focus on analyzing Mi-1-mediated immune signal pathways, however, which plant immune responses are affected at high temperature and what are the key temperature-sensitive genes involved in Mi-1-mediated RKNs resistance are largely unknown. In this study, we found that the hypersensitive immune response mediated by Mi-1 was affected at high temperature (32°C), including decreased reactive oxygen species production, but increased callose deposition in roots. By comparative transcriptome analysis of Mi-1 tomato transcriptomes between 24°C and 32°C, we found that the plant hormones signaling pathways were significantly different at 32°C, the jasmonate (JA) pathway was up-regulated, and the salicylic acid pathway was inhibited. Further, virus-induced gene silencing assays demonstrated that interfering the expression level of differentially expressed genes, including MYB transcription factor (AOS3), JA signaling pathway key gene (JA2), heat stress transcription factor (A-6b), could increase susceptibility to RKNs of Mi-1 tomato. These data and results will provide valuable insights into the regulation mechanism of Mi-1 resistance in temperature sensitivity and support efforts to engineer tomatoes with RKNs resistance at high temperature.

260. Phytophagy by plant-parasitic nematodes impacts the flow of host carbon resources to mutualistic arbuscular mycorrhizal fungi

C Bell[1], E Magkourilou[2], J Ault[3], P Urwin[1], K Field[2]
1 School of Biology, Faculty of Biological Sciences, University of Leeds, UK
2 Plants, Photosynthesis & Soil, School of Biosciences, University of Sheffield, UK
3 School of Molecular & Cellular Biology, Faculty of Biological Sciences, University of Leeds, UK

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: *Globodera pallida*, arbuscular mycorrhizal fungi, symbiosis, resources

ABSTRACT:

Plant roots interact with a myriad of soil organisms including mutualistic arbuscular mycorrhizal (AM) fungi and plant-parasitic nematodes. AM fungi may enhance their host’s access to soil nutrients in exchange for plant carbon, and this exchange is disturbed by the presence of competing symbionts, such as plant-parasitic nematodes. The mechanisms by which plants prioritise carbon allocation to mutualistic AM fungi over parasitic symbionts remain poorly understood. We have shown that host potato roots selectively allocate carbon resources to tissues interacting with AM fungi rather than those interacting with the potato cyst nematode *Globodera pallida*. We found that plants reduce the supply of hexoses but maintain the flow of plant-derived fatty acids to AM fungi when concurrently interacting with *G. pallida*. Transcriptomic analysis suggests that plants prioritise carbon transfer to AM fungi by maintaining expression of fatty acid biosynthesis and transportation pathways, whilst decreasing the expression of mycorrhizal-induced hexose transporters. We also report similar findings from a different plant host species (*Medicago truncatula*) and a phytophagous pest (the aphid *Myzus persicae*). These findings suggest a general mechanism of plant-driven resource allocation in scenarios involving multiple symbionts.

261. Single cell study of root-knot nematode infection at early stages using single cell RNA sequencing

Almudena Gómez Rojas[1], Maite Saura[2], Patricia Abril Urías [1], Thomas Eekhout[3], Bert De Rybel[3], Tina Kyndt[4], Tom Beeckman[3], Carolina Escobar Lucas[1]

1 Facultad de Ciencias Ambientales and Bioquímica, Universidad de Castilla-La Mancha, Toledo, España

2 Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, 9052 Ghent, Belgium

3 VIB centre for Plant Systems Biology, Technologiepark 71, 9052 Ghent, Belgium

4 Department Biotechnology, Ghent University, Proeftiunstraat 86, 9000 Ghent, Belgium

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords:

ABSTRACT:

Root-knot nematodes (RKN), are plant parasitic nematodes (PPNs) characterized by the formation of galls inside the root, causing extensive damage in major crops [1]. During RKN infection, root tip cells undergo transcriptional, epigenetic and also phenotypic changes, i.e., cortex cells suffer hypertrophy, vascular cells proliferate and 5-8 vascular cells of not yet well know origin, differentiate into giant cells (GCs) forming the feeding site (NFs), the gall [2,3,4,5]. Hence, different cell types show differential responses to the nematode infection forming a pseudo-organ, the gall. To unravel these transcriptional changes at single cell resolution, a Single cell RNA sequencing (scRNA-seq) dataset of *Arabidopsis* was generated from roots infected with *Meloidogyne javanica* (*M. javanica*) at early infection stages. The infection protocol was implemented in order to improve their infection rates in *Arabidopsis* in vitro, and the stage of infection analyzed was selected based on the results of reporter lines known to be active in nematode feeding sites [6,7]. Three independent replicates from mock and J2 inoculated samples were collected yielding 35594 and 14153 high quality cells and a median of 1970 and 2005 genes per cell respectively. Using unsupervised graph-based clustering, cells were clustered in 28 major clusters identifying a 'nematode cluster' as a group of cells from the inoculated sample clustered together. These 'rare' nematode-induced cells could be the first cells responding to nematode infection and may already be reprogrammed to form the giant cells. However, further analysis are needed to e*luci*date this question and to study the specific transcriptional changes occurring in these 'rare' cells.

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262. Investigating the genetic basis of constitutive and induced plant defense responses against root-knot nematodes

Farveh Sadat Mostafavi Neyshabouri[1], Damaris Godinez-Vidal[1], Simon Cornelis Groen[1]
1 University of California, Riverside, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: root-knot nematode, *Arabidopsis*, constitutive defense, induced defense

ABSTRACT:

Root-knot nematodes (RKNs) have a significant impact on global agriculture. The current chemical control strategies lack the precision needed to adequately minimize unintended effects on the environment. Despite RKN prevalence, particularly in regions with warmer climates, the defense mechanisms enabling sustained crop yield as well as survival and reproduction of wild plants in regions with high nematode infection pressures remain unknown. Plants have to navigate trade-offs between growth and development versus defenses that arise through a variety of mechanisms. As a result, plants partition their investments in defense between constitutive and induced defenses. How plants coordinate being prepared for nematode infection (constitutive defense) versus being able to respond promptly upon detection of infection (inducible defense) is poorly understood. To understand the molecular basis of the balance between constitutive and induced defense responses, our study focuses on a population of the wild mustard *Arabidopsis thaliana* that shows natural variation in their levels of defense against RKNs. We will treat a collection of *Arabidopsis* accessions with nematode juveniles, NemaWater, nematode egg extract, and purified nematode elicitor molecules (ascarosides and chitin), measuring reactive oxygen species (ROS) and glucosinolate production and root growth as high-throughput measurements for the strength of inducible defenses. These measurements will identify different levels of nematode-induced defenses among *Arabidopsis* accessions. The resulting data will be used for genome-wide association (GWA) mapping to reveal the genetic architecture underlying the phenotypic variation using available genome-wide single nucleotide polymorphism (SNP) data. To further unravel the molecular basis of root defense, we will perform RNA-seq analysis on roots exposed to nematode elicitors. This approach aims to identify differentially expressed genes (DEGs) associated with varying levels of *Arabidopsis* response to elicitors. Combining ROS and glucosinolate production patterns with gene expression profiling and GWA mapping will enrich our understanding of the regulatory network governing constitutive and induced plant defense to nematode infection.

263. Application of grape pomace biochar for the control of *Meloidogyne javanica*

Angela Martínez-Gómez[1], Maria Fe Andrés[2], Angel Barón-Sola[3], Fernando E Díaz-Manzano[1], Ibraheem Yousef[4], Ismael F Mena[6], Elena Díaz[5], Oscar Gómez-Tórres[1], Azucena González Coloma[2], Luis E Hernández[3], Carolina Escobar[1]
1 Faculty of Environmental Sciences and Biochemistry, University of Castilla-La Mancha, Toledo, Spain
2 Crop Protection Department, Institute of Agricultural Sciences CSIC, Madrid, Spain
3 Biology Department, Autonomous University of Madrid, Madrid, Spain.
4 CELLS ALBA, Carrer del Llum 2-26, Cerdanyola del Vallès, Barcelona, Spain.
5 Chemical Engineering Department, Autonomous University of Madrid, Madrid, Spain
6 Chemical Engineering Department, University of Castilla-La Mancha, Ciudad Real, Spain

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: biochar, root-knot-nematodes, tomato

ABSTRACT:

Root-knot nematodes (RKNs) are obligate endoparasites that feed on their host plants to complete their life cycle and represent a major threat to agriculture and the economy worldwide. Due to increasing restrictions on the use of pesticides, it is essential to develop alternative management strategies. Biochar is a carbon-rich material that has already proven effective against different plant pathogens. Here, we obtained and characterised two different biochars (BC350, BC700) derived from the pyrolysis of red grape pomace at two different temperatures (350 °C or 700 °C) and analysed their potential for RKN control. The thermal treatment increased the concentration of C and plant macro-/micronutrients (Ca, K, Mg, P, Fe) in both biochars. These elements were largely present in a water-soluble form and its concentration increased at higher pyrolysis temperature. Both biochars presented a highly porous structure filled with crystals of different morphology and composed of elements such as Ca, K, Mg, P, Al or Si, as revealed by scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. High concentrations of biochar-derived aqueous extracts were phytotoxic to tomato seedlings (toxicity that disappeared upon dilution), but no toxic effect was observed on the nematode's infective stage larvae (J2) or during the plant-nematode interaction in vitro. Tomato plants grown in pots with a sand-lime substrate mixed with biochar washed for 24 hours with distilled water (BC350W) at an application rate of 0.75% (w/w) under controlled conditions and inoculated with *Meloidogyne javanica* juveniles, showed a reduction in their infective and reproductive traits (a 42% reduction of the number of egg masses/plant and a 29% reduction of the number of eggs/plant) compared to untreated control plants. Further research will help identify the mechanism of action responsible for this effect, as well as its efficacy against effect against abiotic stress and other plant-pathogens.

