

# 33<sup>th</sup> Symposium of the European Society of Nematologists

Ghent, 9 – 13<sup>th</sup> September 2018

## Abstract book



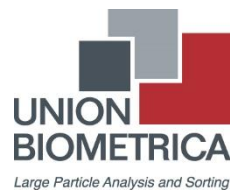




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## A 'worm' welcome to Ghent!

ILVO



For the third time in ESN history the medieval city of Ghent welcomes nematologists for its biennial symposium. When we launched the idea of organising the 33<sup>rd</sup> ESN symposium, memories from 1994, the previous time in Ghent, were shared with us. Vivid memories of a splendid symposium means we now have a challenge to do better.

Ghent has changed a lot since then. Historic buildings have been restored, the bar and restaurant scene gets high ratings in travel guides and the historic city centre is now one of Europe's biggest pedestrian zones. In 2017, Belgian beer culture was acknowledged as a cultural world heritage by UNESCO.

With Ghent University and the Flemish Research Institute for Agriculture, Fisheries and Food (ILVO), the Ghent region can be considered as a hotspot for nematological research and education. Both institutes jointly organise the 33<sup>rd</sup> ESN symposium.

Our team is enthusiastic, the scientific programme looks promising and we hope you will have a fantastic stay in Ghent!

Wim Wesemael

Wim Bert

Godelieve Gheysen

Tina Kyndt

Nicole Viaene

## Scientific committee

Matthew Back  
Wim Bert  
Philippe Castagnone-Sereno  
Sofia Costa  
Danny Coyne  
Etienne Danchin  
Keith Davies  
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Robbie Rae  
Sara Sanchez-Moreno  
Sergei Spiridonov  
Sergei Subbotin  
Soledad Verdejo-Lucas  
Nicole Viaene  
Wim Wesemael

# ORAL SESSIONS



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Convenors: Sergei Spiridonov & Keith Davies



**Plenary session**

[Integrated nematode control solutions are a must in the future](#)

Jaap Smedema

*Bayer AG, CropScience Division, Strategic Business Management, Alfred-Nobel-Str.50, 40789 Monheim am Rhein, Germany. [jaap.smedema@bayer.com](mailto:jaap.smedema@bayer.com)*

Plant-parasitic nematodes are considered to cause an estimated financial loss of over 80 billion EUR per year. Thus, nematode management in agricultural crops is necessary to prevent severe yield losses. However, nematode management is challenging since effective solutions are becoming rare due to regulatory concerns and shrinking societal acceptance of available chemical control products. The complexity of both, the nematode pest and current growing systems, make efficient nematode management even more challenging. Grower's dilemma cannot be overcome by one technology alone. Instead, it requires an integrated set of measures that will help mitigating the impact of nematodes throughout the growing season. This integrated approach could comprise the use of good agricultural practices, cover crops, solarization, resilient varieties, analytical tools and finally the combination of chemical and biological control products. The success of such an approach has already been demonstrated in vegetable crops. Nevertheless, substantial implementation of this concept requires a holistic view and close collaboration of scientists, suppliers and most importantly, the grower to adapt the toolbox to the diverse local conditions and agricultural practices.

Monday, 10 September

[Towards sustainable food systems for the 21st Century: the need for a paradigm shift](#)

Emile A. Frison

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Today's food and farming systems have succeeded in supplying large volumes of foods to global markets, but are generating negative outcomes on multiple fronts: wide- spread degradation of land, water and ecosystems; high GHG emissions; biodiversity losses; persistent hunger and micronutrient deficiencies, the rapid rise of obesity and diet-related diseases; and livelihood stresses for farmers around the world. These problems are tied to the industrial model of agriculture that is increasingly dominant around the world. The uniformity at the heart of these systems leads systematically to negative outcomes and vulnerabilities, and particularly the use of an increasingly narrow pool of animal breeds and plant varieties. The 'Green Revolution' of the post-war period left a dual legacy: huge advances in the productivity of staple crops, and the concurrent marginalization of whole swathes of foods, crop varieties - and the communities depending on them. The low-diversity industrial model is locked in place by a series of vicious cycles. Highly compartmentalized approaches to research, education and policymaking allow one-dimensional productivity-focused solutions to prevail, and obscure the links between healthy ecosystems, a healthy planet and healthy people. Meanwhile, the way food systems are currently structured allows value to accrue to a limited number of actors, reinforcing their economic and political power, and thus their ability to influence the governance of food systems. To break these cycles, a fundamentally different model of agriculture is required, based on diversifying farms and farming landscapes, replacing chemical inputs, optimizing biodiversity and stimulating interactions between different species, as part of holistic strategies to build long-term fertility, i.e. 'diversified agroecological systems'. There is growing evidence that these systems keep carbon in the ground, support biodiversity, rebuild soil fertility and sustain yields over time, providing a basis for secure farm livelihoods and diverse healthy diets.

**S1. Chemical control of plant-parasitic nematodes. Sponsored by Corteva AgriScience**

Convenors: Tim Thoden & Yuji Oka

**Evaluation of a new nematicide for the control of potato cyst nematode (PCN) in the UK**

John I. Keer

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Potatoes are an important crop in the UK, worth over £750 million per annum. PCN is the most damaging pest of UK potatoes and costs the industry around £25.9 million per annum. Scientific surveys in 2002 and 2016 have shown infestation of over 50% of the available potato growing area in England and Wales, predominantly by *Globodera pallida*. UK growers have struggled with PCN control, relying mainly on rotation and granular nematicides. Granular nematicides are under constant scrutiny by regulators. Velum Prime (fluopyram SC400) is a new nematicide which has already shown good activity on free-living nematodes in several countries worldwide. If efficacy against PCN could be shown, it would provide a welcome addition to the PCN control options available to UK potato growers. Velum Prime was compared to standard granular nematicides, for the control of PCN, in a replicated small plot trial in south Lincolnshire, UK. The field was a fine sandy silt loam with a long history of potato growing. Initial PCN densities ranged from 23 – 28 eggs (g soil)<sup>-1</sup> and the species distribution was 100% *G. pallida*. All nematicides were applied to the soil surface and thoroughly incorporated into the top 15 cm soil. Velum Prime was also applied as an in-furrow band spray. The variety 'Maris Peer' was hand-planted immediately after nematicide incorporation. Control of PCN damage to the root system by all the nematicide treatments was indicated by significantly ( $P=0.05$ ) increased crop vigour, compared to the untreated control plots. These foliar effects were reinforced by significant ( $P=0.05$ ) increase in tuber yields. Velum Prime showed similar efficacy levels to Vydate. Nemathorin and Mocap provided the highest yields, although these were not significantly higher than for Velum Prime. No efficacy differences between methods of Velum Prime application were observed. Pf/Pi ratios were similar for untreated and all nematicides. This trial demonstrates a potentially useful new nematicide treatment for the control of PCN in the UK.

Monday, 10 September

## **Fluazaindolizine: a new active molecule for the control of plant-parasitic nematodes**

Tim C. Thoden<sup>1</sup>, Yannis Stamatias<sup>2</sup> and John A. Wiles<sup>3</sup>

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Plant-parasitic nematodes remain a significant threat and source of yield reduction in crop production around the world. Over the last two decades nematicidal products used for protection against these soil dwelling pests have come under significant regulatory pressure due to a range of toxicological and environmental issues. In response, the crop protection industry has initiated an intensive effort directed at the discovery and development of new biological and chemical nematicides, as well as traits in plants. Fluazaindolizine is a new highly effective and selective active ingredient for the control of plant-parasitic nematodes. Specificity for nematodes coupled with absence of activity against the target sites of commercial nematicides suggests that fluazaindolizine has a novel mode of action. It is the first member from the novel chemical class of sulfonamide nematicides. Commercial formulations (Salibro™) primarily include a liquid suspension concentrate (500SC) liquid, with granular formulations also under development for certain markets around the world. Salibro™ has been extensively tested in laboratory, glasshouse, micro-plot and field trials in North America, Latin America, Europe and Asia. In those trials Salibro™ was proven extremely effective against a range of important plant-parasitic nematode species. An introduction to the chemical and biological properties of this new nematicide will be presented.

Monday, 10 September

**CEDROZ<sup>®</sup>, a new terpene nematicide against root knot nematode on Solanaceae and cucurbits**

Emanuele Medico, Eva Van Hende, Khalid El Quadi and Maria De Las Mercedes

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The new product (Cedroz<sup>®</sup>) is an innovative suspension of capsules (CS) based on thymol and geraniol, better known as terpenes. These terpenes have a nematicidal action identified, developed and tested by Eden Research plc and Eastman Chemical B.V. Thymol and geraniol are phenols and alcohols, respectively, with the characteristic of being volatile, lipophilic and insoluble in water. Thanks to the innovative formulation of capsules that promotes suspensibility without the use of solvents, these terpenes can be dissolved in solution and applied to the soil with a slow and gradual release. The mode of action of thymol and geraniol is explained by their synergistic interaction on the lipid substances contained in the cell membranes of nematodes and their eggs. In experimental tests on Solanaceae plants and cucurbits, Cedroz showed an efficacy level comparable to that of the existing post-transplant control solutions. Thymol and geraniol have a favourable toxicological, ecotoxicological and environmental profile. The commercial product will not have pre-harvest interval and will be exempt from LMR.

## **Inhibitor studies and vital dye staining suggest that the nematicide fluensulfone acts against lipid mobilization**

Emily Feist, Matthew Terry, Vincent O'Connor and Lindy Holden-Dye

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Fluensulfone is a novel nematicide with a distinct profile of effects on nematode behaviour (Kearn *et al.*, 2014). It has been shown that its nematicidal activity is associated with a progressive metabolic impairment (Kearn *et al.*, 2017); however, the metabolic fluxes that are impacted are poorly understood. We have used histological staining to investigate the metabolic integrity of *Globodera pallida* second-stage juveniles (J2) following fluensulfone treatment. In particular, the NADH/NADPH dependent precipitation of MTT, a tetrazolium salt that can be used to evaluate cell viability (Mosmann, 1983). In healthy J2, MTT staining is most intense in the head, consistent with a high concentration of mitochondrial activity in this region. In J2 exposed to fluensulfone, we observed an accumulating paralysis that progresses to death, accompanied by a loss of MTT staining. This infers that immotility is associated with loss of metabolism and death. An analysis of the time-course of the effect of fluensulfone treatment of J2 on MTT staining revealed an interesting redistribution of staining prior to complete loss of staining and death. This redistribution was manifest as a transition from predominant anterior staining to predominant posterior staining. This suggests that the fluensulfone-mediated insult that ultimately leads to death is preceded by a redistribution of metabolic activity. This characteristic shift in vital dye staining provided a route to identify compounds that might have similar nematicidal activity. We have investigated the time-course and pattern of MTT staining in free-living J2 following treatment of other known inhibitors of metabolic pathways. Intriguingly, a subset of these inhibitors elicits a similar redistribution of MTT staining as seen with fluensulfone. Among the inhibitors tested, compounds that selectively target mobilisation of lipid metabolism were particularly striking. As lipid stores in J2 are selectively associated with their posterior region, the results are consistent with fluensulfone acting on lipid metabolism and supports wider investigation of lipid metabolism as a new route to selective nematicidal activity.

### References

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- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55-63.

## **Nematicidal or nematistatic! Mode of action of fluopyram in plant-parasitic nematodes**

A. Sylvia S. Schleker<sup>1</sup>, Marc Rist<sup>2</sup>, Christiane Matera<sup>1</sup>, Arunas Damijonaitis<sup>2</sup>, Ursel Collienne<sup>2</sup>, Katja Twelker<sup>2</sup>, Melanie Scharwey<sup>2</sup>, Ute Schlee<sup>1</sup> and Florian M. W. Grundler<sup>1</sup>

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Management of plant parasitic nematodes (PPN) in agricultural crop production is necessary to counteract severe yield losses worldwide but challenging as effective means are rare. Recently, it has been discovered that soil applications of the succinate dehydrogenase inhibitor fluopyram can be effective against PPN. Here we show that fluopyram selectively binds to the target in nematodes but not in mammals and insects. Adenylate energy charge (AEC) values clearly indicate that fluopyram impairs ATP generation in *Meloidogyne incognita*, *Heterodera schachtii* and *Caenorhabditis elegans*. Our investigations reveal that the compound causes paralysis of *M. incognita* and *H. schachtii* second stage juveniles (J2) as well as *C. elegans*. Whereas micromolar concentrations of fluopyram are already nematicidal for *M. incognita* J2, *H. schachtii* J2 completely recover even at about a 100 times higher concentrations of the active substance. Sequence comparison highlights an amino acid exchange at a crucial site in the SDHC target protein that could cause a reversible binding of fluopyram to its target and thus could explain the above observations. Fluopyram efficiently reduces gall formation on lettuce after preincubation of *M. incognita* J2 with the compound. In line with the recovery assays, preincubation of *H. schachtii* J2 with the compound is not effective in inhibiting nematode parasitism of *Arabidopsis thaliana*. Although fluopyram is only nematistatic for *H. schachtii* J2, permanent contact of the nematodes with the compound in a micromolar to nanomolar range completely prevents nematode infection of *Arabidopsis thaliana* or considerably reduces nematode development at the root in a concentration dependent manner. In addition to pure contact activity, fluopyram impacts reproduction of *M. incognita* due to uptake of the compound by plant roots and xylem mobility.

## S2. Nematode responses to soil degradation and restoration

Convenors: Maria Viketoft & Thomaé Kakouli-Duarte

### **Evaluating the threat posed by irrigation water quality to soil ecosystem health**

Gerhard du Preez<sup>1,2</sup>, Mieke Daneel<sup>2</sup>, Victor Wepener<sup>1</sup> and Hendrika Fourie<sup>1</sup>

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Irrigated crop production, which account for 40% of global produce, is threatened by the deterioration of freshwater resources as a result of pollution originating from anthropogenic activities. While the effects of irrigating with low quality water on crop yield and quality is well appreciated, little remains known about the threat posed to soil ecosystems. This can, however, be assessed following the recently standardised soil quality TRIAD approach (ISO19204:2017). The present study represents, with nematodes as bioindicators, the ecological line of evidence (of the TRIAD approach) and was aimed at evaluating the soil health status of farmlands associated with the Hartbeespoort, Crocodile (West), and Marico-Bosveld (reference system) irrigation schemes (South Africa). Irrigation water and soil samples were collected during the winter and summer growing seasons of 2016 and analysed for physico-chemical properties (pH, electrical conductivity, organic content, particle size distribution, and metal, nutrient and salt levels). Results indicated that the Hartbeespoort and Crocodile (West) irrigation schemes utilised water characterised by elevated salt (as indicated by the electrical conductivity) and nutrient (inorganic nitrogen and phosphorus) concentrations. The associated soil ecosystems were classified using nematode-specific indices as either degraded or disrupted. However, the same was evidenced at the reference system, which suggests that irrigation water quality was not the main factor influencing soil ecosystem health. Instead, it is likely that the disruptive effects induced by conventional farming practices (e.g., tillage) induced the observed effects. Redundancy analysis biplots also evidenced a strong correlation between nutrients, crops produced and r-strategists, suggesting that the nematode assemblages responded rapidly, especially to the addition of fertilisers. No significant effect of irrigation water quality on soil ecosystem health was thus evidenced.



Monday, 10 September

## **Nematodes in experimentally polycyclic aromatic hydrocarbons (PAH) and Cu contaminated soils**

Maria Viketoft

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Current environmental risk assessment (ERA) tools suffer from a limited understanding of how contaminants affect soil ecosystems and functions. This frequently leads to expensive dig-and-dump, where all soil masses with levels above guideline values are removed, but with largely unknown benefits for the soil. The Swedish research project APPLICERA aims to develop a site-specific ERA methodology for contaminated land, where relevant soil functions are included. The specific objectives of the project are: *i*) to investigate contaminated sites *in situ* and test a battery of relevant indicators to explore how biotic and abiotic indicators can be used to express soil functions; and *ii*) to explore the relation between soil function and environmental risk using a TRIAD-methodology based on chemical, ecological and toxicological indicators. One of the tested ecological indicators is nematodes. Within the project, a lysimeter experiment with experimentally contaminated soils was performed. The soil came from two unpolluted locations with different soil types, a sandy and sandy loam soil. The soils were either mixed with a strongly polycyclic aromatic hydrocarbons (PAH) polluted soil or a Cu-spiked soil, so that there were three different contamination levels (low, medium, high) for each contaminant and soil type. The experiment was run for two growing seasons. The effect of the contaminants varied between the soil types. In the sandy soil, there was a negative effect of Cu and a positive effect of PAH the first year but these had disappeared during the second year. In the sandy loam soil, there was a stronger negative effect of PAH than of Cu on nematode numbers the first year, and in the second year there was actually an increase in nematode numbers in the Cu treatments.

## **Nematode community structure of rehabilitated surface mining sites in Sibutad, southern Philippines**

Joey Genevieve Martinez<sup>1,2,3</sup>, Kristin Claire Jariol<sup>2</sup>, Giovanni dos Santos<sup>4</sup>, Eric Van Ranst<sup>5</sup> and Tom Moens<sup>1</sup>

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In several developing countries with abandoned and active mining sites, rehabilitation is a crucial post-mining activity to assist in the recovery of damaged ecosystems. In the Philippines, rehabilitation is often carried out through addition of organic amendments (e.g., agricultural wastes) and development of aboveground vegetation. Rehabilitation success is often judged based on the survival rate of plants or their ability to grow. However, this may not be sufficient enough since many of the tree species used in rehabilitated areas, e.g., *Acacia auriculiformis*, can tolerate harsh soil conditions. In the present study, we used soil organisms, i.e., nematodes, to assess the establishment of soil communities in 'rehabilitated' areas. We compared nematode assemblage descriptors (e.g., abundance and diversity indices) and genus composition of the different sites between two time periods (2012 vs 2014). Nematodes were collected in 2012 and 2014 and identified to the genus level using a traditional approach (morphology-based identification techniques). Soil variables in 2012 such as N, P, OM, pH, particle size and heavy metals, such as As, Au, Ba, Be, Cd, Co, Cu, Hg, Ni, Pb, Sr and Zn, were quantified. Results show that there was an overall low abundance in all of the sampling sites in Sibutad, probably due to naturally low pH in the area (highest mean pH was 4.2). The distribution of nematode genera in 2012 was most probably related to high Pb level and lack of OM in soils. Hints of partial soil recovery were, however, manifested by the increase of abundance of 'sensitive' nematodes in rehabilitated areas and increase in nematode descriptors, such as abundance and genus richness, after two additional years of rehabilitation.

Monday, 10 September

## **Development of soil nematode community structure and soil food web during the early pedogenesis of a Mollisol**

Fengjuan Pan, Na Li, Xiaozeng Han and Congli Wang

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Soil nematodes are important components of soil biology, and are known to be sensitive to agricultural practices. However, such effects are largely unknown in the initial stage of soil formation. We measured abundance of soil nematodes and microbes in an ongoing field experiment that was originally established on exposed parent material of a Mollisol taken below 2 m depth. Field management practices were included: natural fallow (NatF), alfalfa (Alfa), and annual cropped fields without fertilisation or crop residue input (FOCO), with fertilisation but no crop residue input (F1C0), with fertilisation and crop residue input (F1C1), and a parent material soil (PM). Our results showed that management practices influenced the community structure of soil nematodes. The abundance of plant parasites was higher in Alfa soil than in NatF soil, and lowest in the soils of FOCO and F1C1. Bacterivores were most abundant in both Alfa and F1C1 soils. Based on phospholipids fatty acids of soil microbes, all the field soils were separated by PM into two groups *via* PC1, the tilled and the no-tilled soils. Redundancy analysis showed that the structure of food web was similar to nematode guild structure rather than to soil microbial community structure among the soils. The food web structure was separated between the soils under perennials and those under cropping *via* Axis 1 and between fertilised and not fertilised soils irrespective of land uses *via* Axis 2. Our results suggest that the ecosystem function of the similar eroded soils can be recovered by agricultural management practices.

Monday, 10 September

## **Trajectories of nematode and plant communities stimulated by liming under nitrogen deposition in subtropical abandoned arable cropland**

Shanyi Tian<sup>1</sup>, Xiaoyun Chen<sup>1</sup>, Yanhong Cheng<sup>2</sup> and Manqiang Liu<sup>1</sup>

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Elevated global nitrogen (N) deposition can induce soil acidification and decrease plant diversity, which suppresses soil community composition and degenerate soil function sustainability. Abandonment of cropland is a common practice for restoring soil ecological function. To our knowledge, however, the effect of liming on acidic soil function restoration of abandoned farmland under N deposition is unclear. We studied the changes of soil nematode assemblage and plant community under simulated N deposition (0, 60 and 90 kg N ha<sup>-1</sup>) and lime amendment (0 and 110 kg Ca(OH)<sub>2</sub> ha<sup>-1</sup>) in an ex-arable field. Results showed that N deposition significantly increased plant productivity, while liming induced the plant productivity convergence under different N deposition. Lime amendment counteracted the negative effect of N deposition on plant diversity, and liming enhanced plant Shannon-Weiner diversity index under N deposition. The abundance of bacterivores and fungivores decreased, while herbivores and omnivores-predators increased with increasing N deposition. Liming significantly reduced the proportion of microbivores and herbivores to a less extent regardless of N deposition. Liming could consistently and significantly enhance the proportion of omnivores-predators. Regression analysis showed that bacterivores, fungivores and herbivores were positively, while omnivores-predators were negatively, correlated to plant above- and below-ground biomass ( $P < 0.05$ ). In conclusion, liming could alleviate harmful effects on plant and nematode diversity of N deposition and accelerate the restoration of soil in subtropical region, and there is a synergic relationship between above- and below-ground communities, which might be references to the future studies of assessing of soil remediation in subtropical area.

### S3. Nematode biocontrol

Convenor: Keith Davies

#### **A hyperparasitic fried egg in a skirt: Genomic sequencing of the nematode parasite *Pasteuria penetrans***

Jamie N. Orr<sup>1,2</sup>, Peter Cock<sup>1</sup>, Vivian C. Blok<sup>1</sup> and Keith G. Davies<sup>2,3</sup>

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*Pasteuria penetrans* is an obligate, and highly specific, parasite of *Meloidogyne* species. Dormant endospores of this bacterium are often found in nematode suppressive soils where they attach to the host nematode cuticle as it migrates. This encumbrance alone is capable of inhibiting directional movement and thus root invasion. However, should the nematode successfully invade the root, the endospore may germinate, penetrating the cuticle and colonising the pseudocoelom. The infected female remains alive and continues to feed but the bacterium alters embryogenesis resulting in sterilisation of its host. *Pasteuria* spp. specificity, formation of endospores, and suppressive activity are attractive *characteristics* for a commercial biocontrol agent. However, the obligate nature of this bacterium presents a significant challenge for in vitro culture and study of its fundamental biology, including the molecular mechanics of its highly fastidious attachment. Using Whole Genome Amplification (WGA) and long read PacBio Sequencing, we have generated a genomic assembly of 2.5Mbp, indicating a reduced genome concurrent with an obligate parasitic lifestyle. Analysis of this sequence provides insights into the metabolic dependencies of this organism, and proteins that may be involved in attachment to the nematode cuticle. Understanding of these processes may lead to improvements in in vitro culture, prediction of efficacy, and ultimately in the effective application of *Pasteuria* spp. as biocontrol agents in the field.

**The role of different host root exudates in enhancing *Pasteuria* endospore attachment to the cuticle of *M. incognita* juveniles**

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The root exudates of cowpea (*Vigna unguiculata*), tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) played a key role in stimulating the surface coat of *Meloidogyne incognita* second-stage juveniles (J2) for attracting *Pasteuria* attachment especially in the aged juveniles. Incubation of fresh J2 of *M. incognita* in the root exudates of preferred hosts, cowpea and tomato, for 24 h at 28°C followed by a spore attachment test resulted in a non-significant 1.96%, and 3.26% increase in the spore attachment, respectively. However, similar incubation of 7 day-old J2 showed a significant increase in the attachment when incubated in cowpea (16.1%) and tomato (31.35%) root exudates which further increased by 57.14% and 102.04% in 14 day-old J2, respectively. By contrast, incubation of the *M. incognita* J2 in the less preferred potato root exudates showed an initial 9.8% reduction in the attachment in fresh J2, followed by 1.69% and 4.08% increase in attachment in 7 and 14 day-old J2, respectively. The GC-MS profiling of the root exudates showed that the majority of the compounds were hydrocarbons followed by ketones, esters and fatty acids; fifteen compounds were common to all three host plants, whilst 18, 13 and 8 compounds were identified to individual host plants of potato, cowpea and tomato respectively. These results will be discussed in the context of crop protection.

**Specificity of microbial attachment to second-stage juveniles of *Meloidogyne hapla* in suppressive soils**

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A number of studies have been reported on the reduction of plant-parasitic nematodes in suppressive soils in the past few decades. The evidence is accumulating that soil microbiota have a key effect on endoparasitic nematodes and their development in the host plant. This encouraged us to screen the soils from different regions in Germany for their suppressiveness against *Meloidogyne hapla*. Several soils stood out with respect to a reduced invasion of the second-stage juveniles (J2) and a decreased reproduction on tomato roots. We hypothesised that certain soil microbes attach to the cuticle of J2 in a highly specific manner while migrating through the soil and affect the J2 by direct or plant-mediated processes. We incubated surface-sterilised J2 of *M. hapla* in soil suspensions of the screened soils and captured the attached bacteria by a culture-dependent approach. Eight bacterial isolates were shown to be strongly attached to J2 in re-attachment assays, which will be confirmed by confocal microscopy. The ability of these bacteria to bind specifically to other nematode species and populations, as well as their mode of action on nematode behaviour is within the scope of current experiments. Strikingly, we found *in vitro* and *in vivo* indications on the involvement of induced systemic resistance in the reduction of *M. hapla* on tomato plants when exposed to these bacteria.

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**Bacterial community assemblages in the rhizosphere soil, endo-rhizosphere and cystosphere of soybean cyst nematode-suppressive soil challenged with nematodes**

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In disease-suppressive soil, plants rely upon mutualistic associations between roots and specific microbes for nutrient acquisition and disease suppression. Notably, the transmission of suppressiveness by the cysts of sugar beet cyst nematodes from suppressive- to conducive-soil has been observed in glasshouse trials. However, our current understanding of the bacterial assemblages in the cystosphere, endo-rhizosphere (interior of the root) and rhizosphere soil in cyst nematode-suppressive soil is still limited. To obtain potential insights into these bacterial microbiota assemblages, the bacterial communities inhabiting the soybean rhizosphere soil, endo-rhizosphere, and cystosphere in soybean cyst nematode (SCN; *Heterodera glycines*)-suppressive soil were characterised by deep sequencing, using soybean grown under growth room conditions with additional juvenile challenge. Clustering analysis revealed that the cystosphere bacterial community was closer to the endo-rhizosphere community than to the rhizosphere soil and bulk soil communities. Interestingly, the cystosphere bacterial community was initially established by the consecutive selection of bacterial taxa from the endo-rhizosphere as the majority of the cyst-enriched OTUs were more abundant in the endo-rhizosphere than in the rhizosphere soil. We also found a set of potential microbial consortia, such as *Pseudomonas*, *Pasteuria*, *Rhizobium*, and other taxa, that were consistently enriched in the rhizocompartments under SCN challenge. Furthermore, these enriched bacterial taxa were also more abundant in the cystosphere than in the bulk soil. Our results suggest that the soybean rhizosphere and cystosphere select a range of specific bacterial taxa likely to suppress *H. glycines* at different life stages of SCN infection in suppressive soil, contributing to plant health.



***Bacillus firmus* strain I-1582, a nematode antagonist by itself and through the plant**

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*Bacillus firmus* strain I-1582 is the active substance of biological based nematicide formulations. Several studies were conducted in order to determine the cardinal temperatures of growth kinetics and biofilm formation; the interaction with tomato and cucumber roots, and with *Meloidogyne incognita* eggs; and the capability to induce systemic resistance against *M. incognita* on tomato and cucumber in a split-root system. The bacterial growth kinetics and biofilm formation were assessed in the range from 10 to 50°C. The interaction with both tomato and cucumber roots, and with nematode eggs were studied under confocal laser microscopy after transformation of the bacterial isolate with the green fluorescent protein. For the split-root system, plant roots were divided into two 200 cm<sup>3</sup> pots, the inducer was inoculated with  $2 \times 10^8$  CFUs of *B. firmus* or not (control) and the responder with 1 juvenile of *M. incognita* per cm<sup>3</sup> of soil and maintained in a growth chamber at 25°C and 16:8h (light:dark) photoperiod for 40 days. At the end of the experiment, the bacterial root colonisation from the inducer, and the number of egg masses and the total nematode eggs from the responder root were assessed. The minimal, optimal and maximal growth temperatures were 10, 35 and 50°C, respectively. The biofilm formation was also related to cardinal temperatures. The transformed *B. firmus* colonised profusely the tomato but poorly the cucumber roots. The bacteria colonised and degraded nematode eggs. In the split root experiments, the number of CFUs in the inducer was higher in tomato than in cucumber. In the responder, fewer numbers of egg masses and total nematode eggs were produced in the tomato roots compared with the control; however, no differences were found in cucumber.

#### S4. Management of potato cyst nematodes: resistance

Convenors: Eric Grenier & Sebastian Kiewnick

##### **New sources of resistance toward *Globodera pallida* in starch potatoes-the “PARES” project**

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Potato cysts nematodes (PCN) are a major threat of the potato production worldwide and in particular for the starch potato industry in Europe. *Globodera pallida* is the most prevalent species found in about 75% of infested fields. Cultivars carrying resistance against *G. pallida* Pa3 are the main measure for control of PCN. Because they are regulated quarantine nematodes in Germany, the use of highly resistant cultivars is mandatory in the official control programme, once an infestation has been detected. The recently discovered highly virulent field populations of *G. pallida* (Pa3), which showed a significant level of reproduction on resistant cultivars, leave producers with little to no effective management options. Therefore, the project PARES, funded by the German Agency for Renewable Resources was initiated as a joint effort between research institutes and breeding companies to tackle this problem. This consortium aims at developing new sources of resistance from wild genotypes of *Solanum* spp. New resistance traits will be identified, characterised using molecular markers and used for breeding programmes of the commercial breeding companies. So far, 5000 clones of wild solanum sources obtained from accessions in two collections (GLKS and CGN) have been evaluated in high throughput glasshouse experiments. This significant number of tests was only possible due to the use of tissue culture plants instead of tubers for resistance testing. In several accessions such as *Solanum andigenum*, *S. gourlayi* ssp. *gourlayi*, *S. oplocense*, *S. vernei* or *S. sparsipilum*, resistance against both, a-virulent and virulent *G. pallida* populations was identified, but not all sources were suitable for follow up testing. However, candidate genotypes with resistance and favourite traits for breeding programmes could be identified and selected for molecular characterisation. The goal is to combine several resistance genes against *G. pallida* to achieve a sustainable and durable plant resistance in starch potatoes.