264. A tool for the biocontrol of the plant parasitic nematode *Meloidogyne* spp. in tomato

Fernando E. Diaz-Manzano[1], Deisy X. Amora[2], Angela Martínez-Gómez[1], Lars Moelbak[2], Carolina Escobar[1]

1 Facultad de Ciencias Ambientales and Bioquímica, Universidad de Castilla-La Mancha, Avenida de Carlos III, 21, 45004, Toledo, Spain
2 CHR Hansen Holding A/S, Boege Alle, 10, 2970, Hoersholm, Denmark.

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: nematodes, tomato, biological control, *Bacillus*

ABSTRACT:

Root-knot nematodes (RKNs, *Meloidogyne* spp.) are obligate plant parasites that constitute a major pest of agriculture worldwide. The genus *Meloidogyne* spp. is one of the most damaging to crops, and particularly in Mediterranean areas, the species *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne* arenaria. They penetrate plant roots, reducing the water and nutrients uptake, causing significant losses on crop yield (Escobar et al., 2015).

An alternative currently being worked on for nematode management is biological control. Rhizobacteria within the *Bacillus* genus show multiple modes of action against plant-parasitic nematodes that can act alone or in combination. Among the bacteria known for their efficacy in RKNs control, Rhizobacteria of the genus *Bacillus*, such as *B. subtilis* has shown great potential (Ahmad et al., 2021). In this context, we have confirmed the effectivity of a combination of two strains (*B. paralicheniformis* FMCH001 and B. subtilis FMCH002) for RKNs control while allowing adequate plant growth in tomato. We also started to explore their mode of action at different infection stages of *Meloidogyne* spp. parasitism. Thus, the combination of both bacterial strains is an efficient agent for the biological control of *Meloidogyne javanica* J2 larvae and during eggs hatching. Moreover, they also interfere during the *Meloidogyne*-tomato interaction at different stages of the nematode cycle decreasing gall formation and nematode development. Our results have shown effective control of *M. javanica* in vitro and in soil trials under controlled conditions (Díaz-Manzano et al., 2023).

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266. Efficacy of different fungi against root-knot nematodes, *Meloidogyne incognita*, on cucumber

Manzoor Hussain[1,2], Miloslav Zouhar[1], Pavel Rysánek[1]
1.Czech University of Life Sciences, Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Plant Protection, Kamýcká 961/129, 165 00 Praha 6-Suchdol. Czech Republic
2.BioAtlantis Ltd., Ireland. Clash Industrial Estate, Co. Kerry, Tralee, V92RWV5. Ireland

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: Nematodes, *Meloidogyne incognita*, nematophagous fungi, *Stropharia rugosoannulata*, cucumber.

ABSTRACT:

Root-knot nematodes (*Meloidogyne* spp.) are some of the most destructive polyphagous pests with a wide host range that includes field crops, vegetables, and ornamental plants. These nematodes contribute to more than 10% of crop production losses worldwide. The problem is increased due to lack of our farmer's knowledge, repetition of the same crops in the fields, and non-awareness of pesticide applications. The efficacy of two fungi, *Stropharia rugosoannulata* and *Clonostachys rosea* was compared. The fungi *Stropharia rugosoannulata* proved to be efficient in reducing the infestation level of *Meloidogyne incognita* up to 90% and providing better growth of tomato as compared to *C. rosea* (75%). *S. rugosoannulata* was not only able to reduce the nematode infestation but also improved soil health by increasing beneficial soil microbiota and microbial activity.

267. Unveiling the molecular basis of parasitism using spatial transcriptomics

Yuhao Wang[1], Anna Pijnacker[1], Stefan van de Ruitenbeek[1], Jaap-Jan Willig[1], Rik Korswagen[2], Jonas Mars[2], Mark Sterken[1], Aska Goverse[1], Geert Smant[1], Jose Lozano Torres[1]

1 Laboratory of Nematology, Wageningen University and Research, Wageningen, The Netherlands
2 Royal Netherlands Academy of Arts and Sciences and University, Hubrecht Institute, Utrecht, The Netherlands

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: plant-parasitic nematodes, spatial transcriptomics, RNA tomography

ABSTRACT:

Plant-parasitic nematodes inflict substantial damage to crops, causing billions of dollars in losses and threatening global food security. Root-knot and cyst nematodes, two of the most damaging sedentary nematodes, feed on plant roots by establishing permanent feeding sites. Despite extensive research, the molecular mechanisms underlying the initiation of feeding sites remain elusive. We hypothesize that this gap of knowledge stems from the lack of spatial transcriptomic data in and around the initial feeding cells during the early stages of parasitism. To address this challenge, we employed RNA tomography, a state-of-the-art spatial transcriptomic technique, to investigate the onset of feeding site formation in *Arabidopsis thaliana* infected with *Heterodera schachtii* and *Meloidogyne incognita*. We developed custom bioinformatic pipelines to analyze and generate the first sub-cellular resolution transcriptomic maps of feeding sites induced by these nematode species. Candidate genes contributing to unveiling the parasitism were found in both nematodes and *A. thaliana* by comparing the transcriptomic maps. Besides, the bioinformatics pipeline is suitable for deciphering the spatiotemporal mechanism of nematode infection with time-series spatial data. Our discovery provides valuable insights into the initiation of parasitism and paves the way for developing novel strategies to breed nematode-resistant crop plants

268. The pathosystem *Pratylenchus penetrans* - *Solanum tuberosum*: exploring potential sources of potato resistance for root-lesion nematode management

Joana Figueiredo[1], Paulo Vieira[2], Isabel Abrantes[1], Ivania Esteves[1]

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

2 United States Department of Agriculture (USDA), Agricultural Research Services (ARS), Mycology & Nematology Genetic Diversity & Biology Lab, Beltsville, MD 20705, USA

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: differentially expressed genes, resistance, susceptibility, transcriptomes

ABSTRACT:

The root-lesion nematode *Pratylenchus penetrans* parasitizes a wide range of crops, including potato, *Solanum tuberosum*. Management strategies based on crop rotation are limited and sustainable strategies to control this nematode are needed. The main goal of this work was to identify potential plant resistance responses of potato cultivars during *P. penetrans* infection. Susceptibility of ten potato cultivars to *P. penetrans* was evaluated under controlled conditions through assessment of nematode penetration, egression and reproduction, with plants inoculated with an initial population density of four *P. penetrans*/g soil. Nematodes/g of root gradually increased with time of infection, followed by tenue or moderate cell browning to advanced root necrotic areas. These potato cultivars showed a diverse range of susceptibility, interestingly, cv. Laura showed the best performance among all the other cultivars, revealing a significant low number of eggs and nematode motile stages established within the roots of this cultivar. Transcriptome analyses of non- and nematode-infected potato roots of cvs Agria and Laura (showing high and low nematode susceptibility, respectively) were assessed at 3 days after nematode inoculation. A total of 12 mRNA-seq libraries were generated, resulting in 86 to 138 million raw reads/replicate. An average of 86.6% of reads were mapped against the potato reference genome. Differentially expressed genes (DEGs) between inoculated and non-inoculated control plants revealed a more complex gene expression profile of cv. Laura. A total of 6,383 DEGs were identified in cv. Laura, contrasting with 2,471 DEGs in cv. Agria. The molecular mechanisms involved in cv. Laura showed significantly enrichment of core molecular pathways often involved in host resistance, such as plant pathogen interaction, phenylpropanoid biosynthesis, MAPK signalling, and plant hormone signal transduction, accompanied by a strong hypersensitive response of the nematode-infected root cells at early time points of infection. These results uncover novel information on potato genes involved in resistance/susceptibility to *P. penetrans*. Furthermore, it provides important insights into the molecular routes involved in various biological processes during potato-root lesion nematode interaction, which may be relevant for selection and breeding programs for the establishment of improved cultivars against this nematode.

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269. The role of peroxidase in plant-nematode interaction

Tevfik Ozalp[1]
1 Bornova Plant Protection Research Instute, Izmir, Türkiye
2 Akdeniz University, Antalya, Türkiye

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: ROS, peroxidase, Mi-1.2, RKN

ABSTRACT:

Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasites that feed on plants. As a result of root-knot nematode feeding on the root tissue, the cells they feed on are converted into enlarged cells called ‘giant cells’. A rapid accumulation of reactive oxygen species (ROS) occurs after infection in compatible and incompatible interactions between nematodes and plants. However, in susceptible plants, the ROS level decreases and infection continues. In plants resistant to root-knot nematodes, reactive oxygen species accumulate in the feeding areas and oxidative burst results in a hypersensitive reaction (HR). As a result of HR, nematodes die without feeding on resistant plants.

The purpose of this study is to investigate the level of peroxidase expression in tomato susceptible and resistant tomato varieties carrying the Mi-1 gene, which is the most commonly used resistance gene. For this purpose, 1000 second-stage juveniles of Mi-1-avirulent *Meloidogyne incognita* were inoculated into susceptible and resistant tomato varieties, and then peroxidase gene expression was determined by qRT-PCR within five days after nematode inoculation. The experiment was conducted with three biological replicates for each group. EF1α was used as a reference gene. Quantification of the relative changes in gene expression was performed using the 2-ΔΔCT method. The statistical difference between the 2-ΔΔCT values was investigated using an analysis of variance (ANOVA) and Tukey s multiple comparison test ($P<0,05$)

In the resistant plant, peroxidase expression began to decrease after inoculation and reached its lowest level, with peroxidase expression down-regulated by almost 2-fold after 4 days of inoculation. Peroxidase expression in susceptible tomato plants, however, reached a maximum on the 2nd day and began to decrease. Peroxidase expression was down-regulated on the 4th and 5th days after inoculation in susceptible tomatoes. In conclusion, the transcription of the peroxidase gene was repressed by nematode infection in the resistant plant, but the expression of peroxidase was upregulated within three days of inoculation in the susceptible plants and then began to decrease. The study could contribute to showing the role of peroxidase in suppressing the occurrence of ISR and HR caused by the Mi-1 gene.

270. Genetic diversity of *Meloidogyne hapla* populations associated to kiwifruit orchards

Rita Gonçalves[2], Isabel Conceição[1], Sofia Costa[2]
1 Centre for Functional Ecology - Science for People and the Planet, University of Coimbra, Coimbra, Portugal
2 CBMA - Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: COI, D2-D3, ITS, intraspecific diversity, *Meloidogyne hapla*, kiwi fruit

ABSTRACT:

Root-knot nematodes (RKN), *Meloidogyne* spp., parasitise a broad range of crops worldwide, with a significant economic impact on agroecosystems. Several RKN species have been reportedly associated with kiwifruit (*Actinidia* spp.), a crop native to China but cultivated in temperate regions across the world. Among them, *M. hapla* stands out as one of the most prevalent soilborne pathogens in the major production regions. In Portugal, kiwifruit has been increasingly cultivated, with a marked expansion of orchards since 2009. Although several RKN species occur in the country, *M. hapla* is widely associated with kiwifruit orchards, being detected in 34 out of 40 orchards sampled (own results). To understand the high incidence of this species, a detailed study was conducted on the intraspecific diversity of 26 *M. hapla* populations through sequencing and analysis of the Internal Transcribed Spacer (ITS), 28S D2-D3 expansion segments within the rDNA and cytochrome oxidase subunit I (COI) genes. To complete these analyses, we used sequences of *M. hapla* obtained from the specified genes available in the NCBI databases.

Among three regions studied, the D2-D3 showed higher genetic diversity compared to the ITS and COI regions, both among kiwifruit isolates and isolates from other host plants. Our study indicates low intraspecific diversity of COI within all *M. hapla* populations, suggesting that this gene may not be a reliable marker for assessing the intraspecific diversity of *Meloidogyne* spp. Taken together, results suggest specialization but also the preservation of some genetic diversity in *M. hapla* within the kiwifruit isolates.

271. Expression of *Arabidopsis* catalase genes during nematode infection

Fatima Zahrae Mchichou[1], Siham Tounssi[1], Ilahm Zahir[2], Noureddine Hamamouch[1]
1 Faculty of Sciences Dhar El Mahraz, University Sidi Mohamed Ben Abdellah, Fes, Morocco
2 Polydisciplinary Faculty, University Sultan Moulay Slimane, Beni Mellal, Morocco

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: Catalase genes, parasitism, cyst nematode, root-knot nematode, *Heterodera* schachtii, *Meloidogyne incognita*

ABSTRACT:

The expression pattern of *Arabidopsis* catalase genes Cat-1 to Cat-3 was examined in the roots of *Arabidopsis thaliana* plants expressing Catp/GUS genes, in response to nematode infection. Plants were infected with beet-cyst (*Heterodera schachtii*) and root-knot (*Meloidogyne incognita*) nematodes, and GUS expression was visualized 15-20 days post nematode infection. GUS histochemical staining showed that expression of Cat1 and Cat3 genes was induced in nematode-formed syncytia, indicating the role of Cat1 and Cat3 in syncytia formation and maintenance. Expression of Cat2 was not conclusive. Similar results were obtained in nematode-formed galls. *Arabidopsis* T-DNA lines impaired in Catalase expression were challenged with H. schachtii and *M. incognita*, and the number of cyst females and nematode-formed galls were counted three weeks post nematode infection. Results show that both the Atcat- and Atcat3-deficient lines were more susceptible to infection by the nematode. Taken together, this study shows that sugar-beet cyst and root-knot nematodes modulate catalase 1 and 3 gens in order to successfully infect host roots.

272. *Heterodera avenae* RXLR effector Ha17370 targets CBSX3 to suppress plant immunity and promote parasitism

Shanshan Yang[1], Qian Liu[1]
1 China Agricultural University, China

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: *Heterodera* avenae, Effector

ABSTRACT:

Heterodera avenae mainly infects cereal crops and causes severe economic losses. Nematodes can secrete effectors to inhibit plant immune response and promote parasitism. However, the effectors secreted by phasmids, or RxLR-like effectors remains unknown. Here, we characterized a RxLR-like effector of *H. avenae*, Ha17370, and identified its target and role in nematode parasitism. *In situ* hybridization showed that Ha17370 was specifically located in phasmids, RT-qPCR showed that this gene had the highest expression level at the J3s of *H. avenae*. The *Magnaporthe oryzae* and barley system was used to validate the secretion activity of the Ha17370. Independent protein absorption experiments in wheat root tips proved that the Ha17370 could enter into wheat root tip cells independently of *H. avenae*. *In vitro* interference of Ha17370 reduced the taxis of *H. avenae* to wheat root. Ha17370 could enhance the growth ability of *P. syringae* expressing in *N. benthamiana*, and it could cause *Arabidopsis thaliana* more susceptible to *Heterodera schachtii*. BSMV-mediated silencing of the Ha17370 in *H. avenae* significantly decreased the invasion capabilities and number of cysts formed in infected wheat. Ha17370 could inhibit plant defense responses such as reactive oxygen species burst, callose deposition, and expression of plant resistance genes. Immunoprecipitation, mass spectrometry and Co-IP assay proved that Ha17370 interacted with AtCBSX3, TaCBSX3 and HvCBSX3. This indicated that *H. avenae* could secret Ha17370 effector to regulate the level of H2O2 to achieve parasitism. All the results showed that Ha17370 effector containing RxLR motif could been secreted by phasmids, transport autonomously. Besides, it could inhibit the plant defense response, promoted parasitism, this was the first report on *H. avenae*.

273. Root architecture plasticity in response to endoparasitic cyst nematodes is mediated by damage signaling.

Nina Guarneri[1], Jaap-Jan Willig[1], Mark G. Sterken[1], Wenkun Zhou[3], M. Shamim Hasan[4], Letia Sharon[4], Florian M. W. Grundler[4], Viola Willemsen[2], Aska Goverse[1], Geert Smant[1], Jose L. Lozano-Torres[1]

1 1 Laboratory of Nematology, Wageningen University & Research, 6708 PB Wageningen the Netherlands
2 Laboratory of Molecular Biology, Cluster of Plant Developmental Biology, Wageningen University & Research, 6708 PB Wageningen, the Netherlands
3 State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China
4 Institute of Crop Science and Resource Conservation (INRES), Molecular Phytomedicine, University of Bonn, 53115 Bonn, Germany

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: *Arabidopsis thaliana*, auxin, damage, ERF109, *Heterodera schachtii*, jasmonates, root architecture, root plasticity.

ABSTRACT:

Plant root architecture plasticity in response to biotic stresses has not been thoroughly investigated. Infection by the endoparasitic cyst nematodes induces root architectural changes that involve the formation of secondary roots at infection sites. However, the molecular mechanisms regulating secondary root formation in response to cyst nematode infection remain largely unknown. We first assessed whether secondary roots form in a nematode-density-dependent manner by challenging wild-type *Arabidopsis* plants with increasing numbers of cyst nematodes (*Heterodera schachtii*). Next, using jasmonate-related reporter lines and knock-out mutants, we tested whether tissue damage by nematodes triggers secondary root formation. Finally, we verified whether damage-induced secondary root formation depends on local auxin biosynthesis at nematode infection sites. Intracellular host invasion by H. schachtii triggers a transient local increase in jasmonates, which activates the expression of ERF109 in a COI1-dependent manner. Knock-out mutations in COI1 and ERF109 disrupt the nematode-density-dependent increase of secondary roots observed in wild-type plants. Furthermore, ERF109 regulates secondary root formation upon *H. schachtii* infection via local auxin biosynthesis. Altogether, we conclude that host invasion by *H. schachtii* triggers secondary root formation via the damage-induced jasmonate-dependent ERF109 pathway. This points to a novel mechanism underlying plant root plasticity in response to biotic stress.