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**The development of potato cyst nematodes in The Netherlands in the last decades, including new virulent populations**

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The occurrence of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, in The Netherlands has been recorded for decades. Sampling techniques, crop rotation and changes in legislation have their effect on the number of findings. Data over the last 15 years show, for example, that the percentages of infestations are higher in the starch potato growing area than in the ware potato or seed potato area. The effect of the new control directive (2000/29/EC, active since 2010) can be seen on the number of findings; in 2004 the percentage of infected samples was around 3.8%, now the percentages of infested samples is about 1.6%. As the major control method is growing resistant potato varieties, this has a profound effect on population dynamics, resulting in the occurrence of new virulent populations of *G. pallida* and possibly *G. rostochiensis*. The Dutch NPPO has developed a strategy of tracing, detecting and testing these populations. The different steps in this process will be explained and results of these tests will be presented.

## Resistance against *Globodera pallida*: Mapping the *H2* gene

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Potatoes (*Solanum tuberosum* ssp. *tuberosum*) originate from South America and were brought into Europe through a limited number of introductions. Because of this, cultivated potatoes grown outside South America have a narrow genetic base and so do not contain the genes required to mount a resistance response to most pathogens that are encountered. However, resistance to many pathogens, including nematodes, viruses, oomycetes and fungi, is present in wild relatives of potato from where it can be introgressed into commercial cultivars. The first major PCN resistance gene identified was the *H1* gene from the wild species *S. tuberosum* ssp. *andigena*. Deployment of *H1*-containing cultivars was hugely successful in controlling *Globodera rostochiensis* infestations, but it caused a shift in species toward *G. pallida* which is not controlled with the *H1* source of resistance. Due to the multiple *G. pallida* pathotypes present in British fields, most potato varieties do not contain sufficiently good resistance for sustained control and suppression of this species. During attempts to identify robust sources of *G. pallida* resistance, the major effect *H2* resistance gene from the wild species *S. multidissectum* was found to provide high levels of control of the Pa1 pathotype. The goal of the research presented was to isolate candidate genes which are responsible for *H2*-mediated PCN resistance. Through the utilisation of a segregating tetraploid F<sub>1</sub> mapping population, as well as targeted genome enrichment to reduce genome complexity, a set of allele differentiating markers were designed which allowed two candidate genes to be identified within a 0.8Mb region on the distal arm of chromosome V.

## Enhancing the breeding for potato cyst nematode resistance through screening of *in-vitro* derived potato clones

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Management of potato cyst nematode (PCN) using resistant potato varieties is more viable, durable and environmental friendly compared to chemical control. A long and resource intensive breeding process and loss of resistance efficacy are some of the challenges facing resistance based management of PCN. This study had two goals, first to develop a resistance screening method that can speed up the selection process in the breeding programme and, secondly, to investigate new *Globodera pallida* Pa3 virulence types overcoming the currently available resistance in potato cultivars. A new screening method for *in vitro* derived plants was developed and validated in glasshouse experiments. Following a study on the reproduction of *G. pallida* on *in vitro* plants grown in small pots, the effects of pot size, inoculum level, timing of inoculation and substrate type on nematode reproduction were studied. *Globodera pallida* was able to multiply on susceptible *in vitro* clones to a level suitable for resistance assessment. Resistance tests of several potato cultivars using the *in vitro* plants and the standard resistance test method reflected their correct resistance ranking. The *in vitro* testing method enables screening of hundreds of potato clones on 1 m<sup>2</sup> glasshouse bench making it resource, labour and cost effective. A study on the recently reported resistance breaking *G. pallida* Pa3 (Emsland population) revealed significant differences in virulence and fitness when compared with *G. pallida* (Chavornay population). A biology study of the population revealed lower hatching rates and slower development in potato roots compared to Chavornay. However, the population produced more females in the roots of a resistance potato variety. Differences in the size of the cysts and the number of eggs per cyst were recorded. In addition, analysis of Vap1 virulence gene confirmed the phenotypic differences recorded in our study.

**Effective population size and implication for durability of plant resistances in the potato cyst nematode *Globodera pallida***

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The effective size of a population is the size of an ideal population that would drift at the same rate as the real population. The balance between selection and genetic drift depends on the effective population size ( $N_e$ ), rather than the real numbers of individuals in the population ( $N$ ). The objectives of the present study were to estimate  $N_e$  in the potato cyst nematode, *Globodera pallida*, using artificial populations and to explore the link between  $N_e$  and the durability of plant resistances. Using a temporal analysis of 24 independent populations, the median  $N_e$  was 58 individuals (min  $N_e = 25$  and max  $N_e = 228$ ).  $N_e$  is commonly lower than  $N$  but in our case the  $N_e:N$  ratio was extremely low because *G. pallida* populations deviate in structure from the assumptions of the ideal population by having unequal sex-ratios, high levels of inbreeding and a high variance in family sizes. The consequences of a low  $N_e$  could be important for their control because *G. pallida* populations will have a low capacity to adapt to changing environments unless selection intensity is very strong. This suggests that durable resistance to cyst nematodes is a truly achievable goal.

## S5. Impact of soil management on soil nematodes in their ecological context

Convenors: Sofia Costa & Sara Sánchez Moreno

### **Diversity of root-knot nematodes of the genus *Meloidogyne* Göeldi, 1892 (Nematoda: Meloidogynidae) associated with olive plants and environmental cues regarding their distribution in southern Spain**

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Root-knot nematodes of the genus *Meloidogyne* are recognised worldwide as a major production constraint in crops of primary economic importance. Knowledge of their diversity and prevalence, as well as the major environmental and agronomical cues for understanding their distribution in specific areas, are of vital importance for designing control measures to reduce significant damage. We provide the first detailed information on the diversity, distribution and levels of *Meloidogyne* species infecting wild and cultivated olive soils in a wide-region in southern Spain that included 499 sampling sites. Overall *Meloidogyne* spp. were found in 6.6% of sampled olive plants, with 6.6 and 6.5% for cultivated and wild olive, respectively. We identified five previously described *Meloidogyne* spp. (*Meloidogyne arenaria*, *M. baetica*, *M. hapla*, *M. incognita* and *M. javanica*) and one new species (*Meloidogyne oleae* sp. nov.) which, characterised using integrative taxonomy, increases the known biodiversity of *Meloidogyne* spp. affecting olive. *Meloidogyne arenaria* and *M. incognita* were only found infecting cultivated olive varieties, whilst *M. baetica* was only found infecting wild olive. Three major parameters drive the distribution of *Meloidogyne* spp. in cultivated olives in southern Spain, cover vegetation on alley, irrigation and soil texture, but different species respond differently to them. In particular the presence of *M. incognita* is highly correlated with sandy loamy soils, the presence of *M. javanica* with irrigated soils and cover vegetation, while the presence of *M. arenaria* is correlated with the absence of cover vegetation on alley and absence of irrigation. These parameters likely influence the selection of each particular *Meloidogyne* species from a major dispersal source, such as the rooted plantlets used to establish the orchards.

Monday, 10 September

## **Effects of 25 years of reduced tillage and crop rotation on soil nematodes and soil quality**

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Conservation agriculture (CA) is used worldwide to reduce soil perturbation, increase soil organic matter and promote water retention in agricultural systems. In cereal production systems in semi-arid regions of Southern Europe CA has widely replaced conventional practices to increase soil fertility. We studied soil properties (C, N,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ), microbial activity (PMN, respiration, MBC,  $\beta$ -glucosidase, urease, FDA), and the diversity, abundance and functioning of soil nematode communities in a CA experiment in central Spain. The experiment was established 25 years ago as a split plot design with four blocks and tillage (standard tillage (ST), minimum tillage (MT), and no-tillage (NT)) as the first factor, and a winter wheat monoculture compared to a 4-year rotation (fallow – wheat – vetch – barley) as a secondary factor. Soil samples were collected at two soil depths (0-7.5 and 7.5-15 cm) at four growing seasons (2013-2016). Most indicators of soil fertility and microbial activity improved under CA, with higher C and N soil contents and increased microbial activity in NT plots. Sampling date greatly influenced soil nematode communities, which showed noticeable temporal dynamics. At the upper soil layer, the effects of crop rotation were evident mainly in the plant-feeding nematode community, whilst at the deeper soil layer the effects of tillage were more manifest. Herbivore nematodes were greatly enhanced by monoculture, and tillage reduction positively affected beneficial nematodes.



**Status of *Pratylenchus coffeae* in different agro-ecological zones growing banana crops in Tanzania**

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*Pratylenchus coffeae* is among the plant-parasitic nematodes that can contribute to yield losses of up to 80% on banana crops. In Tanzania banana is among the major food crops and is used as staple food in some areas. However, information about the status of *P. coffeae* on banana is scant. In 2015, a survey was conducted in 10 regions within the major banana growing areas of Tanzania aiming at collecting basic information on availability and status of *P. coffeae*. This information is useful for improvement of banana production through nematode management. The regions were located in four agro-ecological zones, namely Lake Zone (Kagera region), Southern highlands zone (Mbeya and Ruvuma regions), Northern zone (Arusha and Kilimanjaro regions) and Zanzibar islands (North Pemba, South Pemba, North Unguja, South Unguja and West Unguja). Soil and root samples were collected in addition to geographical data. Morphological features and molecular identification indicated the presence of *P. coffeae*. The survey noted increase in total counts of *P. coffeae* extracted from roots from 74 reported on 1999 to 2996 in the regions of Unguja West, North and South. Additionally, we noted the presence of *P. coffeae* for the first time in Southern highlands, Northern and Lake agro-ecological zones. The number of *P. coffeae* was high in the regions of Southern Highlands (Mbeya and Ruvuma) agro-ecological Zone. The average abundancy of *P. coffeae* on banana roots was significantly five times higher in areas with altitude between 500 and 1000 m where total nematodes were over 1700, whilst at other altitudes (< 500 m and >1000 m) the counts were about 300 or less. The information on status of *P. coffeae* in different banana growing regions is important for development of effective nematode management practices and to prioritise highly infested areas.

## Soil nematode communities in protected tomato cultivation differ among three horticultural management strategies

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Nematode community analysis of free-living nematode guilds can provide indicators of soil fertility and regulation of pests and diseases, whilst data on plant-parasitic nematodes (PPN) can be used as proxy for potential plant damage. Tomato plants, *Solanum lycopersicum*, can suffer root herbivory by several PPN genera, and are considered universal hosts for root-knot nematodes, *Meloidogyne* spp. These nematodes can cause massive losses in fresh-market tomato in protected cultivation, but nematode community data are scarce in these systems. Although PPN are historically managed through chemical control, organic farming can lead to naturally suppressive conditions. Our objective was to compare soil quality through nematode community analyses and to infer on the damage potential of PPN in protected tomato cultivation in three different management strategies: certified organic farms (O), conventional smallholder farms (S), and conventional large farms with good agricultural practices certification (L). As part of the COCOoN Project (POCI/01/0145/FEDER/016611-PTDC/AGR-PRO/3438/2014), nematode communities were sampled and analysed in 45 farms producing tomato in polythene tunnels in Portugal. Taxa abundance and community indices were related through canonical correspondence analysis with gradients obtained through principal components analysis on soil properties, cropping history and plant protection strategies used in each farm. Overall, soil food webs had low structure and denoted high nutrient enrichment across the three strategies, conditions conducive to proliferation of pests and diseases, and indicative of highly impacted cropping systems in all management strategies. Nevertheless, sites O had more mature nematode communities and higher relative fungal decomposition ( $P < 0.05$ ) and, therefore, could be considered more stable systems. Among 12 PPN genera detected throughout, *Helicotylenchus* and *Meloidogyne* were found in population levels exceeding the threshold for tomato plant damage. Together with *Tylenchorhynchus*, these PPN were associated with conditions found mostly in L: continuous cropping of Solanaceae, low soil organic matter content and, notably, chemical nematode control.

Monday, 10 September

**Unravelling the mechanisms underlying health and productivity promoting agricultural practices by fine-mapping rhizosphere communities.**

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The impact of soil pathogens on crops has been studied for decades as if this was a multitude of bilateral interactions. For a long time, it has been realised that crops and soil-borne pathogens are interacting in a densely inhabited environment, the rhizosphere. A more versatile approach was hampered because of technical limitations: it was just impracticable to take major and highly diverse organismal groups such as bacteria, fungi and protists into consideration. Here we monitored the (plant-parasitic) nematode community in the rhizosphere of barley under three distinct soil management regimes, for two developmental stages of the crop, in two different locations. Total DNA and RNA was isolated from rhizosphere samples - 104 rhizosphere samples, for each sample DNA and cDNA was analysed separately - using home-made extraction and purification protocols. Targeted (ribosomal DNA) Illumina MiSeq sequencing was used to characterise the nematode, protist, fungal and bacterial community. First of all, very significant location effects were observed for all four organismal groups. Superimposed on the location effects, clear effects of organic, integrated and conventional soil management could be pinpointed. To the best of our knowledge, this is the first time four major key organismal groups players of the soil food web are simultaneously mapped in order to obtain insight in the effects of soil management practices on plant-parasitic nematodes taking a major part of the rhizobiome into consideration.

## **S6. Systematics and Phylogeography**

Convenors: Wim Bert & Sergei Subbotin

### **Heads or tails? New insights and conundrums in *Aphelenchoides* and close genera**

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Foliar nematodes, *i.e.*, plant-parasitic *Aphelenchoides* species, are part of one of the four evolutionary lineages of plant-parasitic nematodes. Yet, these species reside in a mostly mycophagous genus and their phylogenetic relationships with closely related genera *i.e.*, *Ficophagus*, *Laimaphelenchus*, *Martininema*, *Robustodorus* and *Schistonchus*, remain unsolved. Moreover, morphology-based diagnosis is extremely difficult due to poor descriptions and a generally conserved morphology, while molecular data are available for few identified species and numerous unknown taxa. A combined analysis of molecular and morphological data for several plant-parasitic and free-living species revealed that only the tail terminus shape and the measurements related to the position of the excretory pore relative to the median bulb unequivocally correspond with molecular-defined clades. Moreover, only the tail terminus shape appears to correspond with natural groups; thus, we propose a grouping system to delineate supra-specific groups based on this feature. In addition, we discuss ultra-structural head features and provide a new diagnosis for the genus to include some morphological findings. Our analyses also support recent taxonomic changes within *Aphelenchoides* and its close genera.

## Phylogeography and DNA barcoding of the cyst nematode species of the *Avenae* group from the genus *Heterodera*

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Among the recognised species groups of *Heterodera*, the *Avenae* group is one of the largest with a total of 12 species. Ten of them, *H. arenaria*, *H. aucklandica*, *H. australis*, *H. avenae*, *H. filipjevi*, *H. mani*, *H. pratensis*, *H. riparia*, *H. sturhani* and *H. ustinovi*, are morphologically closely related and represent the *H. avenae* species complex, and the other two, *H. hordecalis* and *H. latipons*, are morphologically more distinct from this complex. In our study we provided comprehensive phylogenetic analyses of several hundred *COI* and ITS rRNA gene sequences from the *Avenae* group using Bayesian inference, maximum likelihood and statistical parsimony. More than 220 *COI* and 11 ITS rRNA new gene sequences from 147 nematode populations collected in 26 countries were obtained in our study. A putatively new cyst nematode species related to *H. latipons* was revealed from the analysis of *COI* and ITS rRNA gene datasets. *COI* gene sequences enabled *H. arenaria*, *H. australis* and *H. sturhani* to be distinguished from each other and other species. The results of the analysis showed that *COI* haplotypes corresponded to certain pathotypes of the cereal cyst nematodes. It is recommended that information on *COI* haplotypes of studied populations be included in research with these nematodes. Based on the results of phylogeographical analysis and age estimation of clades with a molecular clock approach, it was hypothesised that several species of the *Avenae* group primarily originated and diversified in the Irano-Anatolian hotspot during the Pleistocene and Holocene periods and then dispersed from this region across the world. Different geographic barriers, centres and times of origin might explain current known distribution patterns for species of the *Avenae* group. Possible pathways, including a long distance trans-Atlantic dispersal, and secondary centres of diversification were proposed.

## The cuticle ultrastructure of aphelenchoidid predators

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The nematode cuticle structure is extremely variable, not only among different taxa but also between sexes and across the developmental stages within a species, and is believed to reflect its function in adaptation. However, the role of the nematode cuticle in their life history is not yet sufficiently understood, mainly because the relationship between the cuticle structure and the ecological trait is unclear. In the present study, we focused on the Aphelenchoididae family, which is a highly divergent group including mycophagous species, plant parasites, insect parasites and predators. We observed the cuticle structure of several aphelenchoidid nematodes by transmission electron microscope, and compared their structural differences in relation to the life histories and phylogenetic relationships in an evolutionary context. As results, the cuticle structure of five mycophagous species (*Bursaphelenchus xylophilus*, *B. conicaudatus*, *B. luxuriosae*, *B. rainulfi* and *Aphelenchoides xylocopae*) were largely similar, *i.e.*, their cuticle consisted of four parts: a triple-layered epicuticle, electron-lucent cortical and median zones, and a radially striated basal zone. On the other hand, three predator species (*Ektaphelenchoides spondylis*, *Ektaphelenchus* sp. and *Seinura* sp.) had different cuticle structure, *i.e.*, they had osmophilic median zone. In addition, the phylogenetic analysis showed that the emergence of the osmophilic zone was concurrent with the evolution of predatory species from mycofagous species. Furthermore, *Peraphelenchus* sp., an obligate insect parasite derived from predatory nematode species, had no osmophilic median zone, indicating that the zone disappeared with loss of predatism. These results indicate the osmophilic median zone is essential for predacious life history, *e.g.*, avoiding cannibalism.

**Genetic diversity of potato cyst nematode in its native area: a novel phylogeographic study using low and high density markers and different geographic scales**

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Potato cyst nematodes, belonging to the genus *Globodera*, originate from the Andes and are major pests of potato with worldwide distribution. Our knowledge of the diversity of those species in their native areas remains patchy and has to be improved. A previous study based on 42 *Globodera pallida* Peruvian populations revealed a clear South to North phylogeographic pattern with five well supported clades and a maximum of diversity observed in the South of Peru. In order to investigate this phylogeographic pattern more in depth, we have firstly genotyped a large collection of 322 Peruvian populations using a region of the Cathepsin L gene that shows polymorphism between the two species of potato cyst nematodes. These results suggest the occurrence of a third novel genetic species in the South of Peru. Secondly, we have used a set of 13 microsatellite loci to genotype 88 populations all coming from the South of Peru. These investigations allow us both to be precise about the geographic borders of the previously described *G. pallida* genetic clades and to investigate the presence of any novel additional clade using a larger set of populations. Finally, in order to investigate variations at the genome scale and expand the geographic scale investigated, we also conducted a Genotyping-By-Sequencing experiment on a set of populations sampled in different South American countries (Chile, Peru and Mexico) in order to get insight into the maximum genetic diversity present in the native area of *Globodera* spp. The presented results constitute a large set of data corresponding to different genetic markers more or less representatives of the entire genome diversity and improve our knowledge of inter and intra-specific diversity on *Globodera* spp.

**Systematics of the genus *Punctodera***

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*Punctodera* is a genus within family Heteroderidae that include cyst forming nematode species with extensive anal fenestration. Since its creation in 1976, four species have been described: *P. punctate*, *P. chaltoensis*, *P. metadorensis* and *P. stonei*. Type materials of the species were obtained from the Canadian National Collection of Nematodes, and from Museum and Institute of Zoology, Polish Academy of Sciences, and were examined. The morphometric of the second-stage juvenile and of the cyst were compared using regression methods. This review also attempts to provide an update on the systematics, host, and distribution information. The characterisation of *P. stonei* discovered recently in Ontario, Canada using morphological and molecular methods was presented.



## **S7. Plant resistance/Nematode virulence**

Convenors: Philip Roberts & Philippe Castagnone

### **Identification of *avirulence* genes in *Globodera pallida* through target enrichment sequencing**

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The potato cyst nematodes (PCN) *Globodera pallida* (Stone) and *G. rostochiensis* (Woll.) are the most economically important nematode problems of the European and UK potato industry. The most effective way to control PCN is through the use of natural resistance. A number of potential resistance sources and quantitative trait loci (QTL) linked to partial PCN resistance have been identified and mapped. The *H1* gene confers full resistance to a number of *G. rostochiensis* pathotypes but is ineffective against *G. pallida*. During the last decades, the extensive use of *H1*-containing varieties has led to selection in favour of *G. pallida*, which is now the predominant PCN species in the UK. The existence of multiple pathotypes with different virulence behaviour and the fact that different populations may be present in the field simultaneously makes breeding against PCN complicated. Resistance operates through the detection of pathogen-derived effector proteins by plant resistance proteins (R). Upon recognition, strong immune responses are activated in plants and the recognised effectors are characterised as avirulence (*Avr*) genes. We aim to identify *Avr* genes in *G. pallida*. Screening of *G. pallida* populations selected on the commercially important resistance sources from *Solanum vernei* and *S. tuberosum* ssp. *andigena* CPC2802 (or *H3*) confirmed differences in virulence depending specifically on the source used for selection. Single-stranded baits were subsequently used to capture effector-encoding genomic regions followed by short-read enrichment sequencing (on the Illumina MiSeq platform). This, in combination with long-read sequencing using single molecule real-time (SMRT) technology (PacBio), will result in full contig assemblies and an improved reference that will in turn enable us to better study polymorphisms in the sequences of those regions and eventually identify the candidates for *Avr* genes.

Monday, 10 September

## **Stability and heritability of RNAi in generations of transgenic plants and nematodes**

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Plant-parasitic nematodes (PPN) are a major group of crop pests worldwide. The estimated annual losses to grain crops alone in Australia is about AU\$ 300 million. Two genera of sedentary endoparasitic nematodes are major temperate crop pests in Western Australia: these are root-knot nematodes (RKN) (*Meloidogyne* spp.) and cyst nematodes (BCN) (*Heterodera*, *Globodera* spp.). PPNs are difficult to control using conventional methods and many nematicides have now been banned. RNA interference (RNAi, gene silencing) technology is a potential alternative genetic approach for nematode control. One challenge for an RNAi-based transgene approach ('Host-Induced Gene Silencing') is to generate plants that express nematode control transgenes stably over many generations. Therefore, the current study involves the inheritance of RNAi in plants and nematodes conferring resistance to PPNs to underpin knowledge of the application of RNAi as a strategy to confer nematode resistance in crop plants. T2 seeds of different transgenic events in *Arabidopsis thaliana* plants expressing RNAi constructs for nine target genes of RKN and BCN have been studied to determine the segregation patterns in subsequent generations. q-PCR assays have been done to select single copy and multiple copy integrated RNAi constructs in transgenic plants. Selected transgenic plants of seven different genes were challenged with nematodes to determine reduction of infection in two generations. Infection assays of *M. incognita* and *H. schachtii* showed significantly fewer (50%) galls, egg masses and fewer (65%) cysts, respectively, on the roots of infected transgenic plants than on wild type plants. Interestingly, nematodes that survived after feeding on transgenic plants had reduced (70%) hatching compared to nematodes fed on control plants. Our results demonstrate that transgenic plants carrying hairpin construct of nematode target genes can reduce nematode reproduction stably over generations. The outcomes of this study will help optimise a stable RNAi-based nematode control strategy for field application: a vital way to be used to reduce the economic losses caused by PPN.

***Ma* orthologous genes in *Prunus* spp. shed light on an original NBS-LRR cluster conferring differential resistance to root-knot nematodes**

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are considerable polyphagous pests that severely challenge plants worldwide and especially perennials. The specific genetic resistance of plants mainly relies on NBS-LRR genes that are pivotal factors for pathogens control. In *Prunus* spp., the *Ma* plum and *RMja* almond genes possess different spectra for resistance (*R*) to RKN. On one hand, the *Ma* gene, cloned in 2011, displays a peculiar TIR-NBS-LRR (TNL) structure that is unique among plants; on the other hand, previous work showed that the *RMja* gene mapped on the same chromosome as *Ma*. We carried out a high-resolution mapping using a large almond segregating F2 progeny to locate precisely *RMja* on the peach genome, the reference sequence (RKN-susceptible) for *Prunus* species. We showed that the *RMja* gene maps in the *Ma* resistance cluster and that the *Ma* orthologue is the best candidate for *RMja*. This co-localisation is a crucial step that opens the way to unravel the molecular determinants involved in the resistance to RKN. From a BAC library, we obtained a 336-kb sequence encompassing the *RMja* candidate region. Thus, we benefit from three *Ma* orthologous regions to investigate their sequence polymorphism, respectively, within plum (complete *R* spectrum), almond (incomplete *R* spectrum) and peach (null *R* spectrum). We showed that the *Ma* TNL cluster has evolved orthologues with a unique conserved structure comprised of five repeated post-LRR (PL) domains. Our results suggest that the polymorphism contained in the PL domains might underlie differential resistance interactions with RKNs and an original immune mechanism in woody perennials. In addition, our study illustrates how, in the *Ma* cluster, PL exon duplications and losses shaped TNL structure and gave rise to atypical PL domain repeats of yet unknown role.

Monday, 10 September

## Characterisation of root-knot nematode resistance traits in the grain legume cowpea

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Cowpea (*Vigna unguiculata*) is vitally important for food security as a primary source of protein, minerals and vitamins in the human diet in sub-Saharan Africa, parts of Asia and other developing regions, and is also an important animal fodder crop in these areas. Cowpea grain yield is typically only 20% or less of its known yield potential due to abiotic and biotic stresses, including attack by root-knot nematodes (*Meloidogyne* spp.). Natural host resistance deployed in preferred varieties and integrated into rotations is a central nematode management approach for cowpea. Screens for root-knot resistance within the two main cowpea gene-pools have revealed both independent and closely linked species- and population-specific R gene loci. Controlled environment and field phenotyping for nematode reproduction and root-galling were combined with genome-wide genotyping using the cowpea Illumina iSelect 60K-SNP consortium array for genetic mapping. Currently seven specific resistance determinants effective against certain *M. incognita* and (or) *M. javanica* populations have been identified on the consensus genetic map of the diploid genome of cowpea through QTL mapping, and SNP-anchoring enabled analysis of candidate genes in the QTLs regions on the cowpea whole genome sequence assembly. This work provides both marker-tagging of combinations of resistance loci for advanced cowpea breeding and gene candidates for the R genes.

Monday, 10 September

**Genome-wide association mapping of the architecture of susceptibility to the root-knot nematode *Meloidogyne incognita* in *Arabidopsis thaliana***

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Susceptibility to the root-knot nematode, *Meloidogyne incognita*, in plants is thought to be a complex trait based on multiple genes involved in cell differentiation, growth and defence. Previous genetic analyses of susceptibility to *M. incognita* have mainly focussed on segregating dominant resistance genes in crops. It is not known if plants harbour significant genetic variation in susceptibility to *M. incognita* independent of dominant resistance genes. To study the genetic architecture of susceptibility to *M. incognita* in plants, we analysed nematode reproduction on a highly diverse set of 340 natural inbred lines of *Arabidopsis thaliana* with genome-wide association mapping. We observed a surprisingly large variation in nematode reproduction among these lines. Genome-wide association mapping revealed thirty-six quantitative trait loci (QTLs) in the genome of *A. thaliana* significantly associated with reproductive success of *M. incognita*. Mutant analysis of candidate genes located in four QTLs revealed various novel proteins functioning as (co-)regulators of susceptibility to *M. incognita* in *Arabidopsis*. Our data further suggests that breeding for loss-of-susceptibility, based on allelic variants critically involved in nematode feeding, could be used to make crops more resilient to root-knot nematodes.

**S8. Nematode diversity and distribution: from microhabitat to realm scale**

Convenor: Hans Helder

**Living apart-together: Microhabitat differentiation of cryptic nematode species in a saltmarsh habitat**

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Coexistence of highly similar species is at odds with ecological theory of competition; coexistence, then, requires stabilising mechanisms such as differences in ecological niche. In the bacterivore nematode *Litoditis marina* species complex, which occurs associated with macro-algae, four cryptic lineages (Pm I-IV) co-occur in the field along the south-western coast and estuaries of The Netherlands. Here we investigate the temporal and/or spatial niche differentiation in their natural environment using a qPCR-based detection and relative quantification method. We collected different algal species (*i.e.*, two *Fucus* species and *Ulva* sp.) and separate algal structures (*i.e.*, receptacula, thalli, non-fertile tips and bladders) at different sampling months and times (*i.e.*, twice per sampling month), to examine differences in microhabitat use between coexisting *L. marina* species. Results demonstrate that the cryptic species composition varied among different algal species and algal structures, which was also subject to temporal shifts. Pm I dominated on *Fucus* spp., Pm II showed dominance on *Ulva* sp., while Pm III overall had the lowest frequencies. Microhabitat partitioning was most pronounced between the two cryptic species that had similar microbiomes (Pm I and Pm II), and less so between the two species that had significantly different microbiomes (Pm I and Pm III), suggesting that species which share the same microhabitats may avoid competition through resource partitioning. The interplay of microhabitat differentiation and temporal dynamics among the cryptic species of *L. marina* implies that there is a complex interaction between biotic components and abiotic factors, which contributes to their coexistence in the field.

## Nematode diversity of the Telperion Nature Reserve, South Africa

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The Nematology Unit of the Agricultural Research Council – Plant Health and Protection (ARC-PHP), Biosystematics, founded the South African Plant-Parasitic Nematode Survey (SAPPNS) in 1987. It was aimed at comprehensively assessing the nematode biodiversity resources of South Africa. Since the start of the SAPPNS, various surveys have been undertaken in protected areas to record the biodiversity of the plant nematode fauna. The Telperion Nature Reserve, Mpumalanga, South Africa, was chosen to conduct an extensive nematode survey in a protected area in the Grassland Biome. Various samples, including soil, root, water substrate and grass seed, were collected over four consecutive seasons (winter 2015 to autumn 2016). The diversity found during this survey was remarkable. A total of 99 genera were found in the soil and root samples. This included 27 plant-parasitic and 72 free-living nematode genera. Some plant-parasitic species include *Criconema corbetti*, *Hemicriconemoides brachyurus*, *Trichodorus* sp., *Xiphinema capensis* and *X. vanderlinde*. A total of six nematode species were found from various grass seed samples. Four of these species, including *Aphelenchoides lichenicola*, *A. rutgersi*, *A. spicomucronatus* and *Panagrolaimus leperisini*, were found in South Africa for the first time. The remaining two species, *Aphelenchoides africanus* and *Aphelenchoides besseyi*, were previously reported from South Africa; however, this is the first report from seeds. Thirty-six genera were found at four water substrate sites within the reserve. *Chronogaster aspinata*, *Paraphanolaimus behningi* and *Tridentulus* sp. are new reports for South Africa. Four species that are probably new to science have also been found. Plant-parasitic nematodes found at the water substrate sites include *Criconema* sp., *Hemicycliophora poranga*, *Pratylenchus pseudopratensis* (first report for South Africa), *Scutellonema* sp. and *Helicotylenchus* sp.