274. Characterization of ShK domain-containing proteins from the pinewood nematode *Bursaphelenchus xylophilus* suggests involvement in oxidative stress

Madalena Mendonca[1], Cláudia S. L. Vicente[1], Margarida Espada[1]
1 MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, University of Évora, Portugal

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: molecular plant nematode interaction, parasitism, pinewood nematode, oxidative stress

ABSTRACT:

ShK domain-containing proteins are a 35/37-residue peptide toxin capable of blocking the potassium channels and originally found in sea anemones. These proteins are distributed across plant and animal kingdoms and associated with larger multifunctional proteins with several functions. Numerous proteins have been identified in pinewood nematode (PWN), *Bursaphelenchus xylophilus*, during infection of the host, including detoxification enzymes, cell-wall degrading enzymes and ShK domain-containing proteins, and in this last their function is unknown. From the previous PWN transcriptomic dataset, we have identified 96 transcripts encoding ShK domain-containing proteins. This study intended to characterize and understand the role of ShK domain-containing proteins in PWN biology. Therefore, we performed in silico analysis, in situ hybridization to validate the spatial expression of the transcripts in the nematode tissues, and an oxidative stress assay to understand the nematode response to the presence of hydrogen peroxide (H2O2). Nine secreted candidate genes were selected to further studies. In silico analysis of these genes resulted in a lack of similarity to other sequences, indicating that they have only been found in this species so far. Most of these genes were putatively found to be highly expressed during infection of the host, suggesting that they might be involved in parasitism. Moreover, they were localized in different tissues, such as the pharyngeal gland cells, digestive and nervous systems which suggest different functions. The expression levels of these nine candidate genes were upregulated in response to hydrogen peroxide (ROS products), which indicates for the first time that they might be involved in oxidative stress responses. Due to its multidomain architecture and large number, ShK domain-containing proteins may have a diversity of functions in nematodes. Comprehending the function of ShK domain-containing proteins may improve our knowledge of how nematodes modulate hosts during infection and develop new target molecules for nematode control.

Mendonça, M., Vicente, C.S.L., Espada, M. Functional characterization of ShK domain-containing protein in the plant-parasitic nematode *Bursaphelenchus xylophilus*. Plants 2024, 13, 404. https://doi.org/10.3390/plants13030404

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275. Long non-coding RNAs from *Bursaphelenchus xylophilus* involved in the interaction with the pine host.

Madalena Mendonça[1], Jorge M. S. Faria[2], Maria L. Inácio[2], Margarida Espada[1]
1 MED, Mediterranean Institute for Agriculture, Environment and Development & CHANGE, Global Change and Sustainability Institute, University of Evora, Portugal
2 National Institute for Agriculture and Veterinary Research, Quinta do Marquês, Oeiras, Portugal.

Area: Plant-Nematode and Soil Microbiome Interactions
Type: Poster
Keywords: molecular plant–nematode interactions, parasitism, long non coding RNAs (lncRNAs)

ABSTRACT:

Pinewood nematode (PWN), *B. xylophilus*, is a migratory endoparasite that causes major forestry losses worldwide. Due to their function as key regulators of different biological processes, long non-coding RNAs (lncRNA) can be involved in nematode virulence regulation, adaptation and pathogenesis. In this study, we aimed to characterized PWN lncRNAs expression and understand their potential function in parasitism, based on RNA-seq of the PWN-lncRNA libraries from nematodes in interaction with the host. We have found 925 novel lncRNAs, from which, 300 are being differentially expressed (DE) during the interaction with the host. The most enriched GO terms of significant DEGs targeted by lncRNAs in co-expression are oxireductase and peptidase activity, iron and hemme-binding (padj).

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276. Intriguing taxonomic case of *Pratylenchus vandenbergae*: recognition as a separate species and its significant impact on various crops

Cecilia Wanjau[1], Rinus Knoetze[2], , Chantelle Girgan[3], Njira Pili[4], Gerhard Engelbrecht[5], Marjolein Couvreur[1], Wim Bert[1]
1 Department of Biology, Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium
2 ARC Infruitec-Nietvoorbij, The Fruit, Vine and Wine Institute of the Agricultural Research Council, Stellenbosch, South Africa
3 Nematology Unit, Biosystematics Division, Agricultural Research Council, Plant Health and Protection, Queenswood, South Africa
4 Department of Biological Sciences, Moi University, Kenya
5 Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: finger millet, life cycle, molecular, morphology, morphometrics, reproduction

ABSTRACT:

A highly fecund root-lesion nematode parasitizing a variety of crops was discovered and found to be widespread in Kenya and South Africa. These populations were molecularly identical to *Pratylenchus teres teres* and *P. teres vandenbergae* based on D2-D3 of 28S and Hsp90 sequences. However, based on morphological differences with the original description of P. teres and its different geographical distribution, *Pratylenchus* vandenbergae stat. nov., previously known as subspecies *P. teres vandenbergae*, is recognised as a separate species. This species is characterized by a slightly offset labial region with three annuli, en face morphology belonging to Group II, lateral field with four incisures with two outer bands areolated as observed under light microscope and the inner band also partially areolated at the vulva region as observed in scanning electron microscopy view, a robust stylet (14-17 µm) with rounded knobs and subcylindrical tail with annulated tail tip. This important and common species remained largely under the radar, probably due to identifications based only on sequence similarity, including mislabelled *Pratylenchus bolivianus* sequences in GenBank. Pathogenicity of *P. vandenbergae* stat. nov. in finger millet roots was confirmed through acid fuchsin staining, and reproduction and pathogenicity tests in maize, soybean, sunflower and tomato plants demonstrated its capacity to affect crop growth. Life cycle comparison with *P. penetrans* in vitro showed similar life cycle length but significantly higher fecundity. Finally, significant differences in pathogenicity and reproduction of *P. vandenbergae* stat. nov. were found both at genotype level (finger millet genotypes OKHALE-1 & KNE1034) and crop level (maize, soybean, sunflower and tomato plants).

277. Strain improvement of *Trichoderma longibrachiatum* 40418 for raised peptaibol production by CRISPR/Cas9

Huixia Li[1], Ning Luo[1], Bingyan Xie[1], Zhenchuan Mao[1]
1 College of Plant Protection, Gansu Agricultural University, Lanzhou, China
2 Institute of Vegetables and Flower, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: *Trichoderma longibrachiatum*, biocontrol, CRISPR/Cas9, *Meloidogyne* spp.

ABSTRACT:

Trichoderma spp. are crucial biocontrol agents because of their antimicrobial activity against a wide variety of phytopathogens. Among these, peptaibols play a pivotal role in biocontrol. However, the biosynthesis pathway of peptaibols remains largely unknown, as they have a very low content in the secondary metabolites of *Trichoderma* spp. In this study, knockdown and replacement technologies were conducted to investigate whether strong promoters (gpda) could substitute for the natural promoters of non-ribosomal peptide synthetases (NRPS) in *T. longibrachiatum* 40418. Based on genomic information, CRISPR/Cas9 plasmid and recombinant donor DNA plasmid were constructed for enhancement of the tlbA gene expression. Successfully site-directed insertion was confirmed through molecular identification and sequencing. Results from qPCR analysis indicated a significant 15-fold upregulation of the expression of the tlbA gene. The content of peptaibols was markedly increased compared to the wild-type of *T. longibrachiatum* 40418. This study represents the first successful establishment of an overexpression system based on the CRISPR/Cas9 system in *T. longibrachiatum*, which has important scientific significance and practical value for research on the biosynthetic pathway of peptaibols and agricultural production.

278. Rapid detection of the strawberry foliar nematode *Aphelenchoides fragariae* using recombinase polymerase amplification assay with lateral flow dipsticks

Sergei Subbotin[1]
1 Plant Pest Diagnostic Centre, California Department of Food and Agriculture, USA

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: California, molecular diagnostics, recombinase polymerase amplification, strawberry foliar nematode

ABSTRACT:

The strawberry foliar nematode *Aphelenchoides fragariae* is an important pest of strawberry plants, it causes ‘strawberry crimp’ disease. The strawberry foliar nematode is the subject of a regulatory program in California. Rapid and reliable diagnostic methods for *Aphelenchoides fragariae* are critical for facilitating the selection of effective control measures. A diagnostic recombinase polymerase amplification (RPA) assay for *A. fragariae* combined with lateral flow dipsticks (LF) has been developed. Species-specific primers and a probe were designed based on the polymorphism of the ITS ribosomal RNA gene sequences. The assay enables the specific detection of *A. fragariae* from crude nematode extracts without a DNA extraction step, and from DNA extracts of plant tissues infected with this nematode species. The LF-RPA assay showed reliable detection within 18-25 min with a sensitivity of 0.03 nematode per reaction tube for crude nematode extracts or 0.3 nematode per reaction tube using plant DNA extracts from 0.1 g of fresh leaves. The LF-RPA assay was developed and validated with a wide range of nematode and plant samples. *Aphelenchoides fragariae* was identified from rise seed samples in California. The LF-RPA assay has great potential for nematode diagnostics in the laboratory with minimal available equipment.