## **Community structure and ecological niches of free-living terrestrial nematodes in north-eastern Vietnam**

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We investigated the nematode fauna in soils of three Nature Reserves in Cao Bang, Lang Son and Cat Ba provinces in north-eastern Vietnam. First, we identified the nematodes (if possible) to species level in order to estimate the numbers and diversity of known and undescribed taxa. Then, based on the different trophic groups, nematode functional guilds were determined. These data were used to calculate different ecological indices in order to describe how changes in land use affected the functioning of soils in a gradient from primary forest, secondary forests, slash and burn agriculture and intensive agricultural management. In total, more than 14,000 specimens were identified. These nematodes belonged to 105 genera, 42 families and 9 orders, of which 48 genera and 13 families belonged solely to the order Dorylaimida. The Order Dorylaimida is taxonomically particularly challenging because it is highly diverse, but species show only minor differences in morphological characters. More than one third of the identified species of dorylaims were new to science. Also molecular data confirmed that the diversity of dorylaims is little known, only five sequences obtained from GenBank could be assigned to the sequenced nematodes with a similarity higher than 95%. The nematode assemblages and diversity were significantly different between different regions and among four land use intensities. Nematode assemblages in primary forests were composed mainly of large and long-lived predators and omnivores (46-74%), compared to a successive increase with land-use intensification of short-lived bacterivores (5-14%), plant parasites (11-24%) and fungivores (3-15%). Our data indicated that nematode community analysis was a useful tool to predict functional changes in soil ecosystems.



## **Biogeographical distribution of soil nematode community in relation to large-scaled climate and local-scaled soil environment in China**

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Factors driving the biogeography of soil nematodes remained poorly understood. Large-scale climate and local-scale soil environment as well as the consequent vegetation are considered critical. Soil samples of natural forest, natural grassland and agricultural ecosystem across the mainland of China were collected based on annual rainfall and temperature. Soil nematode communities were analysed by microscopy-based morphological and molecular-based high throughput sequencing methods, to quantify what pattern of biogeographic distribution of soil nematode community and the relative importance of determinant factors such as climatic and soil environmental variables. Nematode abundance showed a distinct peak at the grassland of Mongolian plateau (average 34.6 individual g<sup>-1</sup>), much higher than 19.5 individual g<sup>-1</sup> of soil from agricultural ecosystem of North China Plain. Bacterivores and herbivores were the dominant species, ranged from 10%-75% and 9%-81% in grassland and forest ecosystem, respectively. Herbivores were the dominant species, ranged from 25-89% in agricultural ecosystem. The large-scale biogeographic distribution patterns of soil nematode communities were driven more by large-scale climatic factors than local-scale soil environment. In all ecosystem, nematode abundance and communities were affected positively by the latitude ( $P < 0.05$ ), while negatively by the annual average precipitation and annual average temperature ( $P < 0.05$ ). For local-scale soil environment, pH had a positive effect on bacterivorous abundance ( $P < 0.05$ ), while soil organic carbon and total nitrogen had a positive effect only on omnivorous and predators ( $P < 0.05$ ). In conclusion, soil nematodes, affected by climate variability at a large scale, indicate climate change may have a significant impact on nematode communities.

**The remarkably high number of transitions from marine to terrestrial habitats and *vice versa* as an adaptation that could have contributed to the ecological success of nematodes**

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Nematodes have been most successful in colonising soils and marine sediments: it is the only major metazoan group that is persistently abundant and diverse across realms. In marine sediments of shallow waters, nematodes are present at densities between 0.5 and 5.0 million ind. m<sup>-2</sup> (Soetaert *et al.*, 2009). In soil, the number of nematodes under non-extreme environmental conditions ranges from 2-20 million ind. m<sup>-2</sup> (Bongers, 1994). In both soil and marine sediments, the density of other, second most abundant metazoans, including polychaetes and harpacticoid copepods in marine sediments, and mites and springtails in terrestrial soils, are about an order of magnitude lower. These data about nematode abundance and diversity prompt questions about the factors underlying the ecological success of this rather basal Ecdysozoan group. One of the main factors contributing to their ecological success could be their ecological flexibility. Using a phylum-wide SSU rDNA data base harbouring over 3,500 taxa allowed us to pinpoint at least 30 major habitat transitions. In Clades 1-6 (formerly referred to as Adenophorea) these transitions were bidirectional, whereas most likely members of Clades 8-12 (previously known as Secernentea) showed exclusively transitions from terrestrial to marine systems. We relate these transitions to the evolution and diversification of the secretory-excretory (S-E) systems as well as to feeding habits. Their ability to feed on types of food sources that are available both in soils and marine sediments such as bacteria, protists, and other nematodes, and to parasitise organismal groups present in both systems including lower and higher plants as well as a wide range of (in)vertebrates will have contributed to the ecological flexibility and the evolutionary success of nematodes.

Bongers, T. (1994) *De nematoden van Nederland 2<sup>nd</sup> edn*. Utrecht: Koninklijke Nederlandse Natuurhistorische Vereniging.

Soetaert, K., Franco, M., Lampadariou, N., Muthumbi, A., Steyaert, M., Vandepitte, L., Berghe, E.V. and Vanaverbeke, J. (2009). Factors affecting nematode biomass, length and width from the shelf to the deep sea. *Marine Ecology Progress Series* 392, 123-132.

**S9. Nematode biocontrol and animal parasites**

Convenors: Sergei Spiridonov & Keith Davies

**Effects of microbial rhamnolipids on plant-parasitic nematodes**

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Plant-parasitic nematodes (PPN) are a global threat to crop production. Treatment with common nematicides is banned in most countries, whilst crop rotation has often economic limitations. The urge for alternatives raises the awareness of ecologically more friendly approaches such as control agents of biological origin. Biosurfactants (BSFs) are a promising example for such an alternative in crop protection. These secondary metabolites are produced by various ubiquitous microorganisms and show an imposing efficiency against numerous economically relevant pathogens. Here, we demonstrate that rhamnolipids (RLs), one prominent representative of BSFs, counteract the infection of *Arabidopsis thaliana* by the cyst nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita* at concentrations far below the ecotoxicological threshold. Although there is no direct nematicidal activity of RLs the infection is reduced by up to 90% when the host plant is in contact with the compounds. Furthermore, we show that RLs modify the quality of the plant as a host: galls of *M. incognita* are reduced in size and the number of eggs produced is reduced in *M. incognita* and *H. schachtii*. We have evidence that RLs are able to reinforce plant defence already in advance of a pathogen attack enabling the plant to react more appropriate against PPN.

## Isolation of destructive nematophagous fungi using a single-egg plating technique

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The sedentary lifestyle of cyst nematodes may favour the development of their natural antagonists within the micro-environment presented by each cyst. In this context, nematode cysts from monoculture wheat fields of CIMMYT (International Wheat and Maize Improvement Centre) in Turkey were collected and scrutinised for nematode-associated fungi antagonising *Heterodera filipjevi*. Cysts and eggs within displaying symptoms of fungal infections were grouped according to symptoms they exhibited. A single-egg based technique was established to isolate fungal strains directly from the symptomatic eggs. This technique includes collection of diseased eggs from each symptomatic cyst, sufficient surface sterilisation steps, and individually plating single eggs on appropriate cultural media. The fungal pure culture isolated from a single nematode egg was used for DNA-based identification. These sequences were then compared with the fungal DNA sequences obtained directly from the single eggs exhibiting the same disease symptom to assess the identity of the colonising fungal strain. This screening strategy resulted in the isolation of several known and some new fungi, notably some undescribed slow-growing fungi, which could have been outgrown by fast growing strains if several eggs or a whole cyst had been plated. Of the discovered fungi, the species *Ijuhya vitelline*, *Monocillium gamsii*, and *Polyphilus sieberi* were recently described, and several others are currently being studied. Metabolite profiling of the discovered fungi revealed that they produce diverse secondary metabolites especially some new nonribosomal cyclic peptides that showed nematicidal effects in nematode bioassays.

Monday, 10 September

***Severianoia* sp. (Thelastomatidae, Oxyuirida) – intestinal parasites of diplopods in Western Caucasus**

Serge E. Spiridonov and Svetlana V. Malysheva

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*Pachyiulus krivolutskyi* Golovatch, 1977 is a dominant species of diplopods in deciduous forests of the Western part of Caucasus ridge. Nearly each specimen of this diplopods (in early summer prevalence > 95%) is infected with intestinal thelastomatid nematodes of the genus *Severianoia* Schwenk, 1926. A much lower percentage of these hosts (<10%) is infected with the nematomorph parasite *Gordionus alpestris*. Two populations of diplopod hosts separated by torrent river Belaya were studied. As a rule, in spring and early summer the hind gut of *P. krivolutskyi* contained mature females with eggs, and at the end of vegetation season the number of mature female nematodes decreased. Although in June the mean number of females per diplopod was  $3.5 \pm 3.9$  (0-16), in September on the same site it was  $1.1 \pm 1.8$  (0-8). *Severianoia* sp. males were found only in diplopods on a single site (Gruzinka river), when 89 dissected diplopods from another site (Sibirka river) contained only females and juveniles. The morphology of *Severianoia* sp. is typical for thelastomatids: females with short tubular buccal cavity, males with four pairs of genital papillae and a single rod-like spicula. Quite characteristic for the genus are elongated eggshells with several undulating ridges along the shell. Partial LSU rDNA sequences were obtained for four *Severianoia* specimens (three females, one male) collected in 2016 and 2017. All these were identical. In phylogenetic analysis the LSU sequence of *Severianoia* sp. clustered together with basal groups of Thelastomatidae not entering the subclade consisting of *Thelastoma*+*Desmicola*+*Cordonicola*. Under some methods of analysis, *Severianoia* sp. demonstrates the phyletic relationships with such specialised forms of Thelastomatidae as *Travassosinema* from tropical diplopods, *Cameronia* from mole-cricket and *Hystrignathus* from Passalidae beetles.

**WRKYs transcription factors regulating *Solanum lycopersicum* response to root knot nematodes: transcriptomic and metabolomics insights**

Chinnapandi Bharathiraja<sup>1</sup>, Patricia Bucki<sup>1</sup>, Natalia Foitoussi<sup>1,2</sup>, Michael Kolomiets<sup>3</sup>, Eli Borego<sup>4</sup> and Braun Miyara Sigal<sup>1</sup>

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WRKY web of plant defence regulators is implicated in transcriptional reprogramming during plant immune responses. In this study the fluctuation of tomato's WRKYs during infection by the root-knot nematode (RKN), *Meloidogyne javanica*, was analysed, where several upregulated WRKYs genes, among them *SIWRKY3*, *SIWRKY35*, *SIWRKY45* and *SIWRKY72*, were studied in-depth in the context of spatial and temporal expression upon nematode infection, responsive to phytohormone to wounding treatments and functional analysis. Promoter GUS analysis of these WRKYs genes indicate on their involvement during RKN infection with differential expression among the studied WRKYs along feeding site formation, maturation and galls development. Histological analysis of nematode-feeding sites indicated that expression of each studied WRKY TF is associated with feeding site development and formation. Studying the response of *SIWRKYs* promoter to several phytohormones illustrated, that while *SIWRKY45* is highly induced by specific phytohormones, including cytokinin (*N*<sup>6</sup>-Benzyladenine, BAP), auxin (Indole-3 acetic acid, IAA and Indole-3-butyric acid, IBA) and the defence signalling molecules salicylic acid (SA), both *SIWRKY3* and *SIWRKY35* are induced only by SA and IBA. To determine the biological function of *WRKY3* and *WRKY45*, overexpressing tomato lines were generated. Infection tests illustrate that significantly, in roots over expressing *SIWRKY45* substantially increased number of females was measured, indicating *WRKY45* overexpression support a faster nematode development. On the other hand, overexpressing *SIWRKY3* resulted in lower infection of the RKN *M. javanica*, indicating its function as defence signalling regulator. Hormone and oxylipins profile conducted by LC-MS analysis have shown that enhanced resistance observed in tomato roots overexpressing *SIWRKY3* is coupled with increased accumulation of defence molecules parts of the shikimate and oxylipins pathways. Altogether, this comprehensive study shows *SIWRKYs* as a potential transcription factor, which its manipulation by the invading nematode might be critical for coordination of hormone signal genes generating favourable conditions for nematode development in root tissue.

## **A review on taxonomy and phylogeny of Tylaphelenchinae (Aphelenchoidea)**

Zeinab Mirzaie Fouladvand<sup>1</sup>, Natsumi Kanzaki<sup>2</sup>, Robin M. Giblin-Davis<sup>3</sup>, Ebrahim Pourjam<sup>1</sup> and Majid Pedram<sup>1</sup>

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A newly established genus, *Basilaphelenchus* was recently added to the subfamily Tylaphelenchinae. It is characterised by having the distinct apomorphic feature of a more posteriorly located valve in the metacarpus at 72-75%. It is further characterised by having a raised cephalic region, sclerotised vestibule, fine stylet having three elongate posteriorly directed small knobs, three lines in the lateral field, and males lacking the P1 papilla and bursa. It is currently monotypic, but a newly discovered member is being proposed in the present study. The new species can be separated from the type species mainly by its shorter tail and distinct position in both SSU and LSU rDNA phylogenies. In addition to the placement of this genus under Tylaphelenchinae with the genus *Tylaphelenchus*, the genus *Albiziaphelenchus* was also transferred into the subfamily, and new taxonomic placements for three species *Tylaphelenchus grosmanae*, *T. christinae* and *T. sinodendroni* (= *Aphelenchoides sinodendroni*) are proposed. The phylogeny of *Basilaphelenchus* is also discussed in detail using different markers and different methods of molecular phylogenetic inference.

[Tuesday, 11th September](#)

## Plenary session

[Metabarcoding as a tool for monitoring free-living marine nematode communities: can we reach the species level?](#)

Sofie Derycke

[Progress and current challenges in our understanding of the soybean cyst nematode-soybean interaction](#)

Melissa G. Mitchum

## Sessions

### [S10. Nematode parasitism genes](#)

Convenors: Sebastian Eves-van den Akker & John Jones

### [S11. The beet cyst nematode \(Sponsored by Sesevanderhave\)](#)

Convenors: Matthias Daub & Olivier Amand

### [S12. EPN Genomics and breeding](#)

Convenors: Johnathan Dalzell & Carlos Molina

### [S13. Nematode parasitism genes](#)

Convenors: John Jones & Sebastian Eves-van den Akker

### [S14. Systematics and Phylogeography](#)

Convenors: Sergei Subbotin & Wim Bert

### [S15. Model organisms](#)

Convenors: Robbie Rae & Dave Lunt

### [S16. Plant-parasitic nematode biology](#)

Convenors: Etienne Danchin & Catherine Lilley

### [S17. Bioindicators and nematode communities](#)

Convenors: Roy Nielsen & Lieven Waeyenberge

### [S18. Techniques and training](#)

Convenors: Awol Seid





Tuesday, 11 September

[Metabarcoding as a tool for monitoring free-living marine nematode communities: can we reach the species level?](#)

*Sofie Derycke<sup>1,2,3</sup>, Lara Macheriotou<sup>2</sup>, Bjorn Tytgat<sup>2</sup>, Tara Grosemans<sup>1,2</sup>, Ann Vanreusel<sup>2</sup>*

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Species specific responses to environmental stressors and limited dispersal capacity are two important characteristics that make free-living nematode species suitable bioindicators. Although morphological identification of nematodes is too cumbersome for routine monitoring programs, this tedious process may be circumvented using next generation sequencing. Promising studies have shown the usefulness of metabarcoding for meiofauna diversity research at higher taxon level, yet diversity assessments with species-level resolution remain one of the most challenging aspects of metabarcoding. During this talk, the promises and pitfalls of the metabarcoding approach for marine nematodes will be discussed using empirical datasets with mock communities and environmental samples. The resolution of different marker genes (18S and COI), of different bioinformatics pipelines (OTU clustering and sequence variants) and of different taxonomic databases are important considerations when applying metabarcoding for monitoring studies. Equally important is the availability of a high quality reference database with species level taxonomic identification. For the environmental samples, OTU clustering and sequence variant pipelines of the 18S data showed considerable overlap in the number of species detected. In contrast, little overlap between species was found when comparing 18S, COI and morphological data but many species unique to the morphological analysis had congeneric species identified using metabarcode data. Our results further show that metabarcoding data separated impacted areas from control areas in a similar way as morphological data, thereby suggesting that metabarcode data can be used in monitoring studies. Finally, we are also exploring the use of primer-free approaches for nematode community characterization.

Tuesday, 11 September

[Progress and Current Challenges in our Understanding of the Soybean Cyst Nematode-Soybean Interaction](#)

Melissa G. Mitchum<sup>1</sup>

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*Heterodera glycines*, the soybean cyst nematode (SCN) is a major economic threat to soybeans worldwide. SCN management relies heavily on the deployment of resistant soybean cultivars. The roots of plants carrying *Rhg* (for resistance to *Heterodera glycines*) genes are penetrated by infective juveniles, but feeding cells induced by these nematodes to serve as their sole nutritive source degenerate prematurely. The recently discovered identities of several *Rhg* genes in soybean to this nematode reveal that a novel mechanism of plant resistance may be at play. However, the widespread and repeated use of the same type of resistance has selected for virulent nematodes that can reproduce on these resistant cultivars. A multi-pronged approach targeted at both the host and nematode using a combination of omics tools and technologies is underway to develop a mechanistic understanding of plant resistance and nematode virulence, as well as identify ways to both enhance the effectiveness and durability of natural plant resistance and enable the design of novel resistance strategies.

## S10. Nematode parasitism genes

Convenors: Sebastian Eves-van den Akker & John Jones

### **Core effectors of syncytium-forming nematodes**

Kerry Leslie<sup>1</sup>, Sophie Mantelin<sup>2</sup>, John T Jones<sup>1</sup> and Sebastian Eves-van den Akker<sup>3</sup>

<sup>1</sup>*School of Biology, University of St Andrews, North Haugh, St Andrews KY16 9TZ, UK;* <sup>2</sup>*The James Hutton Institute, Dundee DD2 5DA, UK;* <sup>3</sup>*Department of Plant Sciences, University of Cambridge, Cambridge, CB2 3EA, UK.* [kerry.leslie@hutton.ac.uk](mailto:kerry.leslie@hutton.ac.uk)

Plant-parasitic nematodes infect many major food crops worldwide, causing damage globally valued at approximately US\$ 80 billion per year. Some nematodes form a feeding site called a syncytium in the roots of their host. Relatively little is known about how nematodes initiate and maintain these feeding structures. However, specialised proteins and small molecules, known as effectors, secreted into the plant by the pathogen, are thought to play critical roles in these processes. Firstly, a set of novel GH53  $\beta$ -1, 4-galactanase effectors, which may assist in invasion of the host and migration through root tissue, have been identified in *Globodera rostochiensis*, *G. pallida* and *Rotylenchulus reniformis*. These genes have likely been acquired through a horizontal gene transfer event, which has given these nematodes the ability to break down arabinogalactans in dicotyledonous host plant cell walls. Functional characterisation of these effectors will give a greater insight into the invasion process and the co-evolution between the nematode and its host plant. It is equally important to establish a greater understanding of the role of effectors in syncytium formation and maintenance as well as host invasion by the nematode. This project also aims to identify and characterise a subset of core effectors conserved in syncytia-forming nematode species but absent from other nematodes. Exploiting the genomic and transcriptomic data available for four syncytium-forming species, candidate core effectors have been identified using an *in silico* approach. Three of the candidates have been validated as effectors using *in situ* hybridisation to confirm expression in the oesophageal gland cells. Further functional characterisation using *in planta* localisation, silencing by RNAi and yeast two-hybrid analysis for host target identification are undertaken.

Tuesday, 11 September

## **Effector gene birth in plant parasitic nematodes: neofunctionalization of a housekeeping glutathione synthetase gene**

Catherine J. Lilley<sup>1</sup>, Abbas Maqbool<sup>2</sup>, Duqing Wu<sup>1</sup>, Hazijah B. Yusup<sup>1</sup>, Laura M. Jones<sup>1</sup>, Paul R. J. Birch<sup>3,4</sup>, Mark J. Banfield<sup>2</sup>, Peter E. Urwin<sup>1</sup> and Sebastian Eves-van den Akker<sup>5</sup>

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Plant pathogens and parasites are a major threat to global food security. Plant parasitism has arisen four times independently within the phylum Nematoda, resulting in at least one parasite of every major food crop in the world. Some species within the most economically important order (Tylenchida) secrete proteins termed effectors into their host during infection to re-programme host development and immunity. The precise detail of how nematodes evolve new effectors is not clear. Here we reconstruct the evolutionary history of a novel effector gene family. We show that during the evolution of plant parasitism in the Tylenchida, the housekeeping glutathione synthetase (GS) gene was extensively replicated. New GS paralogues acquired multiple dorsal gland promoter elements (DOG boxes), altered spatial expression to the secretory dorsal gland, altered temporal expression to primarily parasitic stages, and gained a signal peptide for secretion. The gene products are delivered into the host plant cell during infection, giving rise to 'GS-like effectors'. By solving the structure of GS-like effectors we show that during this process they have also diversified in biochemical activity, and likely represent the founding members of a novel class of GS-like enzyme. To the best of our knowledge, these are the first three crystal structures of any kind for a plant-parasitic nematode. Our results demonstrate the re-purposing of an endogenous housekeeping gene to form a family of effectors with modified functions. We anticipate that our discovery will be a blueprint to understand the evolution of other plant-parasitic nematode effectors, and the foundation to uncover a novel enzymatic function.

Tuesday, 11 September

**The potato cyst nematode (*Globodera pallida*) effector RHA1B is a ubiquitin ligase and uses two distinct mechanisms to suppress plant immune signalling**

Joanna Kud<sup>1</sup>, Wenjie Wang<sup>1</sup>, Youhong Fan<sup>1</sup>, Aida Duarte<sup>1</sup>, Rachel Gross<sup>1</sup>, Li Huang<sup>1</sup>, Yulin Yuan<sup>1</sup>, Aska Goverse<sup>2</sup>, Louise-Marie Dandurand, Fangming Xiao<sup>2</sup> [aska.goverse@wur.nl](mailto:aska.goverse@wur.nl)

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Plant pathogens, such as bacteria, fungi, oomycetes and nematodes, rely on wide range of virulent effectors delivered into host cells to suppress plant immunity. Although phyto-bacterial effectors have been intensively investigated, little is known about the function of effectors of plant-parasitic nematodes, such as *Globodera pallida*, a cyst nematode responsible for vast losses in the potato and tomato industries. In this study, we demonstrate that a novel *G. pallida* effector, RHA1B, is an E3 ubiquitin ligase that uses multiple host plant E2 ubiquitin conjugation enzymes for catalysation of ubiquitination. RHA1B was able to interfere with effector-triggered immunity (ETI), as manifested by suppression of the hypersensitive response (HR) mediated by multiple nucleotide-binding leucine-rich repeat (NB-LRR) immune receptors, presumably *via* E3-dependent degradation of the NB-LRR receptors. RHA1B was also able to block the flg22-triggered expression of *NbAcre31*, a marker gene of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), but this did not require the E3 activity of RHA1B. Moreover, transgenic potato plants overexpressing the *RHA1B* transgene exhibited enhanced susceptibility to *G. pallida*. Thus, RHA1B is a novel E3-possessing effector that facilitates nematode parasitism not only by triggering degradation of NB-LRR immune receptors to block ETI signalling but also by suppressing PTI signalling *via* an as yet unknown E3-independent mechanism.

Tuesday, 11 September

**Identification of parasitism effectors expressed during plant infection from the transcriptomes of *Meloidogyne incognita* and *M. enterolobii***

Chinh-Nghia Nguyen<sup>1</sup>, Abdelnaser Elashry<sup>2</sup>, Laetitia Perfus-Barbeoch<sup>1</sup>, Andrea Braun-Kiewnick<sup>2,3</sup>, Michaël Quentin<sup>1</sup>, Martine Da Rocha<sup>1</sup>, Pierre Abad<sup>1</sup>, Sebastian Kiewnick<sup>2,3</sup> and Bruno Favery<sup>1</sup>

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Root-knot nematodes (RKN) are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks. They infect roots as microscopic vermiform second-stage juveniles (J2), hatched from eggs in the soil. J2 penetrate the root apex and migrate between cells to reach the plant vascular cylinder. To develop further and moult into a pear-shaped female that will release hundreds of eggs on the root surface in a protective gelatinous matrix, J2 need to successfully establish and maintain specialised feeding structures called 'giant-cells' from which they withdraw water and nutrients allowing their sedentary biotrophic lifestyle. Our project aims to identify RKN genes involved in plant parasitism with an emphasis on genes encoding new secreted effectors of parasitism. Using Illumina RNA-seq technologies, we compared transcriptomes of *Meloidogyne incognita* during its life cycle and identified genes over-expressed in early parasitic stages as compared to pre-parasitic juveniles, eggs, females and males. Once the over-expression of selected genes in parasitic stages was confirmed by RT-qPCR, *in situ* hybridisations were carried out to localise the candidates in the nematode secretion organs. Furthermore, siRNA soaking was used to silence these genes and study their role in pathogenicity. In parallel, we are also comparing the transcriptomes of *M. incognita* with those of another RKN species, *M. enterolobii*, that reproduces by obligatory parthenogenesis. This nematode represents a new threat for the agriculture worldwide because of its ability to reproduce on the majority of known RKN-resistant plants. These transcriptomic analyses provide new insights into the development and host-parasite interactions of these plant-pathogens and highlight differences and similarities between RKN species.

**Molecular characterisation of two new venom allergen-like proteins and parasitism functional analysis of *Heterodera avenae***

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*Heterodera avenae* (cereal cyst nematode, CCN) is a species of sedentary endoparasitic nematode. Venom allergen-like proteins (VAPs) are a type of cysteine-rich secretory proteins that belong to the Sperm Coating Protein/Tpx-1/Ag-5/Pr-1/Sc-7 (SCP/TAPS) superfamily. VAPs have been isolated from plant- and animal-parasitic nematodes. In this paper, we isolated and cloned two VAP-coding sequences, *HaVAP1* and *HaVAP2*, from *H. avenae*. The two encoded proteins, HaVAP1 and HaVAP2, harbour SCP-like domains but share only 38% identity with each other. Both *HaVAP1* and *HaVAP2* are expressed and accumulated in subventral pharyngeal gland cells. *HaVAP1* is expressed mainly at the early stages, whereas *HaVAP2* accumulated principally at the late stages. Both HaVAP1 and HaVAP2 are secreted into extracellular space, but HaVAP1 is delivered into chloroplasts, whilst HaVAP2 is translocated to the nucleus without signal peptides. Knocking-down the expression of *HaVAP1* increased the virulence of *H. avenae*. By contrast, gene silencing of *HaVAP2* hampered the development and generation of *H. avenae*. Both HaVAP1 and HaVAP2 can suppress the cell death induced by BAX in *Nicotiana benthamiana* leaves. Moreover, HaVAP2 physically interacts with a CYP4-like protein (HvCLP) of *Hordeum vulgare* in nucleus. All the obtained results in this study showed that both HaVAP1 and HaVAP2 play important roles in the parasitism of *H. avenae*, but they possess different functions, broadening our understanding about the parasitism mechanism of *H. avenae*.

**S11. The beet cyst nematode. Sponsored by Sesvanderhave**

Convenors: Matthias Daub & Olivier Amand

***Bacillus firmus I-1582* protects plants from *Heterodera schachtii***

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Plant-parasitic nematodes are a serious threat to agricultural crop production worldwide as nematodes cause high yield losses by root parasitism. A strategy to combat nematode parasitism is to utilise antagonistic microbes. One organism in this respect is the Gram-positive rhizobacterium *Bacillus firmus I-1582* which is promoted as a biological nematode control agent. Although *B. firmus* is a known nematode antagonist in general, details about its interaction with nematodes and plants are rare. In order to investigate the impact of *B. firmus I-1582* and its secreted molecules on plant-nematode interaction, a sophisticated agar-based gnotobiotic system was adopted to meet the requirements of the involved organisms. We demonstrate that *B. firmus I-1582* is attracted by *Arabidopsis thaliana* root exudates, particularly by those of young plants. The bacterium colonises the root and develops there in a strictly pH-dependent manner. *Bacillus firmus I-1582* as well as cell-free bacterial secretions stimulate lateral root and root hair growth, thus altering root morphology. Root colonisation by *B. firmus I-1582* significantly protects the plant from infection by the nematode species *H. schachtii*. Interestingly, contact of the root with the cell-free bacterial culture supernatant does not influence nematode parasitism. We therefore conclude that the living bacterium must be present at the root for efficient nematode control. Currently, we study the mechanism(s) leading to the observed results.



**Assessing commercial varieties of sugar beet for tolerance and resistance to *Heterodera schachtii***

Alistair Wright<sup>1</sup>, Mark Stevens<sup>2</sup>, Matthew Back<sup>3</sup> and Debbie Sparkes<sup>1</sup>

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The beet cyst nematode (BCN), *Heterodera schachtii*, is an economically important pest of sugar beet amongst other crop species. It commonly occurs in almost every region of the world where sugar beet is cultivated. Yield losses can reach as high as 60% and the nematode can remain a viable threat for 10 years. Losses attributed to BCN damage have been valued at over £3 million every year in the UK. Research on sugar beet varieties that are tolerant to BCN has been conducted in order to quantify their effect on population multiplication as well as investigate any physiological responses to infestation which may explain the mechanisms behind tolerance. In order to do this, trials were established using large plastic boxes (600 l) filled with top soil and then planted with 16 plants of different varieties of sugar beet that were susceptible, resistant or tolerant to BCN. Half of these boxes were then inoculated using hatched BCN second-stage juveniles (J2), which were pipetted around the sugar beet plants (approximately 2600 J2 per plant in 2016 and 5800 J2 per plant in 2017). The sugar beet plants were grown until maturity, upon which they were harvested and assessed for sugar yield and quality. Whilst the sugar beet grew, differences between the varieties tested were measured in stomatal conductance, chlorophyll density and yield quality and there were significant responses to BCN infestation in some varieties. Population densities of BCN were determined from the soil in each experimental unit (box) two weeks after harvest of the roots. The results indicated a much lower multiplication of BCN populations in response to tolerant varieties than susceptible varieties indicating that they may be partially resistant. It is hoped that this experimental approach can form the basis of a screening technique for sugar beet and assist variety selection for growers.