279. Reference-free nematode mito-metagenomics using deep-learning method

Xue Qing[1], Micha Karlicki[2], Fan Guo[1], Anna Karnkowska[2], Hongmei Li[1]
1 Nanjing Agricultural University, China
2 University of Warsaw, Poland

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords:

ABSTRACT:

The mito-metagenomics (MMG) approach directly sequences the pooled samples, yields numerous mitochondrial reads which can be assembled into full or partial mitogenomes. This method avoids a serial of obstacles in PCR-based metabarcoding and hold great promise in biodiversity and phylogeny study. Unfortunately, reference database was needed to extract mito-reads/contigs, and to provide taxonomic or phylogenetic context, thus limited its application. In present study, we proposed a reference-free pipeline for MMG assembly. This approach assembled raw reads, then used the prebuilt deep-learning model to identify and extract contigs belong to mitochondrial. Finally, the protein coding genes were predicted and annotated. This pipeline was incorporated in snakemake workflow that allows output ready to be used for phylogeny reconstruction in a single run. The performance tests suggested the new approach outperforms reference-based method in soil nematode community profiling. The unrecovered taxa can be attributed to various reasons e.g. low DNA quantity and unsuccessful DNA extraction. We demonstrated assembly quality improves with the increasing of sequencing depth, and an average 1-2 Gb per species is recommended to reach acceptable MMG assembly. Our pipeline offers an opportunity to generate high resolution phylogeny and diversity for those poorly known taxa, e.g. the neglected microscopic eukaryotes.

280. A new interesting dorylaimid, belondirid representative (Dorylaimida, Belondiridae) associated with olive groves of southern Iberian Peninsula

Reyes Peña-Santiago[1], Miriam García-Ruiz[1], Alba Nazaret Ruiz-Cuenca[1], Joaquín Abolafia[1]

1 Departamento de Biología Animal, Biología Vegetal and Ecología. Universidad de Jaén. Campus Las Lagunillas s/n, 23071, Jaén, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords:

ABSTRACT:

Dorylaims, the members of the order Dorylaimida, probably are the most diverse nematode group. Being present in any continental (soil and freshwater) habitat and exploiting a wide spectrum of feeding resources, they are regarded as good bioindicators of environmental health (quality). Dorylaimid fauna of olive groves is poorly explored, but it shows interesting diversity. Actually, an undiscovered form (probably a new genus and species) of belondirid nature was recently found in the course of a survey (*Soil O-live* EU Project) recently conducted in olive groves of Andalusia (southern Iberian Peninsula). It is characterized by its 2.44-2.87 mm long body, cuticle thick and two-layered, lip region narrower than the adjoining body, rectangular in lateral view, and with distinctly sclerotized margins, cheilostom a relatively long truncated cone with thickened walls at its anterior (perioral) part, odontostyle small, robust, slightly fusiform and with aperture occupying up to one-third of its length, guiding ring simple and refractive, odontophore linear, both pharyngeal regions separated by a short, isthmus-like narrowing, basal pharyngeal expansion occupying *ca* two-thirds of the total neck length and surrounded by a conspicuous muscular sheath, female genital system mono-opistho-ovarian, didelphic, without *pars refringens vaginae*, well-developed *pars distalis vaginae* and transverse vulva, caudal region short and rounded, and male unknown. Both morphological and molecular (28S-rDNA) analyses show that the new form is close to members of Belondiridae, especially *Metaxonchium*, but it significantly differs from this in the shape of lip region (vs typically cap-like) and totally absent (vs very distinct) *pars refringens vaginae*.

281. First record of the clover cyst nematode *Heterodera* daverti in Serbia

Violeta Oro[1], Rade Stanisavljevic[1], Marijenka Tabakovic[2]
1 Institute for Plant Protection and Environment
2 Maize Research Institute Zemun Polje

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: clovers, cyst nematode, *Heterodera daverti*

ABSTRACT:

Clovers and other forage legumes are present in Serbia since ancient times. According to archaeobotanical data, clover seeds (*Trifolium* sp.) were found in the Neolithic locality of Starevo-Grad, dated from the Roman (Sarmatian) period. Clovers are valuable for their nutritional value, nitrogen fixation and honey production. *Heterodera daverti* Wouts and Sturhan is a cyst nematode belonging to the Schachtii species group. This species has been reported to cause severe losses to *T. subterraneum* L. Cysts are lemon-shaped, ambifenestrate, bullate, with a prominent vulval cone. The second-stage juvenile (J2) has a well-developed anteriorly concave stylet, oval median bulb and four incisures. The overall morphology, as well as host list are similar to other cyst nematode *H. trifolii* Goffart and perhaps some reports of *H. trifolii* may actually be *H. daverti*. Cysts of *H. daverti* were found in soil samples originating from Bzovik in southern Serbia, and examined at the Nematology laboratory of the Institute for Plant Protection and Environment. Nematodes were extracted using a Spears elutriator. For morphological studies, specimens were placed in lactoglycerol fixative and slides were prepared in glycerol. Photomicrographs were captured using an Olympus BH-2 microscope equipped with Nomarski contrast and Motic digital camera and software. Morphometrics of cysts and J2 were in agreement with the original description by Wouts and Sturhan. Genomic DNA was extracted from cysts with The Dneasy Blood and Tissue Kit and amplified with TW81 and AB28 primers. The obtained sequence of the ITS regions of this population (GenBank accession number PP101636) confirmed the identification of this species with 100% homology to *H. daverti* available in the NCBI database. To our knowledge, this is the first record of *H. daverti* in Serbia.

282. Nematode diagnostics - from microscopy to high throughput long reads sequencing

Maria Belousova[1], Jop Wilten[1], Marieke Beltman[1]
1 ClearDetections, Wageningen, The Netherlands

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: nematodes, identification, PCR, ONS, Nanapore

ABSTRACT:

Diagnosing soil nematodes is crucial as they impact soil health and plant growth. Nematodes contribute to nutrient cycling, organic matter decomposition, and the control of other soil organisms. However, some nematode species can cause damage to plant roots, leading to reduced crop yields and economic losses.

Nematodes diagnostic methods include microscopy and molecular-based diagnostics (PCR and sequencing). Microscopy involves the visual identification of nematodes. However, nematodes have few distinguishable morphological characters on certain life stages, making identification difficult, time-consuming, and often unsuccessful at the genus or species level. On the other hand, PCR detection is quicker and does not require specific morphological knowledge. The most perspective and usable regions for nematode identification are the 18S small subunit ribosomal RNA gene (18S SSU), the 28S large subunit ribosomal RNA gene (28S LSU), the cytochrome oxidase gene I (COI) and internal transcribed spacer regions (ITS) of the ribosomal RNA locus. ClearDetections offers nearly 60 genus- and species-specific primer sets designed using our SSU, LSU and COI databases. PCR diagnostic is the most convenient and cheapest option for answering the target presence/absence question. However, metabarcoding allows for identifying of nematode species not only within the list of available target-specific primers but all species in the sample. Understanding nematode populations and their trophic group ratios is essential for evaluating soil health and making recommendations.

Over the past 40 years, sequencing technologies have undergone significant development and improvement. Initially, Sanger sequencing allowed read lengths up to 1000 bp from a single nematode, which was good enough for detection using SSU, LSU or COI database. However, obtaining many reads from a sample in a single run is highly desirable. Illumina technology offers high read throughput but has limitations on read length. While it provides high accuracy, it is also costly.

Oxford Nanopore (ONS) technology has proven to be an effective tool for nematode diagnostics and allows for efficient long-read sequencing (Knot et al., 2020, van Himbeeck et al., 2024). After evaluating factors such as cost, read quality and length, time to results, and data volume, we have concluded that ONS is the optimal method for nematode community analysis.

Knot, I. E et al., (2020). DNA Barcoding of Nematodes Using the MinION. Frontiers in Ecology and Evolution, 8, 477311. doi.org/10.3389/FEVO.2020.00100/BIBTEX

van Himbeeck, R., et al., (2024). A full-length SSU rRNA-based workflow for high-resolution monitoring of nematode communities reveals direct and indirect responses to plant-based manipulations. Soil Biology and Biochemistry, 189, 109263. doi.org/10.1016/j.soilbio.2023.109263

283. Species diversity of spiral nematode (*Helicotylenchus* spp.) in Portugal.

Tiago Pinho[1], Carlos Gutiérrez Gutiérrez[1]

1 ,MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE, Instituto de Investigação e Formação Avança, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

Area: Taxonomy, Systematics and Phylogeny
Type: Poster

Keywords: DNA sequencing, integrative taxonomy, morphology, plant-parasitic nematodes, rRNA, spiral nematodes

ABSTRACT:

Spiral nematodes (*Helicotylenchus* spp.) currently comprise more than 200 species of polyphagous plant migratory ectoparasites with at least six species that are plant pathogenic emphasizing the need for correct identification to establish an appropriate management strategy. They are able to cause noticeable plant damage when nematode populations are high in number. Identification of *Helicotylenchus* spp. based only on morpho-anatomical data using diagnostic keys is not always reliable, due to high phenotypic plasticity and presence of cryptic species within this genus. Nowadays, sequencing ribosomal and mitochondrial RNA-based markers represent a powerful approach for species-level taxonomic identification within the genus *Helicotylenchus* Steiner 1945 and understanding of the evolutionary relationships within this genus. Since 2023, nematological surveys are conducted from spring to autumn seasons in cereals, grapevine, olive and vegetable crops in Portugal. Thus, the main aim of this research is to evaluate the diversity and distribution of *Helicotylenchus* species in Portugal using an integrative approach. This integrative analysis with both morphological and RNA data has provided an accurate diagnostic of the Portuguese populations of *Helicotylenchus* species. Among them, *Helicotylenchus vulgaris* Yuen, 1964 is the most commonly found spiral nematodes in Portugal.