Tuesday, 11 September

## **Multiplication of *Heterodera schachtii* on cover crops and mixtures of cover crops**

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*Heterodera schachtii*, the white beet cyst nematode (BCN), is the most important plant-parasitic nematode in European sugar beet (*Beta vulgaris*) cultivation. This nematode causes significant reductions in sugar yield. An important measure to control this nematode is the use of resistant cover crops, like oil seed radish (*Raphanus sativus* subsp. *oleiferus*) or white mustard (*Sinapis alba*). For a few years the Common European Agricultural Policy (CAP) has stimulated the use of mixtures of cover crops within the Ecological Focus Areas (EFAs). Farmers can choose out of 32 different cover crops. However, host status is not known for all these different crops, which may result in higher infestation levels of BCN and thereby lower sugar yields. Therefore, climate room trials were conducted to investigate the host status of the cover crops and to investigate the effect of mixtures of a very good host, a bad host crop and a non-host crop on the multiplication of BCN. Our results showed that Ethiopian mustard (*Brassica carinata*) and brown mustard (*B. juncae*) are very good hosts plants for BCN, soybean (*Glycine max*), camelina (*Camelina sativa*), eruca (*Eruca sativa* 'Nemat'), buckwheat (*Fagopyrum esculentum*) are moderate host crops and Egyptian clover (*Trifolium alexandrinum*), niger (*Guizotia abyssinica*), Italian clover (*T. incarnatum*), flax (*Linum usitatissimum*), borage (*Borago officinalis*) and eruca 'Trio' and 'S010' are bad host crops. Furthermore, it was shown that the multiplication of BCN on plants within a mixture not only depends on the different crops, but also on the interactions between these crops. Therefore, it is difficult for farmers to predict multiplication of BCN on cover crop mixtures. The present study does not only give a new insight in host status of cover crops to BCN, but also gives a new perception on the interaction of different crops on the multiplication of nematodes.

Tuesday, 11 September

## **Relevance of *Heterodera schachtii* in the subsoil for sugar beet crops - A three-year field trial following a soil layer specific approach**

Matthias Daub

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The established prediction model on damage thresholds of beet cyst nematodes (BCN), *Heterodera schachtii*, on sugar beets refers to the initial population density (Pi) which usually is detected in soil samples from the top soil prior to the sugar beet crop. In many fields throughout the major sugar beet growing area in central Europe BCN also occurs in soil layers below the top soil and sometimes in considerable densities. Results from experiments using PCV tubes with systematic inclusion or exclusion of BCN are difficult to transfer to the field situation. Hence a three year field trial to investigate the actual damage relevance of BCN in the subsoil was conducted using a sensitive and susceptible standard cultivar. Yield relevant Pi - densities of BCN were adjusted in the topsoil and subsoil independently. A wide range of 13 - 6500 eggs and juveniles (euj) 100 ml<sup>-1</sup> in both layers were achieved by growing of BCN susceptible and resistant oilseed radish cultivars as a pre-crop before sugar beets. After systematic exclusion of yield data from plots with Pi-densities exceeding the known threshold densities of 500 euj 100 ml<sup>-1</sup> in the top soil, no yield effect could be detected in relation to Pi-densities in the sub soil in any year. Damage effects with yield penalties of 2-3 t of white sugar ha<sup>-1</sup> (about 20%) were significant exclusively in relation to the BCN population in the top soil showing distinctive year effects. Multiplication rate of BCN between top and subsoil was distinctively different in relation to the respective Pi densities and annual precipitation. Damage evidently occurred at Pi densities above 500 euj 100 ml<sup>-1</sup>. Thus, results of the field trials could validate the classical damage prediction model for *H. schachtii* in sugar beet crops.

Tuesday, 11 September

**Populations of the beet cyst nematode, *Heterodera schachtii*, exhibit strong differences in their life-history traits across changing thermal conditions**

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It is widely accepted that climate has an essential influence on the distribution of species and that temperature is the major abiotic factor that affects their life-history traits. Species with very restricted dispersal capability associated with a wide geographical distribution are thus expected to encompass several distinct populations adapted to contrasting local conditions. From this point of view, the beet cyst nematode, *Heterodera schachtii*, is a good biological model to study the temperature adaptation of populations coming from different environments. In this study, we have tested that hypothesis using seven field populations of *H. schachtii*, coming from Morocco, Spain, France, Germany, Austria, Poland and Ukraine. On one hand, the hatching and the multiplication of each population were tested at different temperatures, and on the other hand, the hatching of each population was tested after a storage period at different temperatures simulating survival conditions during the intercropping period. Results showed a strong temperature effect on the different explored life-history traits. Regarding hatching (at different temperature and after a storage period at different temperature), this effect was different depending on the origin of populations, separating southern populations from northern populations. Surprisingly, low temperatures influenced hatching of southern populations less. However, for these populations, a storage period at low temperatures strongly affect subsequent hatching. Conversely, our results demonstrate that the nematode multiplication was not differentially affected by temperatures, as favourable conditions for the host are also favourable for the parasite. Moreover, the relationship between the level of specialisation and the genetic diversity was explored, enabling more in depth discussion of our results.

## **S12. EPN Genomics and breeding**

Convenors: Johnathan Dalzell & Carlos Molina

### **B03\_ The effect of *Photorhabdus* (p)ppGpp on *Heterorhabditis* growth and development**

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Bacteria frequently form intimate associations with a eukaryotic host where they may have critical roles in the development, behavior, and/or functions of their animal symbiont. The entomopathogenic nematode (EPN) *Heterorhabditis* is a soil-dwelling organism that utilizes a bacterial symbiont, *Photorhabdus*, to kill its insect targets. *Photorhabdus* cells are carried as symbionts in the gut of the *Heterorhabditis* infective juvenile (IJ), a non-feeding stage and the only free-living stage in the nematode life cycle. Upon entering a suitable insect host the IJ releases its symbiotic bacteria. *Photorhabdus* grows rapidly causing insect death through septicaemia while the IJ recovers, grows, develops, and reproduces by feeding on the high-density of bacterial symbionts in the dead insect. After nutrients are depleted new IJs will emerge from the insect cadaver to search out new insect hosts. In response to nutritional stress bacteria produce the global regulators guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) known together as (p)ppGpp. In *Photorhabdus* (p)ppGpp affects phenotypes such as pigmentation, antibiotic production, luminescence, motility, and fitness inside the insect host. (p)ppGpp in *Photorhabdus* is also essential for the nematode life cycle; however, little is known about which nematode life history traits are affected. In this study, we have demonstrated that (p)ppGpp is important for nematode reproduction, IJ recovery, and body size. Additionally, we used transcriptomics to identify genes that are regulated by (p)ppGpp. We discovered genes that are known to be involved in antibiotic production and nematode development while identifying other potential key symbiotic genes. We selected the Cpx regulon, a two-component pathway, for further study. We investigated the role of the Cpx regulon in various phenotypes that are affected by (p)ppGpp including the symbiotic relationship between *Photorhabdus* and *Heterorhabditis*. Ongoing work will continue to use transcriptomics to elucidate the role of *Photorhabdus* in the growth and development of *Heterorhabditis*.

**Genetic improvement of the oxidative stress tolerance and longevity of the entomopathogenic nematode *Heterorhabditis bacteriophora***

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The entomopathogenic nematode–bacterium complex *Heterorhabditis bacteriophora-Photorhabdus luminescens* is one of the most widespread biological control agents against diverse insect pests. However, poor persistence in the soil, poor product shelf-life and loss of virulence during storage are the major constraints of this entomopathogenic nematode (EPN) species. An in depth understanding of environmental stress factors (e.g., desiccation, high temperature, UV-radiation, hypoxia, oxidative stress) limiting dauer juvenile (DJ) survival and longevity can lead to the identification of better nematode strains. A more targeted approach exploits classical and molecular genetics, making use of the diversity among natural populations for improvement of EPN beneficial traits. In this study, we aimed at the genetic improvement of oxidative stress tolerance and DJ longevity of *H. bacteriophora*. We first screened a total of 40 *H. bacteriophora* wild type strains, inbred and hybrid lines of different geographic origin for their tolerance to oxidative stress and longevity at 25 and 7°C. We determined a high variability among strains of this species and a high heritability for tolerance to oxidative stress ( $h^2 > 0.9$ ). Oxidative stress tolerance was found to have strong positive correlation with DJ longevity. Thus, the oxidative stress can be used as a predictor for DJ longevity, permitting a selection process within a shorter testing period. To enhance further our nematode strains, both cross-breeding and EMS-mutagenesis were carried out. The resulting hybrid (AU1xHU2) obtained a higher tolerance to oxidative stress and longevity compared to their parental lines. In addition, the generated EMS-mutant inbred line (MOX-1L6) had an increased oxidative stress tolerance of about 5 days compared to the non-mutagenised donor line (IL3). The commercial line EN01 had a lower tolerance to stress and a shorter longevity in water. To elucidate the underlying mechanisms in response to oxidative stress, a comparative transcriptomic analysis on the early stage of oxidative stress induction (4 h) in two inbred lines with contrasting stress-tolerance, HU2-IL1 (stress-tolerant) and PT1-IL1 (stress-sensitive) was performed. High throughput molecular approaches have generated the expression from more than 22,000 transcripts in DJ with contrasting longevity during the early stages of oxidative stress. PCR-based KASP markers were derived from relevant transcripts and were tested in natural *H. bacteriophora* materials. Significant correlation between genotype and phenotype was observed for a subset of KASP markers. Further analysis will allow the identification of molecular markers and facilitate breeding for improved lines. Nematode strains with prolonged longevity can provide a longer persistence in the soil after application and thus open a door in larger scale markets, such as for the control of *Diabrotica virgifera virgifera* in maize.

Tuesday, 11 September

### **Genetic improvement of virulence and persistence of *Heterorhabditis bacteriophora***

Bart Vandenbossche, Érika Consoli, Alejandra Centurion, Sitaram Ayril, Mike Barg, Verena Dörfler, Nanette Hope Nellas Sumaya, Olaf Strauch, Carlos Molina and Ralf-Udo Ehlers

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The entomopathogenic nematode, *Heterorhabditis bacteriophora*, is a promising biological control agent of the Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera*, which is one of the most damaging maize pests worldwide. Most European farmers relied on the application of granular soil insecticides and/or chemical seed treatments for several years to control this pest. However, serious sub-lethal side effects on honeybees and other beneficial antagonists, including soil organisms stressed the need for the development of alternative control options. Biocontrol nematodes can close the gap. They are applied into the soil together with the maize seeds at sowing time, when *Diabrotica* eggs are still in diapause. In order to increase control results a genetic improvement of survival and virulence was performed. Wild-type strains were phenotypically characterised for virulence with WCR larvae and larvae of *Tenebrio molitor*. Nematode persistence was analysed by subsequent baiting the soil using *T. molitor* as the trap insect. Results of persistence correlate with longevity and oxidative stress resistance. A wild type strain with best performance in virulence and persistence was selected over several cycles in sand bio-assays with WCR larvae and resulted in an improved performance of the selected strain, thus justifying a reduction of the application rate from currently 2 to 1 billion DJ ha<sup>-1</sup>. The results support the hypothesis that the use of more persistent and virulent strains is likely to enable the reduction of the application dosage and consequently reduce nematode application costs. The outcome of this investigation further documents the potential of breeding programmes for improvement of virulence and persistence of *H. bacteriophora* for use against *D. v. virgifera* and other target pests.

Tuesday, 11 September

**Genetic markers for selection of stress resistant lines of the entomopathogenic nematode *Heterorhabditis bacteriophora***

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The use of the entomopathogenic nematode (EPN), *Heterorhabditis bacteriophora*, in large scale agriculture is often limited by environmental stresses. Breeding for nematode strains with enhanced stress resistance is thus a strategy aimed to make the use of EPN more affordable, *e.g.*, against the corn root borer *Diabrotica v. virgifera*. Here we report on approaches to enhance the longevity and desiccation tolerance of this EPN. In the framework of the BIOCOTES EU project, academic and industrial partners have joined efforts to study in depth the genetic basis of environmental stress-resistance in *H. bacteriophora* dauer juveniles (DJ). Among the major outcomes of this research, a significant correlation between oxidative stress tolerance and survival in storage conditions has been found. A collection of *H. bacteriophora* wild type strains was characterised for their shelf life under oxidative stress and this property has been proposed as predictor to select for longer living nematodes. Hybrid strains and EMS-mutants with extended survival time have been tested for their general performance with satisfactory results. Genomic tools have been applied to gain insights into the mechanisms of DJ-longevity and desiccation stress tolerance in *H. bacteriophora*. Sequence information has been generated comprising of the expression of more than 20,000 different transcripts under infective- and stress-conditions. At least eleven highly-informative desiccation and DJ-longevity expression markers have been identified and validated under oxidative stress, chemical- and hygroscopic-desiccation conditions. In parallel, genotyping by sequencing (GBS) has identified more than 700 reproducible single nucleotide polymorphism (SNPs) for this species. Joining genotype and phenotype information by QTL- and association-analysis has subsequently allowed us to find molecular markers linked to DJ-longevity in *H. bacteriophora*. These markers are being applied in breeding nematode lines with enhanced performance and could be applied in selection of new native lines.



Tuesday, 11 September

## **Transcriptional variation and divergence of host-finding behaviour in *Steinernema carpocapsae* infective juveniles**

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*Steinernema carpocapsae* is an entomopathogenic nematode that employs nictation and jumping behaviours to find host insects. We aimed to investigate the transcriptional basis of variant host-finding behaviours in the infective juvenile (IJ) stage of three *S. carpocapsae* strains (ALL, Breton and UK1). RNA-seq analysis revealed that whilst up to 28% of the *S. carpocapsae* transcriptome was differentially expressed ( $P < 0.0001$ ) between strains, remarkably few of the most highly differentially expressed genes ( $> 2 \log_2$  fold change,  $P < 0.0001$ ) were from neuronal gene families. *Steinernema carpocapsae* Breton displays increased chemotaxis toward the laboratory host *Galleria mellonella*, relative to the other strains. This correlates with the up-regulation of four *srsx* chemosensory GPCR genes, and a sodium transporter gene, *asic-2*, relative to both ALL and UK1 strains. The UK1 strain exhibits a decreased nictation phenotype relative to ALL and Breton strains, which correlates with coordinate up-regulation of neuropeptide like protein 36 (*nlp-36*), and down-regulation of an *srt* family GPCR gene, and a distinct *asic-2*-like sodium channel paralogue. To investigate further the link between transcriptional regulation and behavioural variation, we sequenced microRNAs across IJs of each strain. We have identified 283 high confidence microRNAs, yielding 321 isomiR variants in *S. carpocapsae*, and find that up to 36% of microRNAs are differentially expressed ( $P < 0.0001$ ) between strains. Many of the most highly differentially expressed microRNAs ( $> 2 \log_2$  fold,  $P < 0.0001$ ) are predicted to regulate a variety of neuronal genes that may contribute to variant host-finding behaviours. We have also found evidence for differential gene isoform usage between strains, which alters predicted microRNA interactions, and could contribute to the diversification of behaviour. These data provide deeper insight to the transcriptional landscape of behavioural variation in *S. carpocapsae*, underpinning efforts for functional dissection of the parasite host-finding apparatus.

### S13. Nematode parasitism genes

Convenors: John Jones & Sebastian Eves-van den Akker

#### **Pulling a few strings to facilitate parasitism: *Globodera pallida* is CLASping microtubules**

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The white potato cyst nematode, *Globodera pallida*, is an obligate biotrophic pathogen of a limited number of Solanaceous plants. Like other plant pathogens, *G. pallida* deploys effectors into its host that manipulate the plant to the benefit of the nematode. Genome analysis has led to the identification of large numbers of candidate effectors from this nematode, including the cyst nematode-specific SPRYSEC proteins. These are a secreted subset of a hugely expanded gene family encoding SPRY domain-containing proteins, many of which remain to be characterised. We investigated the function of one of these SPRYSEC effector candidates, *GpSPRY-414-2*. Expression of the gene encoding *GpSPRY-414-2* is restricted to the dorsal pharyngeal gland cell and reducing its expression in *G. pallida* infective second-stage juveniles using RNA interference causes a reduction in parasitic success on potato. Transient expression assays in *Nicotiana benthamiana* indicated that *GpSPRY-414-2* disrupts plant defences. It specifically suppresses effector-triggered immunity (ETI) induced by co-expression of the *Gpa2* resistance gene and its cognate avirulence factor *RBP-1*. It also causes a reduction in the production of reactive oxygen species triggered by exposure of plants to the bacterial flagellin epitope flg22. Yeast two-hybrid screening identified a potato cytoplasmic linker protein (CLIP)-associated protein (*StCLASP*) as a host target of *GpSPRY-414-2*. The two proteins co-localise *in planta* at the microtubules. CLASPs are members of a conserved class of microtubule-associated proteins that contribute to microtubule stability and growth. However, disruption of the microtubule network does not prevent suppression of ETI by *GpSPRY-414-2* nor the interaction of the effector with its host target. Besides, *GpSPRY-414-2* stabilises its target while effector dimerisation and the formation of high molecular weight protein complexes including *GpSPRY-414-2* are prompted in the presence of the *StCLASP*. These data indicate that the nematode effector *GpSPRY-414-2* targets the microtubules to facilitate infection.

**Identification and characterisation of a novel glutathione synthetase gene family in *Rotylenchulus reniformis***

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Glutathione is one of the major antioxidant molecules found in almost all prokaryotic and eukaryotic cells and plays essential roles in many cellular reactions. Glutathione synthetase (GS) is the key ATP-dependent enzyme that carries out the second step of glutathione synthesis: it catalyses the addition of glycine to  $\gamma$ -glutamyl cysteine to produce glutathione. Nearly all animals and plants investigated have only a single gene encoding glutathione synthetase. However, recent genome and transcriptome data have shown that the reniform nematode, *Rotylenchulus reniformis*, one of the most economically important plant-parasitic nematodes, has a massively expanded GS-like gene family with at least 100 members. A phylogenetic tree divides this large gene family into three major clades. Most genes in the largest clade are highly expressed during parasitism and unlike all other GS genes outside plant-parasitic nematodes, the majority are predicted to encode a signal peptide for secretion. *In situ* hybridisation shows that these putatively secreted GS-like genes are specifically expressed within the gland cell of *R. reniformis*, while genes from the other two clades are expressed more generally throughout the nematode. *In planta* expression for complementation of Arabidopsis GS mutant plants indicates *R. reniformis* GS-like genes do not function as typical plant GS enzymes to produce glutathione *in planta*. *In vitro* biochemical assays of recombinant protein reveal at least two distinctive enzymatic activities within ancestral and expanded GS clades. Those in the largest clade have extremely low enzymatic activity compared with those in the other two clades. Site-directed mutagenesis is being used to elucidate the role of specific active site residues in the observed enzyme activities. Taken together, these support a divergence of GS function in each of the three clades and different roles in host-nematode interactions.

Tuesday, 11 September

## **Characterisation of putative effectors from the cereal cyst nematode *Heterodera filipjevi***

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The cereal cyst nematode, *Heterodera filipjevi* is a damaging nematode pest on cereal crops worldwide and causes significant economic yield losses in many countries; however, few molecular details of effectors of *H. filipjevi* parasitism are known. In this study, the transcriptome of *H. filipjevi* at six developmental stages including eggs, J2, parasitic J2, J3, J4 and female were performed using PacBio long read and Illumina sequencing. A total of 136378 transcripts were ultimately obtained, and 1024 transcripts showed best hits to genes putatively encoding carbohydrate-active enzymes in plant-parasitic nematodes that play an important role in the invasion process. A total of 1127 transcripts were homologous to known phytonematode effectors, and 121 putative novel effectors were identified in the *H. filipjevi* transcriptomes. Fifteen transcripts were analysed by qRT-PCR and confirmed to be highly expressed during at least one developmental stage. For *in situ* hybridisation, seven of the 10 tested putative effectors were specifically expressed and located in the subventral gland cells, and five putative novel effectors were specifically expressed in the dorsal gland. Furthermore, 45 transcripts were found to have putative lethal RNA interference (RNAi) phenotypes. These transcriptomic data lay a foundation for further studies of interactions of *H. filipjevi* with cereal and *H. filipjevi* parasitic control.

## **Analysis of the transcriptome of *Heterodera sacchari***

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*Heterodera sacchari*, is an important parasite of sugarcane and rice. Like other cyst nematodes, *H. sacchari* induces a metabolically active syncytial feeding cell system in the stele of its host plants. Initiation and maintenance of syncytium is achieved by injecting protein effectors into the infected root cells. In order for better understanding of the *H. sacchari*-host interaction we have established an *in vitro* culture of *H. sacchari* on rice in pluronic gel. We found that nematodes successfully completed their life cycle in 7-8 weeks at 25°C. Using this *in vitro* material, we performed a transcriptomic analysis of *H. sacchari* second-stage juveniles and parasitic stage nematodes (15 days post infection). Candidate effectors were identified from these transcriptome datasets and showed greater variability than non-effector sequences when compared to other orthologous genes. A wide range of plant cell wall degrading enzymes were present in *H. sacchari*. GH53 enzymes were found to be absent in *H. sacchari* and, since these enzymes are also absent in *H. avenae*, another nematode parasite of monocots, this may reflect an adaptation to parasitism of monocots. Similarly, analysis of CLE-like sequences present in *H. sacchari*, showed that the peptides encoded by these predicted genes are more similar to sequences from rice than to those from other nematodes or other plants. Current work is focused on characterisation of SPRYSEC encoding genes in *H. sacchari*.

Tuesday, 11 September

## **A model of nematode parasitism based on genetic analysis**

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An essential and invariant feature of parasitism by root-knot nematode (RKN; *Meloidogyne* spp.) is the induction of 'giant cells' (GC). GC are stereotypic across *Tracheophyta*. Hence, their induction presumably involves a standardised strategy. Dogma points to proteins expressed in the pharyngeal glands, and purportedly introduced into the host cytoplasm. Various roles have been proposed for these effectors, including locating to the GC nuclei where they apparently interact with host proteins to effect GC development. Effector knock-downs reported in the literature typically report subtle changes to parasitic ability. Using the tools of quantitative genetics, we constructed a linkage map of *M. hapla* to ask questions such as: "what genes in the worm influence gene expression in the host?" and "how does natural allelic variation impact the host response?" Although most of these genes currently remain only as map positions, some have been cloned to identity: none of these obviously encode known effectors. A picture is emerging of two processes at work. One process yields the CG, and is conserved across RKN species. The other process involves a core of genes whose expression is conserved within a species, but which differ between RKN species: We postulate that these genes are responsible for the many phenotypic polymorphisms between individuals.

#### **S14. Systematics and Phylogeography**

Convenors: Sergei Subbotin & Wim Bert

#### **Phylogeography of the vector nematode, *Xiphinema index*, using mitochondrial and microsatellite markers highlights its Eastern origin closely linked to grapevine domestication**

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The economic impact of the dagger nematode *Xiphinema index* is high in Western vineyards by transmitting the damaging Grapevine fanleaf virus. Our phylogeographical study based on mitochondrial sequences and microsatellite loci used more than 80 *X. index* representative samples collected from the Middle- and Near East, the Eastern-, Central- and Western Mediterranean, and the Western countries (Europe and the Americas). In this mainly (meiotic) parthenogenetic species, the mitochondrial marker *CytB* was first considered for comparison of *X. index* with the related amphimictic vector species *X. diversicaudatum*. *Xiphinema index* exhibits a significantly lower intraspecific molecular variability than *X. diversicaudatum*, in agreement with the respective reproduction modes of both nematodes. We then showed that *CytB*, concatenated with additional mitochondrial genes *ATP6*, *ND4* and *COI*, display a robust phylogeographical pattern consisting in three clades grouping Eastern Mediterranean, Near- and Middle Eastern samples and a single clade grouping samples from Western Mediterranean, Europe and the Americas. The highest mitochondrial polymorphism is observed in one clade of Middle- and Near-East samples that overlaps the Transcaucasia and Southern Caspian Sea region from where grapevine has been presumably domesticated and that likely overlaps the nematode native area. East-to-west nematode dissemination appears to match that of its domesticated grapevine host during the Antiquity mainly by the Greeks and then the Romans. In Western Mediterranean, Europe and the Americas, two close and almost exclusive mitochondrial haplotypes were detected. The first haplotype, found in vineyards from the Southern Iberian Peninsula, Bordeaux and Provence, exhibits a high microsatellite polymorphism. By contrast, the second haplotype contains a single predominant microsatellite genotype surprisingly widespread in most Western countries. This is almost certainly due to its recent dispersal during the massive grapevine replants following the 19th century phylloxera crisis. Our data provide an improved knowledge of *X. index* diversity for future pest control strategies.

Tuesday, 11 September

## **Towards unravelling the phylogeny of *Helicotylenchus***

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Morphological and molecular analyses of *Helicotylenchus canadensis*, *H. digonicus*, *H. pseudorobustus* and *H. varicaudatus* were performed. Four investigated *Helicotylenchus* species derived from various localities and diverse natural and anthropogenic environments from Poland. Morphological and morphometric characteristics of the analysed *H. digonicus* were in agreement with the original, and with the other available taxonomic descriptions of the species. However, the 28S rDNA based phylogeny revealed that the corresponding sequences were significantly divergent from the sequences associated with the *H. digonicus* lineage recently proposed as the appropriate one. Newly generated 28S rDNA sequences from *H. canadensis* and *H. varicaudatus* were positioned in the same clade, in separate, closely related, subclades. The observed close positioning of the two species corresponded to the morphological studies as the main differences between these two species relate only to the length of the tail and the width of the annuli at its end. Morphological evaluation of the Polish *H. pseudorobustus* nematode individuals and their positioning at the 28S rDNA tree was in agreement with the type characterisation of this species. Nonetheless, within the mtCOI sequences obtained from this species, unlike the mitochondrial sequences acquired from the other three analysed species, a simultaneous presence of TAG and TAA codons was identified. This may indicate alterations in the mitochondrial genetic code in *H. pseudorobustus* and for an occurrence of certain flexibility in the mitochondrial genomes of nematodes. The overall topology of the obtained mtCOI-based phylogenetic tree was in agreement with the 28S rDNA-based one. As the data acquired in this work delivers new molecular insights for the genus *Helicotylenchus*, phylogenetic relationships within this group are discussed in relation to the use of mtCOI sequences in the study of the nematode evolution.



**Phylogeographic study of the highly invasive sugar beet nematode, *Heterodera schachtii* (Schmidt, 1871)**

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Plant-parasitic nematodes (PPN) threaten crop production worldwide. Yet few studies have examined their intraspecific genetic diversity or patterns of invasion, critical data for managing the spread of these cryptic pests. The sugar beet nematode, *Heterodera schachtii*, a global invader that parasitises over 200 plant species, represents a model for addressing important questions about the invasion genetics of PPN. Here a worldwide phylogeographic study using 15 microsatellite markers was conducted on 231 *H. schachtii* individuals sampled from four continents, and invasion history was reconstructed through an approximate Bayesian computation approach, with emphasis on the origin of newly discovered populations in Korea. Multiple analyses confirmed the existence of cryptic lineages within this species, with the Korean populations comprising one group (group 1) and the populations from Europe, Australia, North America and western Asia comprising another (group 2). No multilocus genotypes were shared between the two groups, and large genetic distance was inferred between them. Population subdivision was also revealed among the populations of group 2 in both population comparison and STRUCTURE analyses, mostly due to different divergent times between invasive and source populations. The Korean populations showed substantial genetic homogeneity and likely originated from a single invasion event. However, none of the other studied populations were implicated as the source. Further studies with additional populations are needed for a better understanding of the distribution of the potential source population for the East Asian lineage.

Tuesday, 11 September

## Uncovering the nematode diversity of mushrooms

Dieter Slos and Wim Bert

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Nematodes are speciose and are present in huge numbers in virtually all marine, freshwater and terrestrial environments but almost nothing is known about nematodes in Fungi. Both Fungi and nematodes are among the most diverse groups of living organisms. In this study we present data on the diversity, insect associations, and host-specificity of Fungi-inhabiting Nematoda, mainly from mushrooms collected in Belgium. Sampling of 36 species of Fungi recovered around 37 taxa of Nematoda belonging to eleven different families, mainly from the families Rhabditidae, Diplogasteridae, Panagrolaimidae, Aphelenchoididae but also Dorylaimidae, Alaimidae, Myolaimidae and several families in the Sphaerularioidea have been found. Twelve taxa appear to be new to science, which is about one third of the recovered species. New taxa were morphologically and molecularly characterised and their life-traits were analysed. Several of these mushroom-associated nematodes appear to be transmitted by insects, either *via* phoresy or as an insect-parasitic life stage. This study shows that nematodes from Fungi are not specific for their mushroom host but for their phoretic or parasitic host insect species. In conclusion, our data show that Fungi are a specious habitat for Nematoda that should not be neglected in nematode taxonomy.

## **New insights on the characterisation of soil nematode communities by DNA-metabarcoding**

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Nematodes are ideal biological indicators to monitor soil biodiversity and ecosystem functioning. With the increasing interest for agroecological studies, nematode communities are receiving more attention as a tool for soil health assessment by a broad range of scientists, increasing the need for widely accessible and quick methods for nematode identification. We investigated the potential use of DNA-metabarcoding based on amplicon sequencing for soil nematode community characterization. Preliminary tests (Illumina MiSeq 2x300 bp) on nematode suspensions from soil samples confirmed the shortcomings reported in previous studies: underestimation of species richness and flaws in quantification. We therefore set up a series of experiments to improve the technique used: *i*) we evaluated a combination of three pre-treatments with five different DNA-extraction methods; *ii*) we designed a new 18S rRNA primer set (V4-V5 region); *iii*) we compared morphological with molecular data obtained from artificial samples made with cultured nematodes (single species and mock communities) and from natural communities *iv*) we used a software package applying exact sequence variants for taxonomic assignment (DADA2); and *v*) we implemented correction factors to improve quantification. Two DNA-extraction methods were selected, one for its high DNA yield, another for its low variation in yield. Results of the selected DNA-extraction methods suggested a large effect of the method on species richness in different ways (inhibition, efficacy). The newly designed primer (510 bp, V4-V5 region) showed a similar taxonomic resolution as a published primer set (490 bp, V6-V8 region). All mock communities were correctly characterised. Correction factors, based on a mock community with equal amounts of different nematode species and applied on another mock community with different amounts of the same nematode species, improved molecular quantification. In conclusion, DNA-metabarcoding of soil nematode communities is ready for monitoring; its precision will be enhanced by further study and analysis.

Tuesday, 11 September

### S15. Model organisms

Convenors: Robbie Rae & Dave Lunt

#### **Development of *Phasmarhabditis hermaphrodita* as a model to study the genetic basis of parasitism and the ability to control host behaviour**

Robbie Rae

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*Phasmarhabditis hermaphrodita* is a lethal parasite of several slug species of economic importance and has been formulated into a biological control agent for farmers and gardeners. It is the only nematode that has evolved to infect and kill slugs and snails. I believe that *P. hermaphrodita* could be an excellent model to study the evolution of parasitism in the Nematoda. I will describe how to isolate, culture, mate and mutagenise *P. hermaphrodita* as well as detail the attributes of this new nematode model system using techniques developed for *Caenorhabditis elegans* and *Pristionchus pacificus*. Interestingly, we have shown that *P. hermaphrodita* has a remarkable ability to manipulate the behaviour of slug hosts through altering levels of biogenic amines such as serotonin and dopamine. As the genome is currently being sequenced and techniques used to carry out forward genetics have been developed, use of this new model nematode could lead to an understanding of the genes involved with parasitism and host manipulation.

Tuesday, 11 September

## **New reference genome sequences for genotyping virulence in continental European field populations of cyst nematodes**

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Plant-parasitic nematodes form an increasingly important problem in agriculture, causing significant crop losses worldwide. The majority of these losses are caused by a small number of species, such as cyst nematodes (*e.g.*, *Globodera rostochiensis*, *G. pallida* and *Heterodera schachtii*), root-knot nematodes (*Meloidogyne* spp.) and stem nematodes (*Ditylenchus* spp.). With a regulatory ban on most nematicidal agrochemicals, the main method of control at the moment is the use of resistant crop cultivars. However, the current spectrum of nematode resistance genes used in major crops is extremely narrow. Prolonged exposure of field populations to a narrow range of resistance genes can result in the appearance of nematode genotypes with modified virulence characteristics. To improve understanding of the genetic mechanisms underlying selection for virulence in cyst nematodes in The Netherlands, we preferred to generate reference genomes from nematode populations with either minimal or well-defined exposure to host plant resistances. For *G. pallida*, we deliberately chose an old isolate that had not been exposed to potato cultivars harbouring the resistance genes that were most widely used over the past thirty years. For *G. rostochiensis*, two near isogenic inbred lines were used that differ in their susceptibility towards the H1 resistance gene in potato. PacBio sequencing technology allowed us to generate new reference genomes for *G. pallida*, two *G. rostochiensis* lines and *H. schachtii*. By comparing these reference genomes with re-sequenced field isolates, we can study the effect of continuous exposure of nematode populations to a limited set of resistance genes on virulence characteristics. The newly generated genome sequences consist of significantly fewer and longer contigs than the publicly available ones from the two potato cyst nematode species. An automated procedure was used to create an initial annotation. After a further manual refinement of the annotation with a particular focus on effector families, these genome sequences will serve as a reference for the elucidation of virulence characteristics in cyst nematode populations from The Netherlands and surrounding countries.

## The sexual and asexual genomes of nematodes

Amir Szitenberg<sup>1,2</sup>, Laura Salazar-Jaramillo<sup>3</sup>, Mark Blaxter<sup>3</sup> and David H Lunt<sup>1</sup>

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The sequencing of the genomes of plant-parasitic root-knot nematodes has given a wealth of information about basic biological processes. The genus *Meloidogyne* shows a wide diversity of reproductive modes including both true asexual reproduction (apomicts) and a range of meiotic sexual reproductive strategies. The rapidly growing genomic resources of the root-knot nematodes, combined with the diversity and number of independent changes of reproductive mode, makes them a very powerful model system in evolutionary genomics. Understanding the forces determining genome content, size, structure and change is fundamental to our understanding of the generation and maintenance of the diversity of life. Here the sexually and asexually reproducing genomes of root-knot nematodes are compared in their rate and pattern of change. In order to study the genome-wide effects of the abandonment of sexual reproduction we use evolutionary models of sequence substitution, examine proteins key to meiosis and sexual reproduction, and carry out phylogenetically-controlled comparisons of genomic characteristics including transposons. The generality of our conclusions are compared across other nematodes, and asexual species from diverse animal phyla. We characterize the effects of the loss of meiosis on the genome and demonstrate that root-knot nematodes are a powerful model system to dissect the contribution of evolutionary forces to genomic change more broadly.

Tuesday, 11 September

## **The genome of the burrowing nematode, *Radopholus similis***

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The burrowing nematode, *Radopholus similis*, is a devastating pathogen of citrus, banana and numerous other economically important crops and ornamentals. Our project is focused on characterising the genome of *R. similis* and identifying genes that distinguish it from its sedentary counterparts. We sequenced the ~65.5 Mb *R. similis* genome and the assembly was assessed using Core Eukaryotic Gene Mapping Approach (CEGMA) and Benchmarking Universal Single Copy Orthologs (BUSCO). CEGMA analysis indicates approximately 93% completeness of genome coverage while BUSCO analysis showed approximately 67% BUSCOs (complete and partial) for the *R. similis* genome. CEGMA analysis revealed approximately two orthologues per CEG, implying that *R. similis* may have undergone a recent genome duplication event. Gene prediction using Augustus and SNAP revealed 13,211 and 15,315 genes, respectively. We performed tblastx, utilising 7,382 EST sequences downloaded from the public database, as query sequences against the *R. similis* assembly and obtained approximately 94% alignment of the transcripts with the genome. *In silico* functional annotation of the genome was performed using the Blast2Go platform, including GO analysis, InterPro Scan, KEGG analysis and PFAM, and manual curation is being employed for deeper analysis. We utilised OrthoMCL to uncover various orthologous groups present in *R. similis* and identified 31 nuclear hormone receptor genes (*NHR*), 63 G protein-coupled receptor genes (*GPCR*) and 22 collagen genes, among other families. We examined the kinome of *R. similis* by coupling OrthoMCL and the Kinomer database. OrthoMCL predicted 411 kinases, which can be grouped into 249 orthologous groups. We identified six kinases in *R. similis*, also occurring in *Brugia malayi*, that were absent in *Caenorhabditis elegans* and *C. brigssae*. This might indicate a role for these kinases in parasitism. Research is on-going to identify possible syntenic regions with other plant-parasitic nematodes and clarify the evolutionary position of this nematode in the phylogenetic tree.

**Feeding of the whiteleg shrimp *Litopenaeus vannamei* larvae with a desiccation-tolerant *Panagrolaimus* nematode to replace *Artemia***

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To replace *Artemia nauplii* during feeding of first larval stages of the whiteleg shrimp, *Litopenaeus vannamei*, a nematode, *Panagrolaimus* sp., was tested as live feed. Nematodes were produced in *in vitro* liquid culture on cells of *Escherichia coli*. In Trial 1, shrimp larvae were fed one of four diets from Zoea 2 to Postlarva 1 (PL1): (A) *Artemia nauplii*, control treatment; (NC) nematodes enriched in docosahexaenoic acid (DHA) provided by the dinoflagellate *Cryptothecodinium cohnii*; (N) non-enriched nematodes and (Algae) a mixture of microalgae supplemented in *C. cohnii* cells. In Trial 2 larvae were fed until Postlarva 6 (PL6), with (A), (NC) and a different treatment (NS) with nematodes enriched in polyunsaturated fatty acids (PUFAs) provided by the commercial product S.presso®. Mysis 1 larvae fed nematodes of the three dietary treatments were 300 µm longer ( $3.2 \pm 0.3$  mm) than control larvae. At PL1, control shrimps were 300 µm longer ( $4.5 \pm 0.3$  mm) than those fed DHA-enriched or PUFAs-enriched nematodes. No differences were observed in length and survival at PL6 between control larvae and those fed DHA-enriched nematodes ( $5.1 \pm 0.5$  mm; 33.1%–44.4%). Shrimp fed microalgae were delayed in development at PL1. This work is the first demonstration of *Panagrolaimus* sp. suitability as a complete substitute for *Artemia* in rearing shrimp from Zoea 2 to PL6.



## **S16. Plant-parasitic nematode biology**

Convenors: Etienne Danchin & Catherine Lilley

### **Host factors influence the sexual fate of cyst nematodes parasitising the roots of *Arabidopsis thaliana***

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Cyst nematodes are biotrophic plant parasites that induce physiological and structural modifications leading to the formation of hypermetabolic syncytial nurse cells in the roots of their host plants. Syncytia are the only food source during their life cycle. Cyst nematodes are sexually dimorphic; however, the sexual phenotype appears only after feeding from the host plant. The sex ratio of males to females is strongly influenced by environmental conditions. Under favourable conditions with plenty of nutrients supplied by the host, more females develop, whereas mainly male nematodes develop under adverse conditions such as in resistant plants. Here, we investigated the host factors influencing the sexual differentiation of cyst nematodes. We developed and validated a method to predict the sex of beet cyst nematode (*Heterodera schachtii*) during the early stages of its parasitism in the host plant *Arabidopsis thaliana*. We collected root segments containing male-associated syncytia (MAS) or female-associated syncytia (FAS), isolated syncytial cells by laser microdissection, and performed a comparative transcriptome analysis. We found that genes belonging to categories of defence, nutrient deficiency, and nutrient starvation were overrepresented in MAS as compared to FAS. Conversely, gene categories related to metabolism, modification, and biosynthesis of cell walls were overrepresented in FAS. We used  $\beta$ -glucuronidase (GUS) analysis, qRT-PCR, and loss-of-function mutants to characterise FAS- and MAS-specific candidate genes. Our results demonstrated that various plant-based factors, including immune response, nutrient availability and structural modifications, influence the sexual fate of cyst nematodes.

**Parasitic success without sex in root-knot nematodes: allopolyploid genomes might help them adapt**

Romain Blanc-Mathieu<sup>1</sup>, Laetitia Perfus-Barbeoch<sup>1</sup>, Jean-Marc Aury<sup>2</sup>, Martine Da Rocha<sup>1</sup>, Jérôme Gouzy<sup>3</sup>, Erika Sallet<sup>3</sup>, Cristina Martin-Jimenez<sup>1</sup>, Marc Bailly-Bechet<sup>1</sup>, Philippe Castagnone-Sereno<sup>1</sup>, Jean-François Flot<sup>4</sup>, Djampa K Kozłowski<sup>1</sup>, Julie Cazareth<sup>5</sup>, Arnaud Couloux<sup>2</sup>, Corinne Da Silva<sup>2</sup>, Julie Guy<sup>2</sup>, Yu-Jin Kim-Jo<sup>1</sup>, Corinne Rancurel<sup>1</sup>, Thomas Schiex<sup>6</sup>, Pierre Abad<sup>1</sup>, Patrick Wincker<sup>2,7,8</sup>, Etienne G.J. Danchin<sup>1</sup>

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Root-knot nematodes (RKN) are devastating crop pests that cause massive damage to the world agriculture annually. Curiously, RKN that reproduce without sex and meiosis (apomictic) are the most devastating. For many years, this parasitic success despite absence of sex and genetic exchange has remained an evolutionary mystery. Recently, we have analysed the genomes of three apomictic RKN and compared them to that of a relative with facultative sexual reproduction (amphimictic). We found that the genomes of apomictic RKN are 3-4 times larger than that of their amphimictic relative and are made of duplicated genome copies with high intra-species nucleic divergence (avg. 8%). The larger genome assemblies coincide with observations of higher chromosome numbers, suggesting polyploidy. Using phylogenomics, we showed that the duplicated and diverged genome copies are most likely the results of inter-specific hybridisations involving unknown amphimictic parents. Because of this allopolyploidization, >90% of the genes are in two or more homoeologous and diverged copies in the apomictic RKN. We profiled the expression patterns of the whole gene set for the apomictic RKN *Meloidogyne incognita* during its life cycle. We showed that >60% of the pairs of gene copies have different expression patterns. Furthermore, a portion of these pairs of gene copies shows signs of positive selection. This illustrates functional consequences to the allopolyploid genome structure and suggests the gene copies resulting from the hybridisation do have different functions. These homeologous gene copies could represent a reservoir for adaptation despite absence of sexual reproduction. As a possible consequence of their allopolyploid origins, the apomictic RKN have higher proportions of their genomes covered by transposable elements (TEs). These TEs might also actively (by moving) or passively (by promoting rearrangements), contribute to the genome plasticity and adaptability of these species despite the absence of sexual reproduction.

**Plant sterol composition: altering the susceptibility of host plant for plant-parasitic nematodes**

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Sterols are essential organic compounds belonging to the family of the isoprenoids. These oligomers are synthesised from the five-carbon precursor isopentyl-pyrophosphate (IPP) and its isomer dimethylallyl-pyrophosphate (DMAPP). Subsequent metabolic reactions and modifications generate an enormous diversity and complexity of sterols. Mammals and other vertebrates contain cholesterol as the main membrane sterol, whereas plants contain a mixture of different sterols, with cholesterol appearing as a minor component. The major plant sterols, sitosterol, stigmasterol and campesterol, differ from cholesterol by an alkylation at C24 at the side chain. Sterols, in particular cholesterol are necessary membrane components and nematodes are not able to synthesise their own sterols *de novo*. However, it is not yet known whether plant-parasitic nematodes have the capacity to modify plant sterols, or whether they can use plant sterols without modification for their own metabolic needs. Our *in silico* investigation revealed that plant-parasitic nematodes lack the ability to modify plant sterols. Hence, the variations of plant sterols are subject for further investigations, since altered plant sterol composition by chemical or molecular means, displayed variations in *Meloidogyne* spp. reproduction. Modifications in the alkylation of plant sterol side chains can lead to proliferation of *Meloidogyne incognita*, whereas direct sterol synthesis inhibition reduces nematode proliferation.

Tuesday, 11 September

**Gene copy number variation as a signature of adaptive evolution to host resistance in the root-knot nematode *Meloidogyne incognita***

Philippe Castagnone-Sereno, Karine Mulet, Mégane Karaulic, Martine Da Rocha, Marc Bailly-Bechet, Etienne G.J. Danchin, Laetitia Perfus-Barbeoch and Pierre Abad

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The obligatory parthenogenetic root-knot nematode, *Meloidogyne incognita*, has a worldwide distribution and is the most devastating plant-parasitic nematode. Despite its asexual reproduction, this species exhibits an unexpected capacity of adaptation to environmental constraints, *e.g.*, resistant hosts. Here, we tested the hypothesis that copy number variations (CNV) may contribute to the ability of *M. incognita* to overcome the tomato resistance gene *Mi-1.2*. For that purpose, we performed an array comparative genomic hybridisation (aCGH) analysis of two pairs of avirulent vs virulent genotypes (*i.e.*, non-adapted vs adapted to the host resistance, respectively), taking advantage of the parthenogenetic reproduction of the nematode to set up near-isogenic lines (NILs) through experimental evolution on susceptible or resistant host plants, respectively. This strategy made it possible to draw up a comprehensive genome-wide landscape of gene CNV associated with the (a)virulence of the nematode, and resulted in a set of 184 genes differentially affected by CNV between avirulent and virulent NILs. The CNV distribution throughout the nematode genome is not random and suggests the occurrence of genomic regions more prone to evolve in response to the selection pressure of the host resistance. We further filtered this list of candidate CNV and identified 33 genes that systematically showed the same highly supported changes in copy number across replicates between virulent and virulent NILs. The fold change values indicated a reduced copy number in virulent nematodes, which was then tested by qPCR. This validation step confirmed a subset of 21 genes exhibiting CNV conserved between the two independent sets of avirulent and virulent NILs. To our knowledge, this study represents the first genome-wide analysis of the distribution of CNV in the genome of a non-model nematode, and provides new insights into the genetic mechanisms that may promote adaptation of a parthenogenetic animal to adverse environmental conditions.

**ABC transporters modulate tomato root exudate composition and parasite behaviour *ex planta***

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Plants manipulate the rhizosphere through the release of water soluble and volatile organic compounds (VOCs) known collectively as root exudate. Root exudate can recruit beneficial soil microflora and fauna; however, it can also attract parasites and pathogens. Plant parasitic nematodes (PPN) are economically important parasites of global agriculture and are highly responsive to root exudates. Understanding the basis of plant-nematode interactions *ex planta* could facilitate the development of aggressive new controls that inhibit hatching and host-finding behaviours. Root expressed transporters have been implicated in the sequestration, mobilisation and exudation of hormones, VOCs and secondary metabolites that influence exudate composition either directly or indirectly, and could represent targets for breeding programmes, rhizosphere engineering, or biotechnological intervention aimed at enhanced crop parasite resistance. Recently it has been demonstrated that VOC release from plants is an active process, mediated by ABC transporters. VOCs have also been implicated in long-range orientation and attraction of plant parasites, implicating them as high value targets for crop modification strategies. Here we present a novel Virus Induced Gene Silencing (VIGS) inoculation method that avoids the characteristic growth stunting impact of blunt syringe leaf inoculation. Using this revised VIGS method, we demonstrate that knockdown of two root-expressed ABC transporters in tomato (ABCG37 and ABCC2) is specific and alters the composition of volatile organic compounds in root exudates according to GC-MS analyses. Following knockdown of ABCC2, we observe statistically significant increases in nonadecane and tetradecanoic acid relative to controls, alongside a reduction in 2-methyl octacosane. Conversely, knockdown of ABCG37 results in the increased exudation of heptadecane, eicosane, octadecanoic acid and 9-O-Pivaloyl-N- acetylcolchinol. These chemical changes suppress PPN hatching, chemotaxis, and invasion behaviours. This study provides the first link between ABC transporters, root exudate VOC composition and parasite behaviours *ex planta*, paving the way for new approaches to parasite control.

**S17. Bioindicators and nematode communities**

Convenors: Roy Neilson & Lieven Waeyenberge

***Steinernema feltiae* exhibits resistance to Cr VI: a candidate organism to detect effects of Cr VI contamination in the environment**

Stephen Boyle and Thomais Kakouli-Duarte

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*Steinernema feltiae* is a commonly occurring nematode in Ireland. Hexavalent chromium (Cr VI) occurs naturally in Irish soils, but can also accumulate due to anthropogenic causes mainly from industrial practices. The work presented here is a set of experiments investigating the suitability of this organism as a bioindicator of Cr VI pollution. Results demonstrate that *S. feltiae* can survive and reproduce in the presence of high concentrations of Cr VI. The nematode was able to produce large numbers of progeny in the presence Cr VI concentrations, within the insect hosts, as high as 1,000 ppm, but an increase in developmental times was observed at 400 ppm Cr VI and above. The presence of the toxicant in sand influenced nematode behaviour in terms of reproduction, developmental times and infectivity. The infective juveniles (IJ) survived and infected hosts for up to 13 days, with an ascending nematode reproduction rate up to 100 ppm Cr VI. Increasing concentrations of Cr VI in sand were observed to reduce IJ infectivity rates, but to increase nematode development times. The capacity of *S. feltiae* to withstand high concentrations of Cr VI and its ability to increase progeny numbers at the early stages of Cr VI exposure may provide a survival advantage for this nematode at contaminated sites. It may also demonstrate the potential for its development as a model organism for ecotoxicity assessments in *in-situ* field sampling.

Tuesday, 11 September

## **SoilBio – assessing soil health using soil nematode communities**

Roy Neilson<sup>1</sup>, Kenneth W. Loades<sup>1</sup>, Katharine Preedy<sup>2</sup>, Bryan S. Griffiths<sup>3</sup>, David M. Roberts<sup>1</sup>, Eric Anderson<sup>4</sup> and Tim J. Daniell<sup>1</sup>

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Providing sufficient food to feed an increasing global population is challenging given limited resources. Soil is a key component of food production providing nutrition and organic matter. However, modern methods of crop production have resulted in degraded soil leading to reduced yields. This contributes to the so-called yield gap, the difference between yield in optimal conditions to that actually achieved. We are developing a test for soil quality that uses measures of the tripartite nexus of soil biology, chemistry and physics. In terms of the biological component, we profile soil nematode community DNA to inform the status of soil quality. This 3-year UK wide project aims to collect approximately 6000 soil samples from different soil textures, agricultural management systems and crops to establish a baseline of soil health and identify the potential drivers of change. Preliminary analysis of year 1 data will be presented and placed in context using exemplar fields.

Tuesday, 11 September

## **Compost impacts on the soil nematode community: insight from two different land management systems**

Gisele L. Herren<sup>1</sup>, Joos Habraken<sup>1</sup>, Lieven Waeyenberge<sup>2</sup>, Annelies Haegeman<sup>2</sup>, Nicole Viaene<sup>1,2</sup>, Mathias Cougnon<sup>3</sup>, Dirk Reheul<sup>3</sup>, Wim Bert<sup>1</sup> and Hanne Steel<sup>1</sup>

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Composted organic amendments have been shown to improve soil quality while increasing crop yield and reducing pests and disease, which can reduce pollution. Readopting traditional methods of fertilisation and soil improvement on a large scale such as composting, has positive effects on soil biology, and is an environmentally safe alternative to today's conventional agricultural practices. Compost additions to managed rangelands (where livestock are managed in rotational grazing) have been shown to stimulate the production of forage, improve soil quality, and lead to carbon sequestration. We present results from ongoing studies to monitor the long- and short-term effects of compost addition (including compost nematodes) to a relatively undisturbed and disturbed soil food web. Samples were taken from a long-term agricultural study site where compost and nitrogen fertiliser treatments were applied within a rotational cropping system and tillage. Sampling was performed in three consecutive years before and after compost applications. In comparison, we studied the nematode community from an ongoing experiment 6 months after compost was applied to the top layer of managed grazed rangeland (with no tillage after compost application). Nematodes were extracted from soil samples using Baermann trays and mounted on slides. At least 100 individuals from each sample were identified to genus level using a light microscope. The morphological data were supplemented and compared with a DNA-metabarcoding approach. Our results show the effects of compost application on the native soil food web structure in the two analysed management systems, including whether nematodes from composts persist in the soil.



Tuesday, 11 September

## **Assessment on the effects of microplastics pollutants using nematodes as environmental indicators**

Loriane Mwana-Scoe, Thomais Kakouli-Duarte, Kieran Germaine and John Cleary

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In the aquatic environment, microplastics (MPs), plastics debris with particle diameter less than 5 mm, have become a global issue due to their persistence and potential adverse effects on biota. The effects of microplastics include adsorption, and potential release into organisms, of persistent organic pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), and nonylphenols. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was conducted to analyse the characteristics and polymer type of the commercial cosmetic products prior to toxicity bioassays. The majority of microplastics research has focused on the marine environment while relatively little research has been undertaken in freshwater. Recently, microplastics contamination has been observed in drinking water. Currently, there is less information on the effects of MPs on soil and benthic nematodes. Nematodes are non-segmented roundworms, occupying nearly every habitat on the planet, with considerable potential as indicators of environmental change. Therefore, this project aims to improve our understanding of microplastic properties and their environmental impacts, including investigation of the toxicity level of those particles using nematodes as test organisms. In this work, the microplastics were tested on several nematode species (*Caenorhabditis elegans*, *Steinernema feltiae* 12 (1) and *Steinernema carpocapsae*) in order to observe their effects on these microinvertebrates. This involved toxicity bioassays exposing nematodes to different pollutants such as polycyclic aromatic hydrocarbons (PAHs) and pesticide at various concentrations, in 96-well plates, over 24 h, 48 h, 72 h and 96 h. So far, results indicate that MPs have an impact on nematode mortality. Our findings will aid in the understanding of the effects of MPs on benthic nematodes.

## **Nematodes play an essential role in the apple replant disease complex**

Xorla Kanfra<sup>1</sup>, Liu Benye<sup>2</sup>, Beerhues Ludger<sup>2</sup> and Holger Heuer<sup>1</sup>

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Apple replant disease (ARD) is a severe problem in apple production worldwide. It is caused by a complex of yet unknown soil biota, leading to small discoloured roots, as well as increased biosynthesis of phytoalexins, total phenolic compounds and antioxidants. We investigated the contribution of nematodes to ARD by dissecting the soil biota from soil infested with ARD and non-infested control soil into a nematode and a microbe fraction. Their separate and synergistic effect on ARD symptoms of susceptible M26 apple plants was analysed in glasshouse assays after inoculation. In three independent experiments, the combination of nematodes from ARD soil with microbes from ARD soil had the strongest adverse effect on the plants, with respect to growth parameters of shoots and roots, total phenolic compounds and phytoalexins in roots, and antioxidants in leaves. Nematodes from ARD soil together with microbes from control soil, or together with the microbiome associated with their own bodies, also had significant effects on the plants but was less pronounced. Microbes from ARD soil alone, or in combination with nematodes from control soil, induced only minor ARD symptoms that were mostly not distinguishable from those of plants inoculated with microbes from control soil or non-inoculated. Overall, a highly significant effect of the source of the inoculated nematodes, ARD or control soil, on ARD symptoms and a strong synergistic interaction between ARD nematodes and microbes was revealed. Microbes from control soil also induced strong but delayed ARD symptoms when inoculated together with nematodes from ARD soil. Nematode communities significantly differed between ARD and control soil as revealed by high-throughput sequencing of 18S rRNA. Plant-parasitic nematodes were too low in abundance to explain root damage. In conclusion, exploring the associations of nematodes and microbes in ARD soils will give the chance to unravel the etiology of ARD.

### S18. Techniques and training

Convenor: Awol Seid

#### **Dityluz: Development of tools for the detection and viability testing of stem nematode, *Ditylenchus dipsaci*, on alfalfa seeds (*Medicago sativa* L.). Development and assessment of a pest threshold**

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In France the stem nematode, *Ditylenchus dipsaci*, is a threat in alfalfa seed production and is a quarantine pest on alfalfa seeds within the European Union (Directive 2000/29/EC); only nematode free-seeds can be marketed. Until 2010, contaminated seeds were exclusively disinfected by fumigation with methyl bromide. Following the removal of this product, seed companies have had to develop alternative techniques to eliminate this pathogen either by industrial processes or thermotherapy. The viability of nematodes is not currently a factor in the certification scheme. Therefore, seed lots are considered positive even if all the nematodes present are dead and pose no risk to the crop, this constrains the application of alternative treatment techniques. The Dityluz project was based on a multiple and innovative partnership involving the personnel from the Seed sector (UFS and GNIS) and Public Research (ANSES, Laboratories of Pathology and Molecular Biology of GEVES) as well as scientific and technical experts (INRA and FNAMS). Using diverse techniques (including molecular biology, morphobiometrics, staining), the Dityluz project has allowed the development and transfer of tools to detect and differentiate live/dead nematodes in seed lots to the seed companies. A detection method on seed extracts by PCR (SE-PCR) was developed. The method consists to pool all the population of nematodes in a small volume and to analyse it by PCR. The method was validated in a ring test where performance criteria were evaluated: the detection threshold was determined at 1 *D. dipsaci* and sensitivity, specificity, accordance and concordance were determined at 100%. In addition, a method for assessing the viability of *D. dipsaci* was also developed and validated according to the evaluation of performance criteria as well. The results of this project were made available to seed companies and the Official Control Service through the organisation of a workshop. The outcome of this programme is intended to implement operational measures to improve the technical regulation and certification scheme for alfalfa seed lots.

Tuesday, 11 September

## **Hyperspectral imaging can detect and differentiate between nematode infestation and water deficiency in tomato plants**

Nik Susič, Uroš Žibrat, Saša Širca, Polona Strajnar, Jaka Razinger, Matej Knapič, Andrej Vončina, Gregor Urek and Barbara Gerič Stare

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As the climate changes the resulting higher average temperatures lead to more severe droughts and facilitate the spread of soil-borne pests like the tropical plant-parasitic nematodes of the genus *Meloidogyne*. Remote sensing applications in agriculture could be used to detect such events earlier and adopt consequent measures more efficiently. Both, root-knot nematodes of the genus *Meloidogyne* (biotic stress) and water deficiency (abiotic stress) lead to similar drought symptoms in the plant canopy. Physiological measurements of photosynthetic rate and chlorophyll *a* fluorescence can be used to distinguish between well-watered and water-deficient plants, but not between nematode-infested and non-infested plants. In this work, hyperspectral imaging was assessed for early detection of nematode infestation and water deficiency (drought) stress in tomato plants. Our results demonstrate that it is possible to utilise hyperspectral imaging data combined with supervised learning classification to discriminate successfully between different types of stresses (biotic or abiotic) on tomato plants. PLS-SVM classification achieved up to 100% accuracy differentiating between well-watered and water-deficient plants, and between 90 and 100 % when detecting nematode-infested plants. Shortwave infrared spectral regions associated with the O-H and C-H stretches were most relevant for the detection of nematode infested plants and severity of infestation. High reliability discrimination was possible in the early stages of symptom manifestation and improved over time. To our knowledge, this study represents the first application of hyperspectral imaging to discriminate accurately between drought-induced abiotic and nematode-elicited biotic stresses in tomato plants. This data could be further implemented for the development of remote sensing applications to detect nematode infestations in the field, which would enable quicker response and more targeted pest management (*e.g.* the targeted use of chemical and / or biological control agents). Technology could be further implemented for breeding purposes for evaluating nematode resistance.

## Disposable qPCR matrices for molecular diagnostics of plant-parasitic nematodes on potato

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Many species of plant-parasitic nematode are able to infect potato causing significant economic losses. Among them, the most devastating are potato cyst nematodes (PCN, genus *Globodera*) and potato stem nematodes (PSN, genus *Ditylenchus*). The first and very important step of the nematode management strategy is a correct identification of the detected parasites. The OEPP/EPPO Bulletins describe in details procedures of identification using morphological and molecular approaches. Molecular identification, such as PCR, is quite sensitive and specific, but usually requires expensive equipment and has some disadvantages, such as the risk of contamination of reaction mixture resulting in false positive or negative results, long duration, and necessity of a highly qualified staff for both analysis and data interpretation. To overcome these issues, diagnostic systems based on disposable long-stored stationary qPCR micromatrices have been developed for prompt detection of two PCN and three PSN species (*G. rostochiensis*, *G. pallida*, *D. destructor*, *D. dipsaci* and *D. weischeri*). Laboratory trials using DNA and cultures of target and related nematode species and the analysis of field samples of PCN and PSN-infected plants demonstrated an excellent diagnostic specificity of the developed test systems. A small reaction volume (1.2 µl) and the earlier developed technology of freeze-drying and stabilisation of PCR reagents within microreactors significantly reduce the total time of analysis (1.5 h) and simplify required manipulations, which can be performed even by unskilled personnel. Two variants of ready-to-use micromatrices with flexible architecture intended for detection and identification of either PCN, or PSN can be stored up to 6 months at room temperature and have a good potential as a rapid and simple method of field diagnostics.