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284. Morphological study of the lip region in cephalobid genera (Rhabditida, Cephalobidae) from xeric areas in the southern Iberian Peninsula based on scanning electron microscopy.

Joaquín Abolafia[1], María-Cristina Robles[1], Alba Nazaret Ruiz-Cuenca[1]
1 Universidad de Jaén, Jaén, Spain

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: Cephalobidae, coastal dunes, lip region, morphology, taxonomy.

ABSTRACT:

Cephalobid nematodes (Rhabditida, Cephalobidae) are characterized by the presence of six lips and oral opening surrounded by three labial probolae. Both the lips and the probolae present different morphological patterns, from simpler to more complex shapes, which have taxonomic value. In the present study, the morphology of the lip region was studied with scanning electron microscopy in nematodes from xeric areas (coastal dunes and deserts) in the south of the Iberian Peninsula. Several morphological patterns of the labial region have been observed in the cephalobids found in the area examined. Thus, there are genera with conoid lips and reduced probolae, which are fused to the adjacent pair of lips, rounded (*Cephalobus*, with subcylindrical female tail with rounded tip, and *Pseudacrobeles*, with conoid female tip with acute tip) or bifurcated (*Eucephalobus*), genera with flattened, smooth or slightly dentate lips, and probolae not fused to the lips, rounded (*Acrobeloides*, with short post-vulval sac, and *Heterocephalobelus*, with very elongate post-vulval sac) or forked (*Chiloplacus*), or a genus with denticulate lips and rounded or slightly forked probolae (*Zeldia*). Other genera show smooth lips with well-developed forked probolae (*Stegelleta*) or with denticulate or lobed lips and reduced probolae (*Cervidellus*, with two axillar processes at primary axil, and *Stegelletina*, with one axillar process at primary axil). The genera with more complex morphology present well-developed and bifurcated probolae with dentate branches, with rectangular (*Nothacrobeles*) or triangular (*Acrobeles*) lips. This increase in the lip region complexity is supported by molecular studies.

285. A new species of the genus *Acromoldavicus* Nesterov, 1970 (Nematoda, Rhabditida, Elaphonematidae) from the southern Iberian Peninsula.

María-Cristina Robles[1], Joaquín Abolafia[1]
1 Universidad de Jaén

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: Coastal dunes, Elaphonematidae, Iberian Peninsula, morphology, new species, taxonomy.

ABSTRACT:

The genus *Acromoldavicus* (Rhabditida, Elaphonematidae) in an infrequent edafic taxon. Currently, this genus contains two valid species: *A. mojavicus*, which was only described in sandy soil from the Mojave Desert (California, USA), and *A. skrjabini*, found in agricultural soils from several countries worldwide (Moldavia, Greece, Iran, Ukraine, Israel, Bulgaria and Spain). In this study, a new species of the genus *Acromoldavicus* has been found in coastal sand dunes and desert soil from two localities of the southeast Iberian Peninsula, both associated with xerophilic vegetation. The new species is characterized by it 557-700 µm body length, cuticle tessellated, lip region with three pairs of expanded lips bearing an acute process at tip bent to the oral opening and a large labial expansion outwards, primary axils bearing axillar guard processes with triangular morphology, larger and free at ventral axil and smaller and fused to adjacent lateral lip at dorsolateral axils, secondary axils lacking axillar processes, amphids oval located apical at lateral lips, labial probolae pentagonal, stoma short and tubular with prostegostom bearing prominent rhabdia directed towards the stoma lumen, female reproductive system monodelphic-prodelphic, spermatheca well developed, post-vulval uterine sac reduced, rectum very large, female tail short with slighly biacute terminus and males unknown. This new species is very similar to *A. mojavicus* but it is mainly distinguished by having lips with larger lobular expansion, longer pharynx (more than 100 µm vs less), vulva located slightly more anterior (V= 51-63 *vs* 60-63), post-vulval sac lacking lumen along most of its length (vs wide lumen), rectum with shorter cuticular part (*vs* more than 50% of its length), tail tip slightly biacute (*vs* finelly rounded), and males absent (*vs* frequent). The difference between both species is also supported by molecular analyses based on 28S rDNA.

286. Long-read genome assembly and population genomic analysis of *Heterodera humuli* from the pacific northwest of the United States

<u>L.A. Núñez Rodríguez</u>[1], C.L. Wram[2], C. Hesse[3], I.A. Zasada[3]
1 Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA
2 USDA-ARS-MNGDB Lab, Beltsville, MD 20705, USA
3 USDA-ARS-HCDPMRU, Corvallis, OR 97331, USA

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: Cyst nematodes, *Heterodera humuli*, hop, long reads

ABSTRACT:

The United States accounts for almost 40% of the worldwide hop production, with the Pacific Northwest (PNW) accounting for ~98% of this production. Several biotic factors are a threat to hop production, among them plant-parasitic nematodes. *Heterodera humuli*, commonly known as the hop cyst nematode (HCN), is the most frequently encountered plant-parasitic nematode in hop yards globally. However, limited information is available on the management or genetics of *H. humuli*. The objective of this study is to generate genomic resources to enabling scientists to explore the genetics of *H. humuli*. A high-quality reference genome of *H. humuli* was produced using PacBio long-read sequencing. The algorithm Hifiasm v.0.16.1-r375 was used for genome assembly and a Blobplot workflow was used to identify and remove contaminants. The assembled genome size of *H. humuli* is 96 MB contained in 1,574 contigs with a coverage of 145X. A total of 1,370 BUSCO genes were estimated with the BUSCO benchmark. To better understand the presence of bacterial endosymbionts, which may be leveraged to control *H. humuli*, a genome skimming tool was implemented. This approach allowed for the detection of the bacterial endosymbiont *Wolbachia* in *H. humuli*. The assembled genome size of this *Wolbachia* endosymbiont is 1.05 MB contained in a single contig, with a total of 178 BUSCO genes. This is the first report of *Wolbachia* associated with any *Heterodera* species. Further analysis will include 20 *H. humuli* populations from the Pacific Northwest (n = 20) and Midwest (n = 5) of the U.S. as well as populations from the Czech Republic, Australia, England, and South Africa for Illumina short read sequencing (2 x 150 bp). A global SNP-based population genomics analysis will be conducted to determine genetic flow between global HCN populations to determine the number of possible introductions of HCN to the PNW. The genome skimming strategy will also be implemented to further explore the presence of *Wolbachia* in *H. humuli*.

289. First report of the genus *Parasitaphelenchus* Fuchs, 1929 (Aphelenchoididae: Parasitaphelenchinae) from Iran

Fariba Heydari[1], Majid Pedram[1]
1 Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2 Department of Plant Protection, Faculty of Agriculture, Isfahan University of Technology, Iran

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords:

ABSTRACT:

The subfamily Parasitaphelenchinae Rühm, 1956, currently contains four genera, *Bursaphelenchus* Fuchs, 1937, *Parasitaphelenchus*, *Ruehmaphelenchus* J.B. Goodey, 1963 and *Sheraphelenchus* Nickle, 1970. Members of this subfamily have a worm-shaped body and are usually associated with insects either as phoretic or parasites. Representatives of two genera *Bursaphelenchus* and *Sheraphelenchus* have already reported from Iran. During present study, several juveniles of *Parasitaphelenchus* sp. were recovered from inside the dissected body of bark beetle *Orthotomicus erosus* dominating handmade coniferous forests of western city of Tehran. The juveniles were characterized by no visible stylet, excretory pore anterior to the well-developed metacorpus and tail conoid with a fine horn-like mucronate terminus. A 1095 bp long small, and two 677 and 719 bp long large subunit ribosomal DNA (SSU and LSU rDNA D2-D3 expansion segments) sequences were made available for this population. Dissecting of several beetles did not yield of recovering of females or males. This is the first report of the presence of occurring of the genus *Parasitaphelenchus* in Iran.