The development of test systems was financially supported by the Russian Science Foundation (project no. 16-16-04109).

**Climate-smart agricultural approach in nematode management: a phytonematicide computer-based dosage model**

Phatu W. Mashela<sup>1</sup>, Kgabo M. Pofu<sup>1,2</sup> and Tieho P. Mafeo<sup>1</sup>

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The 2005 international withdrawal of methyl bromide and most other synthetic chemical nematicides from the agrochemical markets due to the ozone-depleting properties, led to unprecedented research and development on alternatives for managing the notorious root-knot nematodes (*Meloidogyne* spp.). Cucurbitacin-containing phytonematicides had been researched and developed in South Africa for managing nematodes in various crops. The objective of this study is to provide the four empirically-based steps that are essential in the development of the phytonematicide computer-based dosage model for generating appropriate and consistent empirically-based information required for the registration of these sustainable products. The model comprises the development of empirically-based: *i*) mean concentration stimulation point; *ii*) the application interval; *iii*) the application frequency; and *iv*) the dosage model proper. The use of the model eliminates the potential phytotoxicity of phytonematicides, improves consistent efficacy on nematode suppression, with limited chemical residues in plant produce and the soil.

## **Nematology as a training and research discipline in sub-Saharan Africa: progress and prospects**

Laura Cortada<sup>1</sup>, Inge Dehennin<sup>2</sup> and Danny Coyne<sup>1</sup>

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Within sub-Saharan Africa (SSA), nematologists have traditionally been in short supply. The literature frequently presents nematology expertise in SSA as “insufficient”, which is mainly attributed to an overarching lack of capacity across public and private sectors. However, over the past two decades a number of initiatives have been instrumental in building greater nematology expertise and to provide facilities for nematology research. For the first time a structured survey and key informant interviews were undertaken to SSA-nationals that have been (or currently are) involved in nematology training programs and research, employed in national extension services, African universities, or working within the diaspora. The main objectives were to establish a clear understanding on: *i*) the main institutions that provide graduate and postgraduate nematology training; *ii*) where trainees develop professional nematology careers; *iii*) accessibility to nematology-related positions in SSA; *iv*) student return rate after studying outside the continent; and *v*) how all the above influence nematology development, as a science, in SSA. Despite nematology being under-represented in SSA, the study found significant progress and awareness of the science in SSA over the last two decades. The number of nematology positions has steadily increased, whilst increasing numbers of women and men return home to occupy qualified positions in academia, as well as in public and private institutions. These findings help to identify the areas that require sustained support to promote nematology further in the region. Plant-parasitic nematodes are a major threat to crop production in SSA, resulting in substantial economic losses, whilst increasing knowledge of beneficial nematodes supports environmental health initiatives and novel pest management alternatives. This study provides evidence of the positive impact of training initiatives over the past 20 years or so.

Wednesday, 12 September

**WEDNESDAY, 12 SEPTEMBER**

**Sessions**

**S19. Integrated nematode management**

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### S19. Integrated nematode management

Convenors: Danny Coyne & Wim Wesemael

#### **Nematode community management in tomatoes in South Africa**

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Plant-parasitic nematodes (PPN) are a serious problem in tomato farming and ZZ2, the largest tomato farmer in South Africa, has a continuous battle with PPN and more specifically *Meloidogyne*. A decade ago, they started producing tomatoes in net houses where the nematode problem is even more pronounced. Because it is assumed that nematode biodiversity is important in soil health and reducing nematode damage, we sampled eight blocks of 0.82 ha in six net houses for three consecutive years to investigate the effect of biodiversity on these aspects. Sixty nine different nematodes were identified of which 28 were PPN and 41 were non-parasitic nematodes (NPN). However, when the average number of each species was investigated, *Meloidogyne* was by far the most abundant species both in numbers and frequency. Therefore, biodiversity alone does not seem to be enough to reduce damage. Nematode community management should rather be considered where *Meloidogyne* is replaced with another nematode species that can compete with *Meloidogyne* in the root zone but will cause little or no damage to the host plant. In sugarcane studies conducted in South Africa, it was discovered that by changing the balance of PPN, where *Meloidogyne* reduced from 84 to 30% and *Helicotylenchus* increased from 36 to 59%, yield was significantly higher. In a conservation agriculture maize trial, it was observed that higher numbers of *Rotylenchulus* were positively correlated with higher yield, whereas higher numbers of *Meloidogyne* correlated with lower yields. When considering PPN as present or absent in our study, *Meloidogyne* was negatively correlated with *Helicotylenchus*. Also, nematode pathogenicity is inversely related to diversity and relative proportions of the different species. Therefore, if the nematode community can be changed in favour of *Helicotylenchus*, with other PPN such as *Pratylenchus* and *Criconema* also present, it is believed that nematode damage can be considerably reduced.

## Nematode management in strawberry fields of Southern Spain

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Spain is the fourth strawberry producer in the world, where 6,545 ha produced 349,143 tons in 2017, with a commercial value about €412 million annually. A nematological survey was carried out at the end of the growing season in the strawberry growing area of Huelva, South-western Spain in May 2017. Additionally, a poll on perception of nematode-caused diseases was undertaken, by face-to-face interviews to 60 agricultural advisors. *Meloidogyne*, *Pratylenchus* and *Hemicycliophora* were the main plant-parasitic nematodes in the area. Field advisors estimated that nematodes occurred at 63% of the strawberry fields affecting an average area of 5% per field and they estimated 10% yield losses. However, 90% of the surveyed fields were infested with *Meloidogyne*, with *M. hapla* the most frequent species (occurring at 77% of the fields,  $563 \pm 144$  juveniles  $(100 \text{ g soil})^{-1}$ ), but also *M. incognita* and *M. javanica* were present. The most efficient methods against nematodes, according to field advisors' opinion were chemical fumigation combined with soil solarisation. A comparison of the relative efficacies of several techniques and nematicides in controlling nematode populations (*M. hapla* and *P. penetrans*) was carried out in experimental fields for twelve consecutive years. Nematode population densities were reduced by more than 70% by soil fumigation with chemicals such as 1,3 dichloropropene+chloropicrin, dazomet, dimethyl disulfide, and methyl iodide+chloropicrin. The efficacy of metam sodium was about 50% and that of acroleine, chloropicrin, ethyl dinitrile, furfural, ozone, sodium azide, biofumigation and solarisation were under 40%. Combination of solarisation with organic manures (biosolarisation) for at least 6 weeks before strawberry planting reduced soil nematode populations by 75% on average and it is proposed as an effective alternative to chemical soil fumigation for strawberry cultivation in Southern Spain.

## **Integrated management of root-knot nematodes in Florida tomatoes combining host resistance and nematicides**

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Root-knot nematodes (RKN) are one of the major biotic factors limiting tomato production in the United States and the rest of the world. Host resistance, governed by the *Mi* gene, is the only available source of resistance against RKN in tomato, and offers good protection against the most common tropical root-knot nematodes. However, RKN second-stage juveniles (J2) will still puncture the roots of RKN-resistant tomato cultivars, and may make the plant more vulnerable to soilborne pathogens. With the hypothesis that by integrating tomato host resistance with fumigants and/or nematicides, we can improve control of nematodes (and soil pathogens) and increase tomato yields, a field experiment was conducted during autumn of 2017 at the University of Florida's Gulf Coast Research and Education Centre (GCREC). Three tomato cultivars with *Mi* ('Sanibel', 'Skyway687', 'Tasti-Lee' isoline with *Mi*), and one cultivar without *Mi* ('FL 47', were planted in plastic-mulched raised beds and treated with either: *i*) a fumigant (F) (chloropicrin); *ii*) a fumigant + nematicide (FN) (chloropicrin + fluensulfone); or *iii*) no fumigant or nematicide. Root gall damage was significantly affected by treatments, with the highest root gall (%) incidence in untreated beds (28%) > F (16%) > FN (5%). *Mi* cultivars also reduced root galls, with highest gall incidence for 'FL 47' (45%) > 'Tasti-Lee' *Mi* (15%) > 'Skyway' and 'Sanibel' (5%). The FN treatment gave significantly higher tomato yield (176 kg plot<sup>-1</sup>) compared to the fumigant alone (162 kg plot<sup>-1</sup>) and the control (131 kg plot<sup>-1</sup>). 'Sanibel' gave the highest tomato yield (180 kg plot<sup>-1</sup>), followed by 'Skyway' (158 kg plot<sup>-1</sup>), 'Tasti Lee' (144 kg plot<sup>-1</sup>) and 'Florida 47' (142 kg plot<sup>-1</sup>). RKN soil populations at the end of the trial were significantly higher in beds with the RKN susceptible cultivar 'FL 47' (526 J2 (200 cc soil)<sup>-1</sup>) as compared to 'Tasti-Lee' (94 J2 (200 cc soil)<sup>-1</sup>), 'Skyway' (39 J2 (200 cc soil)<sup>-1</sup>) and 'Sanibel' (25 J2 (200 cc soil)<sup>-1</sup>).

## **Vegetable production in Uganda benefits from healthy seedling systems and consequent nematode management**

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Across sub-Saharan Africa peri-urban vegetable production systems are characterised by high levels of pests and diseases, as well as high levels of chemical inputs. Inherent within these intensive production systems are the use of transplanted seedlings that are produced by farmers in a small area of their fields with no attention to soil borne pests and diseases. Such systematic use of 'home-grown' seedlings result in early infection of germinating seedlings and the consequent transfer of infected plantlets to the field. In the field, a high incidence and levels of root knot nematode (RKN; *Meloidogyne* spp.) infections are observed on vegetable crops. In a study of pepper and tomato farmers in Uganda, RKN infection was associated with higher overall levels of pests and diseases. Seed germination rate with sterile media was double than for farmer conditions, with 17% more healthy seedlings surviving transplant than farmer seedlings. By using good agricultural practices (GAP) together with healthy seedlings, the volumes of pesticides applied could be reduced seven-fold. The use of healthy seedlings under farmer conditions increased pepper yields by 50%, and when combined with GAP by 150%. For tomato, healthy seedlings improved yields but less markedly than for pepper. The use of healthy seedlings is viewed as a suitable management option for RKN in intensive vegetable systems, which also leads to lower overall pest and disease infections. By adopting GAP techniques, and creating awareness, pesticide applications can additionally be reduced tremendously, benefitting farmers, consumers and the environment.

## **S20. Host plant responses**

Convenors: Tina Kyndt & Florian Grundler

### **A proteinaceous molecule from plant-parasitic nematodes activates defence responses in Arabidopsis**

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Plant defence against pathogens relies on recognition of pathogen-associated molecular patterns (PAMPs) by surface-localised receptors leading to the activation of PAMP-triggered immunity (PTI). The role of PTI during plant-nematode interaction is not well known. Here we show that treatment of Arabidopsis seedlings with a nematode aqueous diffusate (NemaWater) triggers PTI-like responses including ROS burst, gene expression, and seedling growth inhibition in a manner dependent on the common immune co-receptor BAK1. Treatment of NemaWater with proteinase K or heating abolished the ROS burst activation of NemaWater, indicating that the eliciting capacity of NemaWater is due to the presence of a heat-sensitive proteinaceous ligand. The results showed that a leucine-rich repeat receptor-like kinase, termed NILR1, is essential for the induction of immune responses by NemaWater and for immunity to nematodes. A proteome analysis of NemaWater showed the presence of nematode conserved surface proteins that are or maybe involved in triggering PTI through NILR1. These results demonstrate the relevance of PTI during plant-nematode interaction. The role of various conserved signalling molecules present in NemaWater will be discussed.

**WEE1: A key cell cycle regulator involved in the plant-root-knot nematode interaction**

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Plant-parasitic nematodes are among the most destructive plant pathogens. Root-knot nematodes (RKN; *Meloidogyne* spp.) infect plant roots and trigger the formation of specialised feeding sites by substantial reprogramming root cell development. Both, the plant mitotic cycle and the endocycle, are essential targets for a successful susceptible interaction between the host plants and nematodes. Key cell cycle genes, as well as inhibitor genes are important components to allow the induction and maintenance of the nematode feeding site (NFS) development. Among them, *WEE1* belongs to a family of protein kinases involved in the terminal phosphorylation and inactivation of cyclin-dependent kinase 1-cyclin B complex resulting in G2 cell cycle arrest in response to DNA damage in *Arabidopsis*. *WEE1* is mainly expressed at early nematode infection stages most likely due to stress caused by nematode infection. Morphological analysis shows that the lack of *WEE1* protein induces mitotic activity in galls. Functional analysis of the *wee1-1* line illustrate cumulative mitotic defects possibly due to the lack of the appropriate timing for proper DNA replication and repair in giant cells. Our data suggest a conserved plant *WEE1* function in galls triggering cell cycle arrest in response to DNA damage.

## **Cyst nematode infection triggers a burst of ROS that modulates the activation of systemic responses in host plants**

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Cyst nematode invasion causes tissue damage in roots and triggers a burst of reactive oxygen species (ROS), which leads to a cascade of defence responses to inhibit further growth and development of the pathogens. In addition to the local responses to nematode infection, there are changes in systemic tissues including activation of pathogen-related gene expression, accumulation of defence-related metabolites, and changes in hormone homeostasis. However, the molecular mechanisms that mediate the activation of systemic responses in plants after nematode infection are unclear. Here, we investigated the role of ROS in mediating systemic responses in *Arabidopsis* upon cyst nematode infection. In plants, ROS are primarily generated by plasma-membrane localised Rbohs (Respiratory Burst Oxidase Homologs), and we have previously shown that cyst nematode infection activates RbohD and RbohF to produce ROS. Notably, RbohD/F-deficient plants (*rbohD/F* double mutants) are resistant to cyst nematodes. In this study, we compared wildtype Col-0 and *rbohD/F* to explore the function of Rboh-mediated ROS in systemic responses upon nematode infection. The expression patterns of pathogenesis-related genes, metabolite profiles, and phytohormone level in the shoots of nematode-infected plants revealed a very strong activation of systemic responses in *rbohD/F* as compared with wildtype Col-0. In particular, the systemic tissues in nematode-infected *rbohD/F* plants were more resistant to the bacterial pathogen (*Pseudomonas syringae*) as compared to Col-0. Taken together, our results reveal a novel role for Rboh-mediated ROS in suppression of systemic responses upon nematode infection.

**Role of E2Fa and E2Fb transcription factors in the control of mitosis and the endocycle in root-knot nematode-induced galls in *Arabidopsis thaliana***

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The root-knot nematode (RKN), *Meloidogyne incognita*, infects a large number of plant species and triggers the formation of galls, or root-knots, by inducing vascular cell to reenter the cell cycle. These nematodes infect the host root and usurp the plant cell cycle machinery for their benefit causing the formation of multinucleate giant-feeding cells and surrounding cell division. Therefore, it is known that cell cycle genes are essential for the induction and maintenance of the nematode feeding sites. Gall formation is characterised by an acytokinetic mitosis phase, followed by an endoreduplication phase leading to polyploidisation of nuclei during giant cells expansion. The endocycle is a variant of the cell cycle and it is characterised by the duplication of the genome, without cell division, resulting in polyploidy. The cell cycle is very complex and involves the activation and inhibition of a large number of genes. E2F is a transcription activator factor essential to drive several biological processes including the cell cycle progression in animals and plants. In *Arabidopsis thaliana*, there are eight E2F family members, being *AtE2Fa* and *AtE2Fb* activators of S phase genes, *AtE2Fc* a repressor of the cell cycle, two DP proteins that form heterodimers with E2Fs and three atypical E2Fs (DELs). The goal of this study is to understand the role of the two transcription factors *AtE2Fa* and *AtE2Fb* in nematode feeding sites during cell cycle progression. *In situ* transcript localisation has shown that *AtE2Fa* and *AtE2Fb* are highly expressed in nematode-induced feeding sites. So far, infection tests and functional analysis of *E2Fa* and *E2Fb* knockout and overexpressing lines suggests that despite these genes seems to have slight different timing and spatial localisation during gall expansion, both are essential for gall development.



**S21. Management of potato cyst nematodes**

Convenors: Nicole Viaene & Loes den Nijs

**Characterisation of potato cyst nematode populations in UK fields**

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Potato cyst nematodes (PCN) are an economically important potato pest. Two species, *Globodera rostochiensis* and *G. pallida*, have been described in the UK. Using potato cultivars resistant to PCN is one way of controlling this pest. *Globodera rostochiensis*, which occurs with one pathotype in the UK, Ro1, can be well managed by growing potato cultivars with the resistance *H1*. No single major gene provides complete resistance to all European *G. pallida* populations due to a genetically more diverse introduction to Europe at three occurrences. To get the most effective and durable resistance to *G. pallida* for potatoes on the UK market, knowing which *G. pallida* pathotypes are present in UK fields, their distribution, and how they behave in respect to virulence against resistant potato cultivars is important. Therefore, single cyst lines of UK *G. pallida* populations were generated and characterised phenotypically using six potato cultivars/breeding clones with different levels of resistance and mitotically using a mitochondrial marker to distinguish between the three introductions of *G. pallida* to Europe.

## Widespread occurrence of potato cyst nematodes in Kenyan potato growing regions

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The presence of potato cyst nematode (*Globodera rostochiensis*) in Kenya was first reported in 2015. To establish the status of PCN in the country, field surveys were conducted in 20 potato growing regions (counties) in the highlands of Kenya. The survey included interviews with more than 1200 farmers, geo-referencing and collection of soil samples for extraction and identification of PCN. The results showed that PCN cysts were detected in all the 20 counties sampled, and identity of both species (*G. rostochiensis* and *G. pallida*) confirmed through morphological and molecular analyses. Most of the sites sampled had the pest, with cysts recovered from 82% of the samples. The survey also highlighted the lack of awareness of PCN among farmers and how current farming practices could contribute to its widespread distribution. The problem is being tackled by increasing awareness of PCN among small holder farmers and conducting research on characterisation of Kenyan PCN populations as well as testing known and novel management options. For example, on-going field trials are investigating and verifying the effect of African night shade varieties as practical trap crops to suppress PCN populations.

## Potato cyst nematode management: understanding the practicalities and limitations of control options

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Potato cyst nematodes (PCN) are considered the most important nematodes affecting potato production worldwide. In many areas, PCN have reached population levels where commercial production depends upon the application of nematicides at considerable cost to the grower and environment. In Scotland, populations of PCN are currently less advanced than in other areas of the UK. However PCN, especially *Globodera pallida*, are of increasing concern and current cultural practices are exacerbating the problem, *e.g.*, including turning to ware potato production to avoid the statutory control measures when PCN are found prior to seed production. One of the most effective management options to control PCN is the cultivation of resistant varieties. With *ca* 50% of the Scottish potato crop highly resistant (score of 7-9) to *G. rostochiensis*, this species can be successfully managed. By contrast, only 3% of the area of Scottish potato crop is highly resistant to *G. pallida*. Currently, the amount of land recorded as infested with *G. pallida* (following statutory testing prior to growing seed potatoes) is showing a two-fold increase every six years. A project to discover the attitudes of Scottish farmers towards PCN management was conducted during 2017. This revealed much of the motivation behind growers' attitudes to PCN and some of the practical hurdles that we, as scientists, have to find ways to overcome. We also found that many growers have a poor understanding of the biology of PCN and could be doing a lot more to protect the health of their land. Our existing scientific knowledge could be used far more effectively to manage nematode infestations, but we must improve the communication between science and industry in order to determine appropriate practical solutions that can work on both a local and a national scale.

## **Elucidating the mechanisms of the use of banana paper in “Wrap and Plant” technology in the management of potato cyst nematodes**

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Potato is a widely consumed staple food crop worldwide and the second most important food crop in Kenya, commonly grown by small holder farmers. Potato cyst nematodes (PCN) pose a major threat to potato production, with estimated yield losses of approximately 80% under heavy infestations. The prolonged diapause and quiescence of PCN cysts hinders most management strategies. In Kenya, PCN was first reported in 2014 and is causing substantial losses to production. Recent attempts to assess suitable PCN management options in Kenya demonstrated the effectiveness of the “Wrap & Plant” technology (a field deployable nutrient rich biodegradable matrix produced from banana fibre) impregnated with abamectin (nematicide) (treated paper) as well as without abamectin (untreated paper). When potato seeds were wrapped in the paper, both paper treatments significantly reduced PCN densities in the soil leading to increased potato yields. The present study sought to elucidate the underlying mechanisms by which banana paper affects PCN on wrapped potatoes. We hypothesised that banana paper acts as a physical and/or chemical barrier, disrupting plant-nematode interaction. Using behavioural experiments, we investigated the response of PCN to potato root exudates in the presence of banana paper. Our results showed that banana paper influenced PCN hatching, egg viability and chemotactic response of infective juveniles. Chemical analysis by liquid chromatography coupled to mass spectrometry (LC-MS) of the potato root exudate, exposed and non-exposed to banana paper, revealed interaction between the banana paper and the potato root exudate hatching factors solanoclepin A,  $\alpha$ -chaconine,  $\alpha$ -solanine, and other compounds. These results indicate that further work is warranted to understand how the banana paper interferes with PCN and reduces PCN infestation.

[S22. Cover crops and plant extracts in the management of plant-parasitic nematodes](#)

Convenors: Leendert Molendijk & Matthew Back

**Mixtures of green manure crops; remedy or risk?**

Leendert Molendijk and Johnny Visser

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There is a lot of interest in the growth of mixtures of green manure crops. The use of green manures is an important means of maintaining or improving soil quality. Green manures influence soil structure, nutrients, organic matter and soil life. A large part of the knowledge about population development of harmful nematodes is based on monocultures. How population dynamics change if you grow multiple crops with different host plant status is an important question. A frequently heard theory about the effect of mixtures is that the nematodes are disoriented by the multitude of species, and therefore would have difficulties to find their host. Wageningen University & Research investigates the effect of mixtures of host and non-host plants on the development of nematode populations. Two experiments have been carried out in pots with the root-lesion nematode *Pratylenchus penetrans*. In pots, mixtures of maize (good host), Japanese oats *Avena strigosa* (bad host) and marigold, *Tagetes patula* (active control, catch crop) were sown. Root-lesion nematodes were added to the pots. The final population levels after 7 weeks showed that the mix of maize and Japanese oats, had almost two-thirds of the final population density compared with the monoculture maize. Even in the mix between maize, Japanese oats and marigolds the population of nematodes increased strongly, while the host plant maize only made up 33% of the root mass of the total mixture. There seems to be no hurdle to find the host within a mixture. A field trial (2016) with different green manures, including a mixture of 7 crops caused a strong increase of the *P. penetrans* population, with a negative effect on the subsequent potato crop in 2017. In this case the mixture was certainly no remedy but surely a risk. The choice of green manures and mixtures should be based on knowledge of the specific field situation and therefore the advice can only be tailor made.

**Screening for the capacity of root exudates from selected potato varieties to activate hatching of *Globodera pallida* and *G. rostochiensis* in-vitro**

Bruno M. Ngala<sup>1</sup>; Mélina Ianszen<sup>1</sup>; Laura Egel<sup>1</sup>; Pauline Dewaegeneire<sup>1</sup>; Amélie Beury<sup>1</sup>; Anne-Claire Le Roux<sup>2</sup> and Virginie Gobert<sup>1</sup>

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Potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are ranked second amongst other cyst nematodes, after root-knot nematodes (*Meloidogyne* spp.) in the list of top-10 plant parasitic nematodes species. They are estimated to cause losses of about 9% of total potato production worldwide on an annual basis. Environmental and human health problems over the use of nematicides mean alternative integrated solutions are required. In the framework of the French research project BIODERA, funded by ADEME under the leadership of CMI-Roullier, the capacity of root exudates from selected potato varieties ('Désirée', 'Iodéa', 'Stronga', 'Blanche', 'Magnum' and 'Inovator') to activate hatching of the two PCN species in the absence of host plant (suicide hatching) was investigated. Three methods of root exudates production (plantlets and tubers in tap water as well as plantlets in nutritive solution) were tested. The concentration of carbon (mg (g dry matter)<sup>-1</sup>) in exudates was analysed for each variety as per the method used. *Globodera pallida* responded better in root exudates from all selected potato varieties as compared with *G. rostochiensis*. In exudates produced from tuber roots, *G. pallida* had 98, 95, 89, 62 and 50% in 'Désirée', 'Iodéa', 'Magnum', 'Blanche' and 'Stronga', respectively. The maximum hatch obtained for *G. rostochiensis* was 65% in root exudates produced from 'Désirée' tubers. The concentration of carbon (mg (g dry matter)<sup>-1</sup>) was highest (254) for root exudates produced from plantlets in water, followed by exudates from tuber roots (77.8) and plantlet roots in nutritive solution (4.9). This study shows that *G. rostochiensis* may be a more selective species unlike *G. pallida* that responded better to root exudates from all selected potato varieties. This may explain why breeding for complete resistance against *G. rostochiensis* has been more successful.

***Tagetes patula*; an economic profitable control measure for effective and long-term control of *Pratylenchus penetrans***

Johnny Visser and Leendert Molendijk

*Wageningen University and Research, Business unit Field Crops, Edelhertweg 1, Lelystad The Netherlands*

The root lesion nematode, *Pratylenchus penetrans*, is a common plant-parasitic nematode species in The Netherlands. Especially on sandy soil, *P. penetrans* causes serious damage in cash crops like potato, strawberry, lily and rose nursery. Restriction on chemical control measurements necessitates the development of non-chemical effective and economically feasible control strategies. Marigold (*Tagetes patula*) is known to be a very effective catch-crop for *P. penetrans*. Under Dutch climate conditions maximum effect is achieved when *T. patula* is grown in summer as a main crop, consequently losing the income of a cash crop. In the north east part of The Netherlands, on a peaty soil, naturally infested with *P. penetrans* two field experiments were started to evaluate the agronomical and economic effects of *T. patula* as part of the nematode control strategy. Both long term experiments are based on a four year crop rotation of barley + *Avena strigosa* (green manure) - starch potato - sugar beet - starch potato, a common rotation for this region. In the first experiment, started in 2013, all crops were present each year and *T. patula* replaced barley, and therefore losing the income of a cash crop. In the second field experiment, started in 2016, *T. patula* was grown as a green manure crop after the harvest of barley. In both experiments *T. patula* diminished the *P. penetrans* population more than 99% compared to the control barley + *A. strigosa*. Resulting in an increased yield of the (two) succeeding potato crops of, on average by 15%. Both control strategies were shown to be economic profitable. The strategy, in which the cash crop barley is replaced by *T. patula*, improved net economic income of the four year rotation by almost 5%. The cultivation of *Tagetes* is now promoted as part of the so called 'Boost Year', a year where the farmer does not grow a cash crop but takes the opportunity to maintain and improve soil quality of the field. As showed above this can be profitable even in the short run.

## Borderless plant-parasitic nematodes meet African indigenous vegetables

Oliver Chitambo<sup>1,2</sup>, Solveig Haukeland<sup>2</sup>, Komi K.M. Fiaboe<sup>2</sup> and Florian M.W. Grundler<sup>1</sup>

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African nightshade (*Solanum* spp.) and African spinach (*Amaranthus* spp.) are important African indigenous vegetables in many parts of Africa as nutritionally rich crops and as low input crops. However, their host status to parasitic nematodes remains largely speculative. Our survey revealed that root galling caused by root-knot nematodes (RKN) was severe on *S. villosum*, whereas on *S. scabrum*, *A. cruentus* and *A. dubius* root galling was rare or very low. Moreover, soil collected from *S. villosum* and *S. scabrum* root rhizosphere contained few cysts of potato cyst nematodes (PCN), but no developing female nematodes were observed on the roots of growing plants. Therefore, we studied the dynamics of RKN and PCN on *A. dubius*, *A. cruentus*, *S. scabrum* and *S. villosum* over 2 years in an experimental station in Kenya. The effects of the crop species on RKN and PCN soil infestation was evaluated using susceptible crop species. After the cultivation of *A. dubius* and *S. scabrum* our results showed that RKN soil infestation decreased by 85%, whereas *S. scabrum* and *S. villosum* decreased PCN by more than 80%. Later, the galling index and number of developing PCN females measured on susceptible tomato and potato crop varieties decreased by more than 75%. Moreover, the RKN-PCN co-infection incidences measured on potato crop decreased significantly. Here, we show for the first time the design of a cropping system using African spinach and African nightshade as trap crops for RKN and PCN. This approach creates dynamics in cropping systems by enhancing agro-biodiversity to control RKN and PCN in an environmentally friendly, effective and productive way.



**S23. Plant-parasitic nematode biology**

Convenors: Catherine Lilley & Roland N. Perry

**Molecular characterisation of the eggshells of the potato cyst nematode *Globodera rostochiensis***

James A. Price<sup>1,2</sup>, Terry K. Smith<sup>2</sup> and John T. Jones<sup>1,2</sup>

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Hatching of juvenile PCN occurs in response to host derived hatching factors. Little is known about the molecular mechanisms that underpin hatching in response to these host diffusates. Our work is aimed at developing a better understanding of PCN hatching mechanisms by characterising the nematode eggshell, as little is known about individual protein and lipid components of this structure. We have developed methods that allow extraction of proteins and lipids from large numbers of isolated PCN eggshells. These extraction methods have permitted identification of a range of proteins present in the eggshell, including a lipase, a glucosylceramidase (GH30) and a calcium dependent phospholipid-binding annexin. Focus has been directed towards the annexin due to the apparent role of calcium in PCN hatching. An antiserum raised specifically against this annexin has shown the annexin to be localised to the eggshells of *G. rostochiensis*. To our knowledge, that makes this annexin the first eggshell protein to be identified and localised in any plant-parasitic nematode. Recombinant annexin has been used in lipid binding assays to identify specific lipids with which this protein is able to interact. Mass spectrometry analysis of eggshell lipid extractions suggests that the interacting lipids are present within the eggshell. Further eggshell lipid data will be used to give the first insights into the total eggshell lipidome for PCN. A transgenic potato line that expresses double-stranded RNA specific to the eggshell annexin has been produced. Our future work will use these transgenic lines to examine the effects of silencing annexin expression on eggshell development and/or hatching.