290. Morphological and molecular characterisation of Avenae group cyst nematodes from Greece

Andrea Skantar[1], Zafar Handoo[1], Maria Kormpi[2], Emmanuel Tzortzakakis[3]
1 USDA ARS NEA Beltsville Area, Beltsville, MD
2 Benaki Phytopathological Institute, Kifisia, Athens, Greece
3 Institute of Olive Tree, Subtropical Crops and Viticulture, Department of Viticulture, Vegetable Crops, Floriculture and Plant Protection, ELGO-DIMITRA, Heraklion, Crete, Greece

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: taxonomy, cyst nematodes, Greece, *Heterodera*, potato

ABSTRACT:

Cyst nematodes of the genus *Heterodera* comprise more than 60 species of economically important plant parasites, with the Avenae group one of the largest, consisting of 12 species. Sampling for cyst nematodes was carried out from multiple locations in Greece from 2015 to 2021. Several soil samples from potato fields were investigated for presence of the potato cyst nematode (*Globodera* spp) at the Nematology Laboratories of the Benaki Phytopathological Institute, Athens, Greece and of the Institute of Olive Tree, Subtropical Crops and Viticulture, Heraklion, Crete, Greece. Additional specimens were recovered from athletic stadium turfgrass and from garlic fields. Cysts were extracted from dried soil samples using a Fenwick can. From these samples, cysts of *Heterodera* species were recovered, preserved in DESS and subjected to morphological and molecular identification. Nematode DNA was extracted from individual juveniles, and sequences from 28S, ITS1&2 rDNA, mitochondrial COI and nuclear Hsp90 markers were analyzed by molecular phylogenetic comparison to other Avenae group species. Species identified by integrative taxonomic approaches include *H. filipjevi* from potato fields and turfgrass, *H. hordecalis* from potato fields and *H. mani* from a garlic field, representing new records for Greece.

291. First report of *Hemicriconemoides kanayaensis* Nakasono & Ichinohe, 1961 from tea plantation of Guilan province, Iran

Negin Mirghasemi[1], Elena Fanelli[2], Alberto Troccoli[2], Salar Jamali[1], Francesca De Luca[2]
1 Plant Protection Department, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran
2 Istituto per la Protezione Sostenibile delle Piante (IPSP), Consiglio Nazionale delle Ricerche (CNR), Bari 70126, Italy

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: migratory nematode, *Camellia sinensis*, identification, ribosomal DNA, mitochondrial COI, *Hemicriconemoides kanayaensis*

ABSTRACT:

In 2020, a soil survey was performed in tea gardens in Guilan province. The aim of this study was to assess the abundance, distribution and identity of plant parasitic nematodes associated with tea. Plant-parasitic nematodes were extracted using standard protocols and a consistent migratory ectoparasitic population was isolated. Morphological identification of nematodes revealed the occurrence of *Hemicriconemoides kanayeansis*. The females have a slightly bent cylindrical body. The labial region has two rings, the first ring from the labial region separated from the body, usually with a rounded edge and sometimes with irregular lateral edges. The female nematode with spermatheca full of sperms and average body length 494 (409-560), stylet length 71 (65.9-78.2), tail length 34 (26.6-40) micrometers, total number of body rings 152 (141-179) and indices V=1.89, RX=35 (35-35), VL/VB= 2.26 (1.95-2.7) and RV=16.4 (15-19) is distinguished from close species such as, *H. strictathecatus* and *H. mangiferae*. In the sample, male specimens and juveniles were found. Molecular characterization, using ribosomal and mitochondrial markers, of the Iranian population the presence of *H. kanayeansis*. Phylogenetic and phylogeographic relationships will be presented.

292. Bibliometric and sequence review of *Helicotylenchus* species

Ebunoluwa Ijeoma Ajobiewe[2], Alessio Vovlas[1], Elena Fanelli[1], Alberto Troccoli[1], Francesca De Luca[1]
1 Institute for Sustainable Plant Protection - CNR, Via Amendola 122/D, Bari, Italy
2 Department of European and Mediterranean Cultures, University of Basilicata, Via Lanera, 10, 75100 Matera, Italy

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: nematodes, bibliometrics, sequencing, *Helicotylenchus*, VOSviewer

ABSTRACT:

The genus *Helicotylenchus* consist in many species complexes as they show conservative morphology complicated by their phenotypic plasticity (intraspecific variability and minor interspecific differences), leading to potential misidentification of the species. To explore the global scientific literature on *Helicotylenchus* species and to study the trend and research hotspots, the current study used bibliometric analysis over the last decade (2010-2023) in the Scopus database, by using VOSviewer software, and sequences, released in GenBank Database.

The Scopus Database was selected as literature repository for its professional standards, international visibility, broad coverage, and controlled-vocabulary thesaurus available for indexing and retrieving documents. The GenBank database collects nucleotide sequences and relevant bibliographic and biological annotations for sequence analysis. This study aims to highlight the specific complexities of working with this genus, molecular techniques used for characterization and explore the intraspecific and interspecific variability that exists among the populations of the main pathogenic species, thereby informing the scientific community on the relevance of the genus.

293. Plant parasitic nematodes associated with maize in North Italy

Elena Fanelli[1], Alberto Troccoli[1], Alessio Vovlas[1], Trifone D Addabbo[1], Lorenza Michelon[2], Daniele Della Torre[2], Francesca De Luca[1]

1 Istituto per la Protezione Sostenibile delle Piante-CNR, SS di Bari, Via Amendola 122/D, 70126 Bari, Italy
2 Servizio Condifesa Lombardia Nord-Est, Via Malta 12, 25124 Brescia, Italy

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: maize, *Heterodera elachista*, PPN, Lombardy

ABSTRACT:

From 2020 to 2023, surveys were carried out in maize fields in Lombardy (Brescia and Pavia provinces). The objective of this study was to assess the abundance, distribution and identity of plant parasitic nematodes associated with local maize crops. A total of 242 soil samples were collected from 4 different maize crop farms. Plant-parasitic nematodes were extracted, counted and identified using standard protocols. Morphological identification of nematodes revealed a consistent presence of nematode belonging to the genus *Heterodera*. Molecular characterization, using ribosomal and mitochondrial markers, confirms the presence in North Italy of the Japanese rice cyst nematode *Heterodera elachista* and several other plant parasitic species. The most important genera of plant-parasitic nematodes found to be associated with maize were *Helicotylenchus*, *Mesocriconema*, and *Pratylenchus*. Noteworthy is that, in the same area was also recorded the presence of *Meloidogine graminicola* in 2016. Experimental tests for *Pratylenchus* sp. are in progress to clarify the possible pathogenicity of this species and to develop effective management strategies. The aim of this work is to contribute to understanding the ecology and diversity of nematodes associated to maize.

Study funded by Lombardy Region, Progetto ‘Nemagest’.

294. Morphological and molecular variability among *Xiphinema rivesi* populations from Bosnia-Herzegovina and Italy

Fanelli E.[1], Vovlas A.[1], Nježić B.[2], Vasilic A.[2], Đekanović R.[2], Troccoli A.[1], De Luca F.[1]
1 Istituto per la Protezione Sostenibile delle Piante-CNR, Via Amendola 122/D, 70126 Bari, Italy
2 University of Banja Luka, Faculty of Agriculture, Bulevar vojvode Petra Bojovica 1A, 78000 Banja Luka, B&H

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: *Xiphinema rivesi*, molecular identification, phylogenetic relationships

ABSTRACT:

In 2020, a survey was carried out in vineyards in North Italy to determine the distribution of *Xiphinema* species. In most of soil samples three *Xiphinema* species were recovered in vineyards and identified, at morphological level as *X. index*, *X. pachtaicum* and *X. rivesi*. Molecular characterization revealed high nucleotide variability at ribosomal and mitochondrial level within Italian populations of *X. rivesi*. Phylogenetic analyses showed that Italian populations formed a well-supported subgroup separated from other European *X. rivesi* populations. In 2020, survey in Bosnia-Herzegovina in apple and populus orchards, two *Xiphinema* species were recovered. Molecular characterization revealed the occurrence of *X. rivesi* and *X. incertum* in both orchards. Morphological and molecular comparison of all European populations of *X. rivesi* is in progress.

295. *Bursaphelenchus arthuri* from *Pinus pinea* in Portugal

Hugo Silva[1], Joana M. S. Cardoso[1], Ricardo da Costa[2], Isabel Abrantes[1], Luís Fonseca[1]
1 University of Coimbra, Associate Laboratory TERRA, Centre for Functional Ecology - Science for People and the Planet, Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
2 University of Coimbra, Molecular Physical-Chemistry Unit, Department of Chemistry, Rua Larga 3004-535 Coimbra, Portugal

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: LSU rDNA sequencing, morphology, PCR ITS-RFLP, phylogenetic analysis.

ABSTRACT:

In a survey for *Bursaphelenchus* species in a centennial *Pinus pinea*, with significant wilting symptoms, in Coimbra, Portugal, some nematodes having the main morphological characters of the genus *Bursaphelenchus* were extracted from wood and transferred to *Botrytis cinerea* cultures for the establishment of a nematode isolate. Subsequent morphological and molecular characterisation were carried out. The morphological data of both females and males identified these nematodes as *Bursaphelenchus arthuri*. The main morphological characters described for this species were observed: lateral field with four lateral lines, females with a long post uterine branch, vulva without flap and tail with ventrally bent terminus, males with spicules with a sharply pointed rostrum without cucullus and with a distinct oval dorso-ventral terminal bursa. Morphological identification was confirmed by molecular characterisation performed by DNA extraction and amplification of ITS rDNA regions containing partial 18S and 28S and complete ITS1, 5.8S and ITS2 sequences followed by restriction fragment length polymorphism analysis by AfaI, AluI, HaeIII, HinfI and MspI endonucleases (PCR ITS-RFLP), displaying a restriction pattern specific to *B. arthuri*. Furthermore, the sequencing of the D2-D3 expansion region of the large subunit (LSU) of rDNA confirmed the identification and phylogenetic analysis by the multiple sequence alignment of selected D2-D3 sequences revealed that the Portuguese isolate forms a clade with other *B. arthuri* isolate. According to our knowledge, this study represents the first report of *B. arthuri* from P. pinea in Europe.

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296. Morphological and molecular characterization of *Paratylenchus holdemani* in Puglia, South Italy

Alberto Troccoli[1], Elena Fanelli[1], Alessio Vovlas[1], Stefano Convertini[2], Nicola Tinella[2], Francesca De Luca[1]
1 Istituto per la Protezione Sostenibile delle Piante - CNR, via Amendola, 122/D, 70126 Bari, Italy
2 Agrin Soc. Cons. A R.L., Via XXV Aprile 13, 36055 Nove (VI), Italy

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: Identification, Morphology, Molecular markers, *Paratylenchus holdemani*

ABSTRACT:

During 2018-2020, in countryside farms in Brindisi province, southern Italy, a widespread vegetative post-transplant degeneration on celery and parsley was observed, characterized by aspecific symptoms such as rapid loss of vigour, rosette growth, chlorosis, desiccation, with consequent total yield loss. A large population of pin nematodes (*Paratylenchus* sp.) has been isolated from the rhizosphere of diseased crops.

Paratylenchus genus is characterized by high intraspecific character variability and overlapping morphological characters. Molecular identification, using 18S rRNA, 28S rRNA and ITS region as markers, revealed that the nematodes recovered from infected Apiacee crops belong to *P. holdemani*. This record represents the first occurrence of *P. holdemani* in Italy. The correct identification of the species has allowed to adopt proper management strategy, by considering the severity of the symptoms and the rapid spread of the population.

297. Discovery of a newly identified plant-parasitic nematode (Tylenchida: Anguinidae) infesting western sword fern leaves in Washington state rainforests, USA

Sergio Álvarez-Ortega[1], Sergei A. Subbotin[2]
1 Universidad Rey Juan Carlos, Madrid, Spain
2 Plant Pest Diagnostic Center, California, USA

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: fern, leaf necrosis, new genus, new species, phylogeny, taxonomy

ABSTRACT:

A newly discovered genus and species of anguinid nematodes were found inhabiting leaves of the Western Sword Fern, *Polystichum munitum*, in various locations within Olympic National Park, Washington State, USA. These nematodes induce distinct non-specific necrotic and chlorotic symptoms on fern leaves, marked by yellowing and light to brown areas between veins. The new species is characterized by a long and slender body, six incisures in lateral field, robust stylet (12.0-13.5 µm) with small and rounded knobs, pyriform to elongate and abutting basal pharyngeal bulb, and long conical tail with pointed terminus. Females having posteriorly located vulva (V = 74-80%) and well-developed post-vulval uterine sac (26-59 µm) and males having spicules of 20-23 µm long. Phylogenetic analysis of the partial 18S rRNA and the D2-D3 expansion segments of the 28S rRNA genes showed that this anguinid nematode formed a separate evolutionary lineage different from all other Anguinidae taxa. Additionally, the new species was characterized by sequencing the ITS rRNA and COI genes.

298. Prevalence of cereal cyst nematodes in Sweden

Zahra Saad Omer[1]

1 Zahra Saad Omer, Rural Economy and Agricultural Society, R&D: P.O. Box 412, 751 06 Uppsala, Sweden
2 Maria Viketoft, Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-750 07 Uppsala, Sweden
3 Eva Edin, Rural Economy and Agricultural Society, R&D: Brunnby Gård 1, 725 97 Västerås, Sweden
4 Ulf Axelson, Vilske-Kleva Anfastorp 1, 21 94 Falköping, Sweden
5 Ann-Charlotte Wallenhammar, Rural Economy and Agricultural Society, R&D: Gamla vägen 5 G, 702 27 Örebro, Sweden

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: *Heterodera avenae*, oats, soil sampling, morphological analysis

ABSTRACT:

Cereals are crops grown worldwide, and they are an important component of food security. However, a threat to production is yield loss due to infection of cereal cyst nematodes (CCNs). In recent years, Swedish farmers and crop advisors have experienced an increase in incidences and injuries of CCNs, especially in the western part of the country. To date, the status of CCNs has not been fully investigated in all cereal-dominated areas in Sweden. Investigations carried out in oat fields during the 1980s in one region in western Sweden showed a wide occurrence of CCNs, especially *Heterodera avenae*. Follow-up surveys in 2018-2019 revealed high prevalence of CCNs, occurring in approx. 50% of the randomly sampled fields. The aim of this study is to close the knowledge gap on the status of CCNs in Swedish cereal production. The hypothesis is that CCNs are abundant in most of the cereal districts, but different CCN species dominate in different geographical regions. Cereal fields located throughout the cereal districts in south central Sweden were selected in consultation with crop advisors. Soil samples were collected in a W-pattern into one pooled sample per field. In total, 85 different fields were sampled in the autumn of 2023. Soil properties, such as clay content, were obtained from SGU (Swedish Geological Survey), Digital field maps https://www.sgu.se/. Information about crop rotation and other cultivation practices were obtained from respective farmer. The nematodes in the soil samples were analysed morphologically for the occurrence of CCNs at the Vildrosen jord och hund AB, Löberöd, Sweden. The cysts were extracted from soil using centrifugation floatation followed by enumeration under microscope. The cysts were then crushed, and the number of eggs and juveniles were counted. We will present the current distribution of CCNs in cereal districts in Sweden revealed through our sampling.

299. *Darmera peltata* found as a new host of *Aphelenchoides fragariae* in the UK

Phougeishangbam Rolish Singh[1], Maggie Correia[1], Valeria Orlando[1], Chloe Macey[1], Joseph Rotifa[1], Zachary Kershaw[1], Rebecca Lawson[1], Thomas Prior[1]
1 Fera Science Ltd., Sand Hutton, York YO41 1LZ, UK

Area: Taxonomy, Systematics and Phylogeny
Type: Poster
Keywords: *Aphelenchoides*

ABSTRACT:

Darmera peltata (umbrella plant or Indian rhubarb) is a perennial plant that generally prefers moist soil with partial to full shade. It is often grown as an ornamental plant in the UK. At an outdoor nursery in York, UK, this plant was found exhibiting heavy symptoms typical of *Aphelenchoides* infestation. Majority of nursery stock and established plants at the nursery showed interveinal leaf chlorosis and necrosis, and malformation of the foliage. A high density of *Aphelenchoides* specimens were extracted from the leaf samples. Morphological, molecular, and phylogenetic analyses of the detected nematodes confirmed the identity of the nematodes as *A. fragariae*. This is the first report of *A. fragariae* infestation to *D. peltata*.

300. Investigating nematode biodiversity in the Irish boglands using morphological features

Tilman Klappauf[1], Anusha Pulavarty[1], Douglas MacMillan[2], Thomais Kakouli-Duarte[1]
1 Molecular Ecology and Nematode Research Group, enviroCORE, Department of Applied Science, South East Technological University
2 Green Restoration Ireland Cooperative Society Ltd (GRI)

Area:
Type: Poster
Keywords: peatlands, nematodes, biodiversity, nematode communities, morphological analysis

ABSTRACT:

Approximately 80% of Ireland s 1.5 million hectares of peatlands have been subject to historical damage from peat extraction, predominantly for burning as a heat source. This practice disrupts natural carbon sinks, impacting climate change and biodiversity. Peatland disturbance releases stored carbon dioxide, thus contributing towards climate change. To counter this, Europe initiated rewetting and restoration projects. Green Restoration Ireland Coop (GRI) is actively restoring Ireland s peatlands, aiming to revive their carbon storage capacity while providing farmers with diversified income opportunities. In collaboration with GRI, the Molecular Ecology and Nematode Research Group of SETU contributes by assessing this peat restoration program. Focusing on nematode diversity and communities, 13 distinct peat habitats have been studied. Soil from each habitat underwent meticulous processing, providing homogenous representative samples for both molecular and morphological analyses. The Cobbs method for extraction of nematodes from soil was optimised for peat soils. Formaldehyde, ethanol, and glycerol were used for permanent fixing of individual nematodes on slides (Seinhorst, 1959). A high-power light microscope (Euromex Delphi-X Observer) was used for morphological identification and image capture. The NINJA tool was used to calculate relevant biodiversity indices for each peat habitat (Sieriebriennikov et al. 2014, https://shiny.wur.nl/ninja/). Preliminary findings indicate specific nematode associations with distinct peatland ecological conditions. Healthy bog lawns and degraded bog hummocks were dominated by algal feeders (Achromadoridae), whereas plant parasites were abundant in degraded bog lawns. A wasted peat habitat studied with diminished peat depth was dominated by nematodes belonging to Cephalobidae. Initial analyses of ten habitats at the first sampling site indicated them to be in a maturing or matured state based on metabolic footprints, with rewetted habitats trending towards maturity. The NINJA investigation, utilising approximately, 40-70 nematode individuals from each habitat, offers an initial preliminary perspective on the success and impacts of the peatland restoration efforts. Further insight into the ecological status of restored and non-restored peatlands will be gained through ongoing nematode community analyses via molecular methods.

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