## Surface coat biosynthesis in cyst nematodes

Philipp Gutbrod<sup>1</sup>, Lars Melcher<sup>1</sup>, Katharina Gutbrod<sup>1</sup>, Alexandra Paul<sup>2</sup>, Sapun Parekh<sup>2</sup> and Peter Dörmann<sup>1</sup>

<sup>1</sup>*Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany;* <sup>2</sup>*Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany.* [Gutbrod@uni-bonn.de](mailto:Gutbrod@uni-bonn.de)

Plant-parasitic nematodes are a global threat to food security due to considerable crop yield losses. Economically, sedentary cyst nematodes including the genera *Heterodera* and *Globodera* are among the most damaging plant-parasitic nematodes. *Heterodera* sp. infect various plant species such as soybean, sugar beet and Arabidopsis. After invading a host plant they establish a permanent feeding site that will provide all the nutrients required for their development. During their development, *Heterodera* sp. secrete a white coat onto the surface of their cuticle that has been suggested to contain fatty acids and their calcium salts. Nematode surface coats have been implicated in diverse physiological functions including abiotic stress tolerance, antigen masking, dispersal, immunity as well as protection from predators and nematicides. These important features make the nematode surface coat an attractive target for the development of novel control strategies. However, little is known about composition, biosynthesis and function of cyst nematode surface coats. Using lipid-specific stains, spectroscopy, mass spectrometry and small molecule inhibitor studies, we show that the surface coat of *H. schachtii* is almost exclusively composed of straight very long chain fatty acids (VLCFAs) and their respective salts. These fatty acids are only synthesised during the sedentary stage and their synthesis is malonyl-CoA-dependent. Interestingly, soluble VLCFAs were solely found as free fatty acids and CoA-thioesters. This suggests a mechanism for surface coat biosynthesis in which long chain fatty acids are elongated to VLCFAs as CoA-thioesters using malonyl-CoA, and release of free fatty acids onto the cuticle. Once outside of the nematode, these VLCFAs may get bound by metals forming the corresponding insoluble salts. This work establishes key steps for surface coat biosynthesis of an economically important plant-parasitic nematode. This will help to identify the involved enzymes that may be utilised as novel targets for nematode control.

Wednesday, 12 September

**Temperature modifies transcription and root exudate composition of tomato, altering parasite host-finding behaviour**

Steven Dyer, Neil D. Warnock, Deborah Cox, Johnathan J. Dalzell

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Climate change is predicted to alter crop – parasite interactions; however, we have little insight to the molecular and physiological basis of these changes. Here we aimed to characterise the impact of temperature on developmentally-matched tomato seedlings grown at 23°C and 28°C. Transcription of protein-coding and small RNA genes is significantly altered between temperature regimes. GC-MS reveals that root exudate composition is also modified, resulting in the elevated attraction of *Meloidogyne incognita* second-stage juveniles (J2) to root exudate collected from seedlings grown at 28°C relative to those grown at 23°C. Transcriptional changes between experimental groups highlight a variety of biochemical pathways that may contribute to modified root exudate composition. In particular, Ethylene Response Factor (ERF) genes are implicated. As ethylene signalling has been shown to alter nematode attraction to crop plants in the literature, we used Virus Induced Gene Silencing (VIGS) to silence two ERF genes, and find that knockdown of ERF-A3 alters root exudate composition, resulting in the elevated attraction of *M. incognita* J2 to collected root exudates. These data provide insight to the impact of temperature on tomato biology and parasite behaviour ex planta, underpinning efforts to develop climate-smart resistance.

## Tricky parasites: How nematodes take their vitamins from plants!

Shahid Siddique<sup>1</sup>, Zoran S. Radakovic<sup>1</sup>, Muhammad Shahzad Anjam<sup>1</sup>, Clarissa Hiltl<sup>1</sup>, Esther Riemer<sup>1</sup>, Sebastian Eves-van den Akker<sup>2</sup>, Julia Holbein<sup>1</sup>, Divykriti Chopra<sup>1</sup>, Miroslaw Sobczak<sup>3</sup>, Alexander Graf<sup>4</sup> and Florian M. W. Grundler<sup>1</sup>

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Vitamin B5 (VB5) is an essential nutrient that is synthesised *via* a three-step process in plants. In *Arabidopsis*, *AtPANB1* and *AtPANB2* encode the enzyme for the first step and *AtPANC* the enzyme for the last step of the pathway. In comparison to plants, multicellular animals absorb VB5 from their diet. Cyst nematodes are biotrophs, and parasitism is based on the formation of a syncytium in the roots from which nematodes withdraw their nutrients. Here we investigated the role of VB5 during cyst nematode interaction with *Arabidopsis*. We found that expression of *AtPANB1* and *AtPANB2* is strongly induced upon infection, and this upregulation is essential for nematode development. In comparison to *AtPANB*, *AtPANC* is not upregulated and does not play a role in parasitism. Notably, we identified a nematode PANC gene (*HsPANC*), and showed that the nematodes are able to perform the last step of VB5 biosynthesis using *HsPANC*. A comprehensive, biochemical, molecular, and genetic analysis revealed that compartmentalisation of VB5 biosynthesis between plants and nematodes is necessary to avoid feedback/feed-forward inhibition and ensures a continuous supply of VB5 to the rapidly developing nematodes.

**S24. Ecology and evolution of plant-parasitic nematodes**

Convenor: Hans Helder

**Impact of native plant-parasitic nematode communities on the establishment of *Meloidogyne chitwoodi***

Nathan Garcia<sup>1,2</sup>, Eric Grenier<sup>2</sup>, Corinne Sarniguet<sup>1</sup>, Alain Buisson<sup>1</sup>, Fabrice Ollivier<sup>1</sup> and Laurent Folcher<sup>1</sup>

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Among plant-parasitic nematodes (PPN), the species *Meloidogyne chitwoodi* constitutes an important agricultural issue and is listed as a quarantine species in the EU. Here we investigate whether PPN communities can affect the establishment and expansion of *M. chitwoodi* during a simulated introduction. We developed an original experimental design on potato in glasshouse involving four PPN communities derived from a single natural one that was initially grown on different host plants. *Meloidogyne chitwoodi* was inoculated in two different densities (50 or 1000 juveniles per pot). After 4 months, *M. chitwoodi* was more abundant in the community showing a low overall abundance of PPN, decreased in the other PPN communities and failed to establish in five pots out of 80. Results showed that establishment - even starting from a very low inoculum - can occur in all communities even if indigenous PPN in the community affect the expansion through interspecific competition. The reverse interaction was also observed as PPN taxa were generally less abundant in the 1000 *M. chitwoodi* juvenile inoculation treatments. The proportion of males of *M. chitwoodi* produced was higher in the community showing low overall PPN abundance suggesting a strong sensitivity of *M. chitwoodi* to intraspecific competition. The presented results suggest that fields with a low abundance of indigenous PPN present a higher risk of expansion of *M. chitwoodi* even if the introduced inoculum is low. Maintaining a certain abundance of non-harmful taxa in natural PPN communities is therefore useful to prevent high multiplication of *M. chitwoodi* in the community.

Wednesday, 12 September

**Microbial ecology, community dynamics and gene flow in the *Meloidogyne incognita* species group and their habitat**

Amir Szitenberg<sup>1</sup>, Hana Dimeretz<sup>1</sup> and Rivka Shani Alexander<sup>1</sup>

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Microbes in the rhizosphere and root play both supportive and antagonistic roles throughout the life cycle of root-knot nematodes (RKN; genus *Meloidogyne*). For example, they promote root penetration and reproduction *via* cell wall degradation, or inhibit RKN mobility towards the root through trap and repulsion mechanisms. However, most of our knowledge of the microbial diversity in RKN infested fields is derived from culture-based techniques, with only a few limited studies using culture-free methods such as microbial metabarcoding and metagenomics. Using a metabarcoding analysis of field samples, we have studied the microbial diversity and the factors shaping it, in rhizosphere, root, gall and second-stage juveniles (J2) samples, throughout the RKN life-cycle in eggplants, and found unique microbial communities at each niche and time-point. Temporal changes are characterised by shifts in consortial biological function, with possible consequence to RKN. We have further analysed the core microbiome of the various niches and found that while the environmental seed bank may differ among location, the core microbiome of J2 is similar. Interestingly, a few J2-specific bacterial species are consistent in all the samples, but with no apparent gene flow among sites, suggesting coevolution. OTUs highlighted by the metabarcoding analysis were isolated and their function studied experimentally and through metagenomics. This research represents the first step in our effort to characterise RKN associated microbiomes across species and geographic locations in order to understand their function and the factors shaping them. Hopefully, a system in which the host (RKN) has low genetic diversity across large geographic area, will serve as a useful model in the study and assessment of hologenome evolution concepts. Additionally, we seek to improve biological control solutions efficacy in real world scenarios, building on knowledge of the multifactorial interplay in such systems.

***Meloidogyne incognita* shows surprising parallel adaptations to different ranges of host plants despite clonal reproduction and low variation at the genome level**

Georgios Koutsovoulos<sup>1</sup>, Eder Marques<sup>2</sup>, Marie-Jeanne Arguel<sup>3</sup>, Andressa C.Z. Machado<sup>4</sup>, Regina M.D.G. Carneiro<sup>2</sup>, Philippe Castagnone-Sereno<sup>1</sup>, Etienne G.J. Danchin<sup>1,\*</sup> and Erika V.S. Albuquerque<sup>2,\*</sup>

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Root-knot nematodes (RKN, genus *Meloidogyne*) are one of the most ubiquitous and polyphagous plant-parasitic species. Interestingly, the most damaging RKN species, e.g., *M. incognita*, are parthenogenetic that have abandoned meiosis. Although reproducing clonally, it has become apparent that the entire host range of *M. incognita* is not present in every specimen, but different populations or isolates of the species have a different range of plant targets. To that effect, the North Carolina Differential Host Test is being used to categorise *M. incognita* into four races based on the compatibility/incompatibility of six standardised host plant species. To investigate whether there is a genetic determinism of host-races, we have sequenced the genomes of eleven isolates representing the four host-races from populations parasitising six different crops from geographically different locations across Brazil. By analysing the genomic variance between these eleven isolates and the reference genome, we found no genealogical signal supporting the host-races. Thus, the capacity of infecting a particular set of plants seems to have resulted from multiple independent and convergent adaptations. Furthermore, there was no structuration according to the nature of the host plant or the geographic location of the isolates. Overall, we found that despite its phenotypic diversity, the rate of variable positions in the *M. incognita* genome across different populations (~0.27%) was lower than in *C. elegans* (~0.63%). This is concordant with previous studies that showed little variation in the protein-coding sequences between strains from widely different geographic origins. Finally, we show that the Brazilian isolates contain almost all of the divergence of *M. incognita* strains that have been sequenced to date. Our analysis represents the first genome-wide characterisation of the intra-species genetic variability coupled with phenotypic data in RKN and paves the way for further phenome/genome research.

**Coevolution for the hatching trait between *Globodera pallida* populations and *Solanum* species, in Peru**

Camille Gautier<sup>1,2</sup>, Sylvain Fournet<sup>1</sup>, Christophe Piriou<sup>1</sup>, Florence Esnault<sup>3</sup>, Lionel Renault<sup>1</sup>, Eric Nguema-Ona<sup>2</sup> and Josselin Montarry<sup>1</sup>

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Plant-parasitic nematodes are among the most harmful pests of cultivated crops causing important economic losses. For cyst nematodes, hatching, which is the first step of the development cycle, is stimulated by root exudates released by the host plant into the rhizosphere. The removal of chemical nematicides requires development of alternative approaches to protect crops against nematodes and especially in the case of quarantine species such as *Globodera pallida*. For this purpose, root exudates may constitute an effective and innovative biocontrol method that could be used in the absence of the host plant to induce a ‘suicide hatching’ of nematode juveniles and to control cyst nematodes pressure on crop. Thus, the aim of this study was to explore the level of dependence between root exudates from wild *Solanum* species and *G. pallida* populations by a co-phylogeography approach, in its native area. This study will allow to test the hypothesis of a pattern of differential adaptation between the plant and the nematode and, thus, to illustrate the coevolution through a life-history trait depending on the plant: the hatching from the cyst. The phylogeography of *Solanum* species and *G. pallida* populations were well described over the South American continent. Sixteen *Solanum* species and fourteen populations of *G. pallida*, all originated from Peru, have been selected. Root exudates from these *Solanum* species have been produced by leaching at 3 and 4 weeks after transplantation of the seedlings and were used to study the hatching dynamics of each of the fourteen *G. pallida* populations. The results will be discussed according to both the ‘home vs away’ and the ‘local vs foreign’ criteria of local adaptation.



**THURSDAY, 13<sup>TH</sup> SEPTEMBER**

**Plenary session**

**Current challenges in the control of nematode infections in humans and animals**

Peter Geldhof

**Lethal fighting in entomopathogenic nematodes**

Christine T. Griffin

**Sessions**

**S25. Plant nematode effectors**

Convenors: Melissa Mitchum & Aska Goverse

**S26. EPN Application**

Convenors: Raquel Campos-Herrera & Branimir Nježić

**S27. Host plant status and population densities**

Convenors: Nicole Viaene & Misghina Teklu

**S28. Host plant responses**

Convenors: Godelieve Gheysen & Shahid Siddique

**S29. EPN Ecology and biology**

Convenors: Christine Griffin & Bart Vandenbossche

**S30. Management of plant-parasitic nematodes**

**Convenors:** Soledad Verdejo Lucas & Johan Desaeger

## Current challenges in the control of nematode infections in humans and animals

Peter Geldhof

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Infections with parasitic worms still have devastating effects on man and animals worldwide. Control of such infections still relies almost entirely on treatment with chemical drugs. However, consequences of the intensive treatment programs, especially in the veterinary field, are the high selection pressure for resistance and increased drug residues in animal products and the environment. In recent years, worm isolates resistant against one or multiple drugs have appeared worldwide, in particular in sheep and cattle. Fortunately, the situation in humans is different. So far there are only some occasional reports on treatment failure in humans. However, one of the objectives of the WHO is to eliminate morbidity due to worm infections in children by 2020. To achieve this goal, 75 % of the children in endemic areas would need regular treatment, which obviously increases the risk for the development of resistance. As very few new anthelmintic compounds have been marketed in the last decades, medical doctors and veterinarians are left with very few options to combat these infections. For these reasons, research into alternative control strategies, novel drugs and new methods to diagnose those individuals that need treatment is of crucial importance. The aim of this presentation is to provide an update on the progress made in these areas.

[Lethal fighting in entomopathogenic nematodes](#)

Christine T. Griffin, Apostolos Kapranas, Annemie N.R. Zenner and Kathryn M. O'Callaghan

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Males of certain species of *Steinernema* fight and kill each other. In combat, one male coils around and squeezes another, and this frequently results in paralysis and death. Fatal fighting is rare amongst animals; we investigated factors influencing its expression in steinernematids, including the developmental pathway, the number of competitors present, and their relatedness. The probability of death occurring in pairs of males was affected by the developmental pathway followed, being much higher in males that had developed from the infective juvenile stage (analogous to dauer stage) than in males that developed directly without passing through an arrested stage. We tested the effect of relatedness on aggression in groups of *Steinernema longicaudum*, using nine inbred lines. Higher average relatedness led to progressively lower rates of male mortality, as predicted by kin selection theory, and results indicated that male steinernematids recognise their kin. Males also killed females of species other than their own. The occurrence of this unusual behaviour in *Steinernema* spp may be at least partly explained by their lifestyle as entomopathogens. The combat takes place within a host insect that was previously colonised by infective juveniles and killed with the aid of their bacterial symbiont. The host cadaver is a valuable resource, potentially yielding hundreds of thousands of descendants for successful colonists, or even one male colonist and his consorts, and this may have favoured the evolution of this extreme form of competition.

## **S25. Plant nematode effectors**

Convenors: Melissa Mitchum & Aska Goverse

### **Proteomic profiling of syncytia induced by cyst nematodes reveals new candidate effector proteins**

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Cyst nematodes induce the formation of hypermetabolic syncytial nurse cell systems in the roots of host plants, which serve as their sole source of nutrients throughout their weeks-long life cycle. The formation of syncytia is facilitated by the release of a cocktail of proteinaceous secretions known as effectors inside the host cell. Considering that effectors constitute only a minority among nematode secretions, it remains challenging to establish which secretory proteins are *bona fide* effectors. Here, we performed proteomic profiling of syncytia induced by the beet cyst nematode, *Heterodera schachtii*, in *Arabidopsis thaliana* roots using a Quadrupole-Orbitrap mass spectrometer. The nematodes were carefully detached from syncytia and analysed separately. We found that plant proteins related to metabolic processes and energy production were particularly enriched in syncytia as compared to control roots. Notably, we identified a repertoire of 100 proteins of nematode origin within the syncytia proteome. We further shortlisted these candidate effector proteins based on the following criteria: *i*) presence of a signal peptide; *ii*) absence of a transmembrane domain; *iii*) function not known; and *iv*) homologues conserved in cyst nematodes but absent in all other organisms. In this way, we selected eight novel candidate effectors, which we termed SPEs (Syncytial-isolated Putative nematode Effectors). *In situ* hybridisation confirmed that the corresponding genes for SPEs were expressed in nematode gland cells. Detailed further characterisation revealed roles for selected SPEs in the modulation of host immune responses. Investigation into interactions of SPEs with plant proteins by immunoprecipitation is currently underway and will provide new insights into the nematode parasitism process.

## **STATAWAARS: a new promoter DNA motif associated with pharyngeal gland cell expression in *Bursaphelenchus xylophilus***

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The migratory endoparasitic nematode, *Bursaphelenchus xylophilus* (pinewood nematode) is a quarantine pathogen that infects some species of pine trees (*Pinus* spp.) and has a huge negative economic impact on the wood industry and natural forest resources. Using an approach pioneered in cyst nematodes, we have analysed the promoter regions of a small panel of previously validated pharyngeal gland cell effectors from *B. xylophilus* to identify an associated putative regulatory promoter motif: STATAWAARS. The presence of STATAWAARS in the promoter region of an uncharacterised gene is a predictor that the corresponding gene encodes a putatively secreted protein, consistent with effector function. Additionally, we were able to validate experimentally that a subset of STATAWAARS-containing genes were specifically expressed in the pharyngeal gland cells. Finally, we independently validate the association of STATAWAARS with tissue representation by directly sequencing the mRNA of pharyngeal gland cells. We combine a series of criteria, including STATAWAARS predictions and abundance in the gland cell transcriptome, to generate a comprehensive effector repertoire for *B. xylophilus*. The genes highlighted by this approach include many previously described effectors and a series of novel effector genes. Our data, coupled with those from previous studies, suggested that lineage-specific promoter motifs are a theme of effector regulation in the phylum Nematoda.

## **A root-knot nematode effector targets the spliceosomal plant machinery facilitating the giant cells formation**

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Root-knot nematodes are phytoparasites that trigger a long-lasting and intimate relationship within their host plant. To complete their life cycle, nematodes first invade the root system and secondly induce the reprogramming of 5 to 7 vascular root cells into a feeding site built-up of giant cells. *Via* their stylet, *Meloidogyne* species secrete in the host cells effector proteins synthesised in pharyngeal glands. *In planta*, these effectors manipulate some important processes such as cell cycle, cytoskeleton remodelling, plant defences, transcriptional regulations or phytohormones signalling, leading to the formation of the giant cells. MiEFF18 (Minc18636) was identified as such a putative effector secreted to favour parasitism (Rutter *et al.*, 2014; Nguyen *et al.*, 2017). The MiEFF18 effector carries a signal peptide for secretion and nuclear and nucleolar localisation signals. MiEFF18 localised into the nucleus, and particularly within the nucleolus, when transiently expressed *in planta*. Because MiEFF18 does not have any known function, a yeast two hybrid approach was used to search for plant partners of this effector using a tomato root cDNA library. We found the spliceosomal protein SmD1 as a high scored target of MiEFF18. We validated this interaction *in planta* using Bimolecular Fluorescent Complementation (BiFC). Pathogenicity tests using VIGS-silenced *Nicotiana benthamiana* plants and *Arabidopsis thaliana* KO mutant lines showed that SmD1 protein is an essential protein in the nematode parasitic success. Staining of galls showed that *M. incognita* is not able to develop correctly in plants missing SmD1. We are investigating the outcomes of MiEFF18 interaction with its target SmD1, and the cellular functions, including alternative splicing, RNA quality control or PTGS modulation that may be hijacked by this effector.

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

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Despite causing considerable damage to host tissue during parasitism, nematodes establish persistent infections in both animals and plants. An elaborate repertoire of nematode effectors modulates damage-triggered immune responses of the host. However, the nature and mode of action of most of nematode immunomodulatory compounds is not well understood. We discovered that the nematode effectors named the venom allergen-like proteins (VAPs) selectively suppress host immunity during the onset of parasitism in plants. VAPs are uniquely conserved in secretions of all animal- and plant-parasitic nematodes, but their role in parasitism has remained elusive. Knocking-down the expression of Gr-VAP1 hampered the infectivity of the potato cyst nematode *Globodera rostochiensis*. By contrast, heterologous expression of Gr-VAP1 and VAPs from the beet cyst nematode *Heterodera schachtii*, in *Arabidopsis*, resulted in the loss of basal immunity to multiple pathogens. Surprisingly, VAPs only affect the defence responses mediated by surface-localised immune receptors. The modulation of basal immunity by ectopic expression of VAPs involves extracellular protease-based host defences and jasmonic acid responses. Crystal structures of VAPs revealed lipid binding motifs. In these cavities VAPs can bind palmitate and sterol both *in vitro* and *in vivo*. The delivery of VAPs into host tissue coincides with large modifications in the extracellular matrix by migratory nematodes. We, therefore, conclude that parasitic nematodes most likely utilise VAPs to suppress the activation of defences by immunogenic breakdown products in damaged host tissue.

## **Effector GpRpb-1 from *Globodera pallida* targets E3 ubiquitin ligase complexes to promote nematode infection**

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Endoparasitic plant-pathogenic nematodes manipulate plant cell morphology and physiology to establish sophisticated feeding structures. Modifications to plant cells are achieved through the activity of nematode secreted effectors. SPRYSECs are a remarkably expanded family of effectors identified initially in potato cyst nematodes. While SPRYSECs have been implicated in suppression of plant immunity, their intrinsic role in nematode virulence remains unexplored. GpRpb-1 is a 'type' SPRYSEC from *Globodera pallida* with virulent and avirulent variants present in field populations of the nematode. Y2H screening of a nematode-infected susceptible potato library yielded interacting candidates for a virulent GpRpb-1 that are involved in post-translational modification in the plant. We have independently confirmed that E3 ubiquitin ligase UPL3 can interact with GpRpb-1 *in planta*. Transcriptomic profiling of *upl3* mutant plants shows that Skp-like and F-box-like E3 ubiquitin ligases are regulated upon nematode infection. Furthermore, upon silencing of the corresponding ligase genes in *A. thaliana*, we observed significant differences in the amount of developing females present in the roots of nematode-infected plants. The interaction of GpRpb-1 with UPL3 and the transcriptional regulation of other E3 ubiquitin ligases suggest that the intrinsic role of the effector is carried out through manipulation of the plant post-translational modification machinery. Our findings suggest that nematodes are able to use the SPRYSEC family of effectors to control different aspects of the plant cell to establish a feeding site. Therefore, our results may provide further insight into the basis of virulence of nematodes in plants.



## S26. EPN Application

Convenors: Raquel Campos-Herrera & Branimir Nježić

### **Foliar application of entomopathogenic nematodes for controlling *Tuta absoluta* on tomatoes**

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Two experiments were performed to develop foliar application for controlling *Tuta absoluta* on tomatoes. The first experiment was conducted to investigate the efficacy of *Steinernema carpocapsae* (Weiser) [BA2 isolate from Egypt] against *T. absoluta* 4<sup>th</sup> instar larvae on tomato plants. Nematodes were applied in four concentrations (2500, 5000, 10000 or 20000 infective juveniles (IJ) ml<sup>-1</sup>) and applied once or twice within 24 h. The results showed that applying *S. carpocapsae* in 5000 IJ ml<sup>-1</sup> twice caused 68.8% larval mortality. This concentration was used in the second experiment. Here the ability of seven adjuvants to increase the nematode efficacy against *T. absoluta* on plants was tested. The seven adjuvants were Nemaperfect®, Addit®, Squall®, Sorbitol, Xanthan, Chitosan and Carboxymethyl cellulose (CMC). The nematode isolate BA2 was applied with the different formulation additives in a concentration of 5000 IJ ml<sup>-1</sup> and repeated after 24 h on tomato plants infested with *T. absoluta* larvae. The results showed that all the tested formulations increased the nematode efficacy compared with water. However, only three formulations increased the nematode efficacy significantly. These adjuvants were Nemaperfect (88.8%), Addit (86.8%) and Xanthan (83.8%). Further studies are underway to develop the use of these nematode biological control agents on tomato crops in practice.

## Factors affecting foliar applications of entomopathogenic nematodes

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Entomopathogenic nematodes (EPN) have been used in foliar applications for the control of several pests with varying results, which could be in response to the effects of different factors such as the spraying pressure or the kind of adjuvants with which they were mixed. Mixing with adjuvants is considered necessary when using EPN for foliar application as they protect them from desiccation, thus increasing the likelihood of infecting the target pests by increasing their lifespan. Consequently, these factors could have a big influence on the effectiveness of EPN, and thus their effects must be well known in order to optimise foliar application of EPN. In order to clarify the effects of these factors on the commercially available EPN *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* we conducted two experiments, of which the first aimed at evaluating the effects of different spraying pressures on the survival and infectivity of the above mentioned EPN. Spraying pressures varied from 5 to 26 bars, and we found they did not affect either the survival or the infectivity, thus revealing that EPN could be sprayed with all application equipment operating under 26 bars. In the second experiment we tested the effects of eight different adjuvants and revealed varying effects on the survival and infectivity of the three EPN species. Thus, our results serve to improve foliar application of EPN by adapting the spraying pressure, using the most harmless adjuvant and correcting the concentration according to the expected impact on the EPN.

**Entomopathogenic nematodes for the control of striped flea beetle, *Phyllotreta striolata*, in a cabbage field**

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The striped flea beetle, *Phyllotreta striolata*, is a key pest of crucifer vegetables in southern China and causes year-around damage on the cabbage production. Heavy use of chemical insecticides to control *P. striolata* has resulted in pest resistance and serious residue on cabbages. Entomopathogenic nematodes (EPN) of the genera *Steinernema* and *Heterorhabditis* have the ability to control the larvae of *P. striolata*. Virulence of different EPN isolates against different larval stages and pupae of *P. striolata* was compared. Four EPN isolates, *S. carpocapsae* All, *S. pakistanense* 94-1, *H. indica* LN2 and *H. indica* 212-2, showed the potential to suppress the *P. striolata* larvae. Production feasibility, yield and optimum storage temperature for the four EPN isolates were evaluated. Tolerance to environmental stress (heat, osmosis, desiccation, cold, UV radiation and hypoxia) of the four EPN isolates was also studied. *Steinernema pakistanense* 94-1 showed greater tolerance to heat exposure and UV radiation, while *S. carpocapsae* All was more resistant to osmotic pressure, desiccation, cold treatment and hypoxia than the other isolates. Performance of the four EPN isolates to control the *P. striolata* larvae in cabbage field was studied in different cabbage-planting fields. All the four EPN isolates were able to suppress the soil-dwelling stage of *P. striolata* in the fields. An integrated management of *P. striolata* by combining EPN with environmental friendly insecticides and physical control methods was developed. The results further demonstrate the efficacy of EPN against *P. striolata* larvae, and establish a novel integrated strategy to control the striped flea beetles in cabbage fields.

## Control of adults of European cherry fruit fly *Rhagoletis cerasi* by entomopathogenic nematodes

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European cherry fruit fly *Rhagoletis cerasi* is the most damaging pest of sweet and tart cherries. Methods for the pest control are mostly based on insecticides. The phase-out of old insecticides challenges management of this pest, since there is still a lack of environmentally sound products. Entomopathogenic nematodes have shown high efficacy against soil dwelling stages of some pests. Since *R. cerasi* spends the majority of its life cycle in soil it is potentially a good target for entomopathogenic nematodes. The objective of this study was to determinate efficacy of *Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* against adult stage of *R. cerasi*. In laboratory conditions 25, 50, 100, 200 and 400 nematodes per pupa were released in cell wells (24 cells filled with sterilised silver sand) in time before anticipated adult emergence. Number of dead adults infested with the nematodes was assessed. In field conditions tests were performed in 2015 and 2016 in an orchard with high infestation of *R. cerasi*. Cherry trees were covered with insect proof net before the estimated start of adult emergence. The tunnel was divided into four compartments. Treatments included commercial products of *S. carpocapsae*, *S. feltiae* and *Neemazal* and non-treated control. The nematodes were applied at a rate of 0.5 million m<sup>-2</sup>. First application of nematodes was at the time just before the anticipated start of adult emergence and the second one followed 15 days later. In laboratory conditions the highest adult mortality caused by nematodes was in treatments with *S. carpocapsae* (up to 83.6%). In field conditions the lowest percentage (0-0.3%) of infested fruits was with the same nematode in both years. This study reveals that the entomopathogenic nematode *S. carpocapsae* has potential as a control agent of *R. cerasi*.

## Improving the biocontrol potential of slug-killing nematodes

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Slugs are important pests of a broad range of crops, causing serious economic losses. Slugs are conventionally controlled using chemical based products that may have significant non-target effects. For this reason, the development of alternative, less harmful control methods is of great importance. The application of slug-killing nematodes could be such an alternative, as they have great potential as biocontrol agents. Our current research aims *i*) to identify new populations of slug-killing nematodes from soil samples and field-collected slugs; *ii*) to identify slug attractants and feeding stimulants; and *iii*) to encapsulate slug-killing nematodes together with attractants/feeding stimulants in polymer-based beads. Slug killing nematodes normally enter a slug host *via* the respiratory system and migrate to the shell cavity. We envisage facilitating this infestation process by applying beads with slug-killing nematodes, which will be consumed by slugs due to the addition of the attractive and enticing substances. This should greatly increase the chance of infection. So far, there is only one species of nematode, *Phasmarhabditis hemaphrodita*, commercially available to control slugs. To obtain additional species to be implanted in the beads, we developed qPCR based techniques to identify and quantify slug-killing nematodes from soil and host samples. To identify attractants and feeding stimulants and to test the effectiveness of the bead application strategy we have conducted experiments with two slug pests, *Arion* sp. and *Deroceras reticulatum*. Beads were supplemented with various grounds up leaf material or other known attractants. Both slug species will readily feed on some of the extract-treated beads types and their survival and feeding activity was significantly reduced when feeding on beads contained slug-killing nematodes.

## S27. Host plant status and population densities

Convenors: Nicole Viaene & Misghina Teklu

### ***Paratylenchus* sp. in intensive lettuce production in Flanders: prevalence, damage threshold and host status**

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Butterhead lettuce (*Lactuca sativa*) is an important crop for the fresh vegetable market in Belgium. It is mainly produced in glasshouses as a monoculture with up to five harvests per year. In 2017, the production of butterhead lettuce generated revenue of €24.4 million, with a production of 74.5 million heads. In a survey of 38 Flemish commercial glasshouses, *Paratylenchus* was found in 47% of the glasshouses. The damage on butterhead lettuce consisted of small, unmarketable heads, resulting from roots injured by the ectoparasitic feeding habit of this nematode. We wanted to investigate why *Paratylenchus* can multiply to the very high densities observed (up to 23 000 nematodes (100 ml soil)<sup>-1</sup>). Therefore, we monitored population densities of a same *Paratylenchus* sp. (species still to be confirmed) in two soil layers, 0-30 cm and 30-60 cm deep, of five commercial glasshouses for 2 years. Plant type and lettuce cultivar had an important influence on the population dynamics. This was shown in a host status experiment where we found that lamb's lettuce, wild rocket and parsley are poor hosts. A pot experiment to determine damage threshold densities of *Paratylenchus* sp. on butterhead lettuce 'Cosmoplia' was set up with naturally infested soil and repeated twice. Different densities from 0 to 35 000 nematodes (100 ml soil)<sup>-1</sup> were tested in 8 or 12 replicates. Roots were damaged starting from 450 *Paratylenchus* (100 ml soil)<sup>-1</sup> onwards, while root weight decreased from 2000 *Paratylenchus* (100 ml soil)<sup>-1</sup> and higher. No reduction in lettuce weight was observed in the pot experiments. In the field, however, growers are advised to take action as soon as the population density exceeds 2000 *Paratylenchus* (100 ml soil)<sup>-1</sup>. One alternative to chemical soil disinfestation could be growing lamb's lettuce, wild rocket or parsley.

**Effect of initial population densities, temperature and varieties on *Meloidogyne haplanaria*: an emerging threat to tomato production in Florida**

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Root-knot nematodes are one of the major plant-parasitic nematodes causing important economic losses in a wide range of crops. The use of resistant crops is among others, a key tool to manage these nematodes; however, several researches have reported the emergence of resistance breaking populations of root-knot nematodes. One of these resistance-breaking populations is *Meloidogyne haplanaria*; the first report of this root-knot nematode was in 2003 in Texas causing significant damages in peanut fields. In 2015, the identification of this nematode was on tomato fields in Naples, Florida on Mi- resistant cultivars. The Mi-gene is widely used to provide resistance to several species of root-knot nematodes like *M. javanica*, *M. incognita* and *M. arenaria* in different tomato cultivars that are widely used in fields. However, factors such as temperatures, high initial population densities and gene dosage can interfere with the expression of this gene. This study evaluated the effect of several initial population densities of *M. haplanaria* on tomato plants at in vitro conditions, it also established initial damage threshold of *M. haplanaria* at in vitro conditions and analyzed the impact of temperature and genetic background on the resistance breaking in tomato cultivars. Results showed a damage threshold of three eggs J2/ per cm<sup>3</sup> of soil. Additionally, our results propose that at high temperatures *M. haplanaria* could reduce its life cycle, in comparison to the virulent *M. enterolobii*. This study also determined that *M. haplanaria* was able to infect and cause severe damage on homozygous or heterozygous resistant tomato plants. Therefore, this study have demonstrated the importance and potential threat that this nematode can become to tomato industry in Florida.

## Population dynamics of *Meloidogyne chitwoodi* on resistant potato genotypes and 'Desiree' during plant growth

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Potato cultivars are divided into groups: early, mid-early and late, according to their time of maturing. As population dynamics of *Meloidogyne chitwoodi* is related to plant growth, estimates of potato root and tuber resistance might be biased if final population densities ( $P_f$ ) are estimated at different harvest times. Therefore the population dynamics of *M. chitwoodi* related to plant growth was studied in a time series. Three resistant potato genotypes 'AR04-4096' (mid-early), '2011M1' (early) and 'Ka-2006/2217' (late), and the susceptible 'Desiree' (mid-early) were grown in a glasshouse at initial population densities ( $P_i$ ) of 0 and 16 J2 (g dry soil)<sup>-1</sup>. At 0, 28, 42, 56, 64, 70, 76, 84, 90, 97, 104 and 111 days after planting, six plants were harvested and plant weights, height and tuber quality were estimated. At the same time, nematodes were extracted from roots, soil and tubers and their numbers estimated. Plants with nematodes were taller than plants grown at  $P_i = 0$ . Tuber growth was logistic and the time of tuber setting differed between genotypes and 'Desiree'. Increase and decline in nematode numbers was closely related to root weight and the maxima,  $P_{f_{Max}}$ , on all parameters were reached at 70-80 days after planting. Tuber infestation and quality damage increased in time despite the decline in nematode numbers. Crucial for resistance testing is the time of  $P_{f_{Max}}$ , the subsequent decline pattern and the time of harvest when  $P_f$  values and relative susceptibilities are estimated.



## **A re-appraisal of the abundance and identity of *Meloidogyne* spp. associated with maize crops in South Africa**

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Maize is a staple food in South Africa and also an important feed source for livestock. In South African crop production areas maize crops are damaged by various pests, including plant-parasitic nematodes. In the 1970s and 80s, *Pratylenchus* spp. was regarded as the economically most important plant-parasitic nematode genus that infected local maize. However, improvement of the efficacy of a NaOCl-method for extraction of *Meloidogyne* spp. from maize roots during 1995 proved otherwise. The objective of this study was to conduct a survey to re-assess the abundance and identity of nematode pests associated with local maize crops. Root and soil samples were obtained from 78 commercial maize fields (irrigation and rain-fed) in local maize-production areas during the 2015 and 2018 summer growing seasons. Plant-parasitic nematodes were extracted, counted and identified from the samples using standard protocols. Molecular identification of *Meloidogyne* spp. was done using the sequence-characterised amplified region (SCAR) - polymerase chain reaction (PCR) and NaDH5-gene sequencing methods. *Meloidogyne incognita*, followed by *M. javanica*, *M. arenaria* and *M. enterolobii* (in descending order of predominance) were identified. Association of *M. enterolobii* with maize, which according to the literature is a non- or poor host crop of this nematode pest species, is the first report for South Africa. Crops locally used in rotation with maize (*e.g.*, dry bean, potato, soybean) are highly susceptible to *M. enterolobii* and will allow the build-up of high population densities of the species that will be difficult to manage in order to enable sustainable crop production.

## Zeros

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Zero numbers of plant-parasitic nematodes found in a farmer's fields after statutory soil sampling; nematode multiplication rates equaling zero on resistant plants. These are often the results of nematode investigations. Do these results really indicate the absence of nematodes or full resistance in plants? It seems to be not that simple. Sampling studies by Been & Schomaker (2000) demonstrate that zeros, or rather probabilities of finding zeros, depend on sampling methods and techniques. From pot experiments by Heve *et al.* (2015) and Teklu *et al.* (2016), we learn that multiplication rates of nematodes depend on pot size and plant density. Yet, eradication of quarantine nematodes is high on the agendas of governments as is finding full resistance against plant-parasitic nematodes in plants. The questions we should ask ourselves are: *i)* can we prove that farmer's fields are truly nematode free; *ii)* is it possible to make sure that infestations are indeed eradicated after repeated methyl bromide and/or di-chloropropene treatments; *iii)* can we, without any doubt, demonstrate in tests that plants are fully resistant against a certain nematode species; and *iv)* can we discriminate between bad hosts and non-hosts? On the other, rather cynical, hand: how can governments and scientists mend their methods to assure 'nematode-free areas' or 'fully resistant crops'. Answers to these questions can be found in probability analysis, based on variables from experimental methods and techniques, and also by studying quantitative patterns in population dynamics.

### References

Been & Schomaker (2000)

Heve *et al.* (2015)

Teklu *et al.* (2016)

## **S28. Host plant responses**

Convenors: Godelieve Gheysen & Shahid Siddique

### **Enhancement of resistance in rice against *Meloidogyne graminicola* by phosphite and azelaic acid**

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Systemic acquired resistance (SAR) is a phenomenon manifesting enhanced resistance in a plant earlier exposed to a pathogen infection. This increased resistance is usually broad spectrum, long lasting, and associated with the induction of a wide range of genes so called 'pathogenesis-related genes'. SAR has also been shown to be mimicked by a number of chemicals and to be effective in reducing the development and reproduction of plant-parasitic nematodes in both monocots and dicots. In our studies, phosphite (a salt of phosphorus acid), azelaic acid and acibenzolar-s-methyl (BTH) were tested for their ability to induce SAR by foliar spraying onto 19-day-old 'Nipponbare' rice plants grown in the glasshouse. Two days later, the rice plants were inoculated with 300 second-stage juveniles (J2) of *Meloidogyne graminicola*. Twelve days later, the number of galls, number of nematodes successfully infecting the rice root system, and the percentage of nematode females in each rice root system were determined. The result demonstrated that phosphite at the concentration ranging from 50 to 100 mM, azelaic acid at 100-1,000  $\mu$ M, and BTH at 25-250  $\mu$ M reduced by about 50% the number of galls, nematodes and females in rice roots as compared to the control (without chemical treatment). Furthermore, phosphite, azelaic acid and BTH did not pose direct toxicity to J2 of *M. graminicola in vitro*. Expression analysis of rice genes confirmed that plant defence was induced upon the treatment with these three compounds.

## Comparing the defence responses to root-knot nematode (*Meloidogyne graminicola*) infection in resistant vs susceptible rice (*Oryza sativa*) cultivars

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The root-knot nematode *Meloidogyne graminicola* causes substantial yield losses in rice (*Oryza sativa*). Recently, it has been reported that the rice cultivar 'Khao Pahk Maw' ('KPM') is resistant to *M. graminicola* infection. To investigate the underlying mechanisms of that resistance, the physiological, molecular and biochemical responses of 'KPM' were analysed and compared with a susceptible cultivar ('Nipponbare') with and without nematode infection. Using an *in vitro* choice assay, we observed that nematodes are equally attracted to both resistant and susceptible roots. During the nematode infection assay the resistance in 'KPM' is linked with reduced nematode penetration, development and reproduction. Interestingly, hypersensitive response (HR) like necrosis was observed in the root tissues infected by nematode in 'KPM' indicating a strong defence response. We also found a significant increase in reactive oxygen species (ROS) content in 'KPM' upon nematode infection but not in 'Nipponbare'. The levels of plant hormones salicylic acid and jasmonic acid were significantly higher in resistant 'KPM' compared to 'Nipponbare'. Lignin biosynthesis intermediates were also quantified as well as expression of genes involved in this pathway. Higher accumulation of phenolic acid metabolites and transcripts of lignin biosynthesis genes were associated with the resistance trait in 'KPM'. Furthermore, a higher diversity and quantity of diterpenoid phytoalexins (*e.g.*, phytocassanes, oryzalexins) were found in 'KPM' roots upon nematode infection compared to 'Nipponbare'. These results suggest that the resistance trait of 'KPM' to *M. graminicola* infection could be linked to multiple layers of defence responses. We discuss our results in line with the existing literature and conclude that the accumulation of ROS, defense hormones, phytoalexins, phenolic acids, and activation of defence-related genes all might play a cumulative role in resistance of the 'KPM' cultivar against *M. graminicola* infection.

## **Characterisation of small RNAs involved in the plant response to parasitic nematodes, genus *Meloidogyne***

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The redifferentiation of root 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. Our work aims to investigate the role of plant small non-coding RNAs, the microRNAs and the siRNAs, in the control of the massive transcriptional reprogramming observed during formation of RKN induced feeding cells. The role of microRNAs and siRNAs in plant response to RKN was firstly investigated by analysing infection rate of *Arabidopsis thaliana* mutant lines. Then, the small RNAs of uninfected *A. thaliana* WS roots and of root galls induced by the RKN *Meloidogyne incognita* were sequenced by high throughput sequencing technologies. A catalogue of microRNAs expressed in *A. thaliana* uninfected roots and root galls was established. We identified 24 *A. thaliana* microRNAs that are differentially expressed in root galls and we showed a role for the miR159 family in the regulation of a plant MYB transcription factor in response to *M. incognita*. Beyond analysis of miRNAs, we used Shortstack algorithm (Axtell MJ, 2013) to analyse siRNA populations from galls and uninfected roots. We identified siRNA producing clusters that are differentially expressed in infected roots and evidenced an over-representation of the 23-24nt siRNAs in galls. This size corresponds to the canonical size of heterochromatic siRNAs (hcsiRNAs) that are known to regulate expression of transposons and genes at the transcriptional level by inducing DNA methylation. Comparison of expression of siRNA clusters with previous transcriptomic data identified a list of protein coding genes differentially expressed in galls that may be repressed indirectly *via* transcriptional gene silencing of neighbouring transposable elements.

## **Nematode infection redirects hormonal homeostasis via Rboh-mediated ROS to facilitate parasitism of host roots**

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Cyst nematodes are obligate parasites that establish syncytial-feeding sites in roots of their host plants. Their invasion and feeding causes tissue damage in the host roots triggering an oxidative burst. In plants, ROS is mainly produced by plasma membrane-bound NADPH oxidases, named respiratory burst oxidase homolog (Rboh). Surprisingly, Arabidopsis mutants lacking ROS production by Rboh (rbohD/F) have been shown to be less susceptible to cyst nematode attack. A comprehensive microscopic, biochemical and molecular analysis has demonstrated that Rboh-dependent ROS are not required for Arabidopsis root invasion by cyst nematodes; however, the absence of Rboh-mediated ROS impairs syncytium establishment and development. To understand the role of Rboh-mediated ROS in syncytium formation, we performed a genome-wide transcriptome analysis of Col-0 and rbohD/F upon nematode infection. Several genes involved in auxin transport, synthesis and/or homeostasis were down regulated in rbohD/F as compared to wild type. Notably, we identified WAT1, an auxin transporter, as one of the downstream targets of ROS. Hormone quantifications, metabolic profiling, genetic complementation and mutant analysis suggest that it regulates the pathways linking Rboh-mediated ROS to downstream responses. In summary, our work provides a first mechanistic understanding of the role of ROS in promoting infection of nematodes and other pathogens.

## **P45\_ *Meloidogyne graminicola* induces DNA hypomethylation in rice**

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There is growing evidence that epigenetic processes play an important role in plant response to adverse environmental conditions. DNA methylation, one of the most important epigenetic phenomena, plays a key role in plant development and growth but its role in plant-nematode interactions remains poorly investigated and needs to be further elucidated. An in-depth study to investigate the role of DNA methylation in the interaction between *Meloidogyne graminicola* (Mg) and rice (*Oryza sativa* ssp. *japonica*) using an ELISA-based global DNA methylation assay, whole genome bisulfite sequencing and analysis of mutants in the RdDM (RNA-directed DNA methylation, the major pathway of *de novo* methylation in plants) pathway and a DNA methyltransferase inhibitor was conducted. The global DNA methylation assay on 3 days post infection galls and plants treated with NAMP (nematode associated molecular pattern) showed that both of them induce DNA-hypomethylation in rice. A genome-wide study on the pattern of DNA methylation using bisulfite sequencing confirmed that Mg induces DNA hypomethylation in rice plants, mainly in a CHH context. Several mutants in the RdDM pathway were used for infection assays and revealed that loss of DNA methylation confers slightly enhanced defence to nematode infection, which is in agreement with data from plant-fungi/bacterial interactions. Also, inhibition of DNA methyltransferases by spraying plants with Azacytidine makes them slightly less susceptible to nematodes. Taken together, Mg infection induces DNA hypomethylation in rice plants, most likely as part of a basal defence response of the plant to the infection.

## **S29. EPN Ecology and biology**

Convenors: Christine Griffin & Bart Vandenbossche

### ***In vitro* liquid mass culture of indigenous nematodes for the control of invasive slugs**

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European slugs have become significant pests in South Africa, targeting a number of crop types, including arable, ornamental and vegetable crops. Current methods for controlling slugs rely heavily on chemical molluscicide pellets; however, such methods are often overused, ineffective and toxic to non-target organisms and the environment. The use of slug-parasitic nematodes is a possible environmentally-friendly alternative. To date, *Phasmarhabditis hermaphrodita* is the only slug-parasitic nematode that has been developed as a biological molluscicide. However, due to current legislation, Nemaslug® can neither be sold or used in South Africa. Nematode surveys were conducted in the study and produced four local *Phasmarhabditis* species. *Phasmarhabditis papillosa* was selected as a biocontrol candidate, based on pathogenicity tests, as well as its ability to be grown under *in vivo* and *in vitro* conditions. *In vitro* monoxenic culturing is important in the commercialisation of nematodes, as it allows for large scale production, but reduces contamination risks. *Kluyvera* sp., a highly pathogenic bacteria, was isolated from the intestine of the slug, *Deroceras reticulatum*, and used for all *in vitro* cultures thereafter. Furthermore, nematode cultures containing *Kluyvera* sp. were optimised using temperature-range experiments, with the results demonstrating that 15°C was the optimum growth temperature for the nematode. Current work is focused on the optimisation of the mass-culturing protocol, and on testing the pathogenicity of the monoxenic combination on different slugs species. Results from the study could lead to the development of a new biological control agent for slugs in South Africa.



**Soil texture and olive cultivar determine natural occurrence and assemblage of entomopathogenic nematode in Southern Spain: consilience with main drivers for plant-parasitic nematode community**

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Entomopathogenic nematodes (EPN) are biological control agents widely distributed in natural and agricultural soils throughout the world. Recent evidences support the hypothesis that stable rhizosphere as those presented in perennial crops can favour the occurrence of EPN. Hence, conservation biological control tactics can be promoted under these agroecosystems. Cultivated olive (*Olea europaea* L. subsp. *europaea* L.) is a key perennial crop in Mediterranean basis. Herein, for the first time, we investigate whether the EPN presence and assemblage to target soil organisms can be affected by soil and orchard management associated with the olive agroecosystem. By using real time qPCR approaches, we screened the presence of nine species of EPN, six nematophagous fungi (NF), and five free-living nematodes (FLN, competitors against the EPN) in 92 olive groves from Andalusia (Southern Spain). In this survey, prevalence of EPN was 23%, comprising only two of the nine EPN screened: *Steinernema feltiae* (67%) and *Heterorhabditis bacteriophora* (33%). Overall, the agronomic variables olive orchard management (organic, conventional) and soil treatment (tillage, cover crop) did not affect the EPN presence nor their natural enemies. EPN recovery frequency and abundance varied significantly depending on soil texture and olive variety, with the highest values for *H. bacteriophora* in sandy clay loam soils and 'Lechin' cultivar, respectively. Similarly, the FLN and NF were mainly affected by texture type and olive variety, as previously shown for plant-parasitic nematodes. Seven environmental explanatory variables (P, N, K, clay content, organic matter, elevation, and precipitation) explained 60% of the EPN, NF and FLN species variation in multivariate analysis. This information settles the basis for developing strategies to enhance EPN prevalence in olive groves and further to enhance their capacity as biocontrol agents against soil dwelling insect pest.

**Influence of *Photorhabdus luminescens* natural and mutant strains on virulence, reproduction, and survival of the nematode symbiont *Heterorhabditis bacteriophora***

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The entomopathogenic nematode (EPN), *Heterorhabditis bacteriophora*, is symbiotically associated with the bacterium *Photorhabdus luminescens*. This partnership made it an efficient biocontrol agent against a wide range of insect pests in agriculture. The free-living dauer juvenile stage (DJ) carries a few bacterial cells that are released into the insect haemocoel after invasion of the insect. The influence of different natural and mutant strains of *P. luminescens* on the reproduction, virulence and longevity of *H. bacteriophora* was investigated. The native bacteria from natural nematode strains have been isolated and combined with the homozygous nematode inbred line IL3. Reproductive and longevity assays have been carried out with six bacterial wild type strains. In parallel, the influence of nine *P. luminescens* TT01 deletion mutants defective in the stilbene and GameXPptides biosynthesis, as well as a global transcriptional regulator, has been evaluated. Our results indicate a broad range of nematode phenotypes ranging from the full interruption of the nematode development to a slight increase in the DJ virulence and recovery. The acquired information is extremely valuable not only for production purposes, but also for further understanding of the role of the bacterium in the nematode life cycle.

## Evidence that phoretic bacteria and free-living nematodes regulate an entomopathogenic nematode in nature

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A non-pathogenic *Paenibacillus* sp. in Florida produces endospores that adhere specifically to the cuticle of the entomopathogenic nematode (EPN) *Steinernema diaprepesi*. The bacterium reproduces inside the nematode-infected insect cadaver without apparent effect on nematode fecundity or development. However, spore encumbered EPN do not move through soil to infect insects as readily as do spore-free individuals. To study whether these bacteria, and/or free-living nematode competitors of EPN for cadaver resources, regulate *S. diaprepesi* in nature, we monitored the abundance of naturally occurring *S. diaprepesi* and *Paenibacillus* sp., along with that of other fungal, bacterial and nematode natural enemies of *S. diaprepesi* during two years in four Florida citrus orchards. Nematodes were extracted from two soil depths at ~ monthly intervals, DNA extracted, and target organisms measured using qPCR. Potential regulation of EPN by the bacterium or nematodes was assessed in multiple ways. *Acrobeloides* group species were inversely related to the monthly population flux of *S. diaprepesi* at two of three sites containing the nematode. *Paenibacillus* sp. exhibited significant phase-space (predator-prey) dynamics at two of those sites. The initial abundance of both *S. diaprepesi* (positive effect) and *Paenibacillus* sp. (negative effect) explained significant variation in population changes of *S. diaprepesi* in a multiple regression model at a lag of 3 months. Soil pH in these surveys was directly related to the infestation rate (encumbrance) by *Paenibacillus* sp. and inversely related to the abundance of *S. diaprepesi*. Complementary laboratory experiments showed that pH is directly related to adherence of the bacterial endospores to the *S. diaprepesi* cuticle. These results suggest that soil pH management can be used by growers to conserve the demonstrated services of *S. diaprepesi*. They also suggest a need for better understanding of potential non-target effects of practices favourable to bacterivorous nematode competitors of EPN.

**Life history table and video on the life cycle of the entomopathogenic nematode *Heterorhabditis bacteriophora***

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*Heterorhabditis bacteriophora*, symbiotically associated with the bacterium *Photorhabdus luminescens*, is commonly used against insect pests. Dauer juveniles (DJ) develop into self-fertilising hermaphrodites, which lay eggs until juveniles hatch inside the uterus and feed on the body content of the mother (*endotokia matricida*). The life history traits (LHT) of *H. bacteriophora* were studied at  $2.5 \times 10^9$ ,  $5 \times 10^9$ ,  $10 \times 10^9$  and  $20 \times 10^9$  cells ml<sup>-1</sup> of *P. luminescens* at 25°C using a hanging drop technique. The number of offspring produced per hermaphrodite increased from 50 at  $2.5 \times 10^9$  cells ml<sup>-1</sup> to 269 at  $20 \times 10^9$  cells ml<sup>-1</sup> of *P. luminescens*. At 25°C and  $20 \times 10^9$  cells ml<sup>-1</sup> of *P. luminescens* approximately 40% of the offspring of the hermaphrodite originated from *endotokia matricida* and the others from eggs laid before entry into *endotokia matricida*. Almost 100% of the offspring of amphimictic females originated from *endotokia*. Data on LHT will be provided. Larvae of the invasive pest Western Corn Rootworm (*Diabrotica v. virgifera*) were infested with DJ and the development was recorded daily by video. The occurrence of hermaphrodites, egg laying, the development to second amphimictic adults and *endotokia matricida* in the cadaver of last instars of the beetle will be commented on based on the video.

### S30. Management of plant-parasitic nematodes

Convenors: Soledad Verdejo Lucas & Johan Desaegeer

#### ***In vitro* susceptibility of root-knot nematodes (*Meloidogyne* spp.) and entomopathogenic nematodes to Alltech crop science formulations**

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This work is part of larger collaborative project between IT Carlow and Alltech, on sustainable nematode management alternatives. Two commercial formulations of Alltech, one a proprietary blend of fermentation products, plant extracts with micronutrients (ACS-5075) and second microbial based product (ACS-3048), were evaluated against two species of root-knot nematodes (RKN), *Meloidogyne incognita* and *M. javanica*. Preliminary bioassays were conducted to determine lethal and sub-lethal concentrations (LD<sub>50</sub>) of these products against three strains of entomopathogenic nematodes (EPN): *Steinernema feltiae* (SB12 (1)), a wild enviroCORE strain and a commercial (e-NEMA) and *Steinernema carpocapsae* (commercial, e-NEMA). The sensitivity of EPN was tested by estimating percentage mortality of infective juveniles (IJ) after 24 h treatment with dilutions of the products (ACS-5075 & ACS-3048). Subsequent experiments with RKNs were conducted at dilutions below EPN LD<sub>50</sub> concentration to determine RKN sensitivity to the products. In laboratory tests, effectiveness of both products was recorded in terms of RKN hatching and juvenile motility at different concentrations compared to non-treated controls. ACS-5075 was found safe for EPN upto 4% concentration. However, a reduction in hatching of RKNs from 100% in non-treated, to 10, 0, 0 and 0% hatching at 0.5, 1, 2 and 3% concentrations of product, respectively, was observed. A 10-fold reduction in juvenile motility was observed in 0.5% treatment, and motility of *M. javanica* dropped to zero in concentrations 1% and above. Juvenile motility of *M. incognita* was zero in all treatment concentrations. Survival of 100% was found in all strains of EPN treated with up to 80% of ACS-3048 product, whereas, 100% mortality of *M. incognita* juveniles occurred when treated with a 10% concentration. Our results indicate that RKN were more sensitive to ACS-5075 compared with ACS-3048. However, both products could provide sustainable solutions for RKN management and potentially prove to be safe for EPN.

## **Plant-parasitic nematode management in sub-Saharan Africa through ‘Wrap & Plant’ technology**

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Plant-parasitic nematodes are one of the major constraints on production and yield of food crops throughout the world. We present an innovative and cost effective ‘wrap & plant’ methodology for crop protection from plant-parasitic nematodes using abamectin as a model pesticide. In this approach, we have developed banana paper based matrices loaded with very low amounts of abamectin, as wraps for the seed pieces to be planted in the soil. To understand the effect of various processing parameters on the final properties of the matrix, we have produced banana paper *via* mechanical refining of the pulp for different time periods. The controlled release of abamectin is then used as one of the functional assays of the resulting paper. Pulp refining time as well as various properties of the matrix are found to affect its tendency to: *i*) allow germinating root penetration; and *ii*) the release rate of abamectin. We perceive that by varying processing conditions of the pulp, we can obtain matrices that facilitate the slow and sustained release of abamectin, which results in long term protection to the growing plant roots. We have used these results to develop pilot scale paper rolls which are currently used in yam and potato field trials in Benin and Kenya, respectively. Initial field trials demonstrate promising results: better crop protection in nematode infested soils with significantly improved yield, and reduction in tuber spoilage during storage.

## Opportunities and constraints for nematode management in Florida strawberries

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Florida ranks second in the USA in the production of strawberries. The state produces between 10 and 15% of the total USA crop, and 100% of the domestically produced winter crop. Nematodes are a major constraint to strawberry production in Florida. Sting nematode, *Belonolaimus longicaudatus*, is the most widespread plant-parasitic nematode in Florida strawberry fields, but several other damaging nematodes (*Meloidogyne hapla*, *Pratylenchus penetrans* and *Aphelenchoides besseyi*) have become more prevalent as well. Increasing nematode problems in Florida strawberries are probably linked to the loss of methyl bromide, and warmer temperatures favouring nematodes in nurseries and production fields. Fumigants remain the primary nematode management tool in Florida strawberries. However, because of the long growing season, and the lack of post-plant crop rescue practices, plant-parasitic nematode populations often rebound after fumigation later in the season. In addition, fumigant labels have changed significantly in recent years, and with residential developments rapidly expanding around Florida's strawberry fields, the use of fumigants will become more difficult in the future. Increased occurrence of nematodes such as *M. hapla*, *P. penetrans* and *A. besseyi* (as well as other pests and diseases) in transplants coming from strawberry nurseries is also a growing concern for Florida strawberry growers, and raises the need for new methods to prevent further introductions of these nematodes in Florida. Until recently no nematicides were registered on strawberries in Florida for at- or post-plant use. Fortunately, nematology is now back on the radar of many crop protection companies, and new chemical and biological non-fumigant nematicides (e.g., fluensulfone, fluopyram and *Burkholderia*) have recently become available to growers in Florida and elsewhere. These new nematicides are currently being evaluated in a wide range of crops, including strawberries, and will hopefully provide Florida strawberry growers with some much-needed new options.

## Accession of wild Cicer species (*Cicer reticulatum* and *C. echinospermum*) resistance for plant-parasitic nematodes

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The root lesion nematodes (*Pratylenchus thornei*, *P. neglectus*) and *Ditylenchus dipsaci* are an economically important pest described as one of the major limiting factors in chickpea production. RLN produce severe lesions on chickpea roots, and can be managed effectively by the use of resistant cultivars. In this study 273 Accession of wild *Cicer* species (*Cicer reticulatum* (n=63) and *C. arietinum* (n=210) were conducted against to root lesion nematodes and *Ditylenchus dipsaci* for resistance genotype. All genotypes of chickpea were sown within the same trial condition and experiment were assessed on the basis of either final population density of nematodes or multiplication rate in soil. The wild relative and chickpea cultivar experiments had a range of nematode densities the soil and roots after growth under greenhouse conditions. Observation of root and soil indicated that almost of all chickpea lines were affected by the *D. dipsaci* nematode and there were differences between lines of *Cicer echinospermum* and *C. reticulatum* indicated that *C. echinospermum* was more resistant than *C. reticulatum* to *D. dipsaci*. As a result of this research showed for us, both species of chickpea (*C. echinospermum* and *C. reticulatum*) have similar responses and were sensitive to *P. neglectus*. However, there was considerable genotypic variation for these responses in *C. reticulatum*, such that some genotypes may be more resistant than *C. echinospermum* to *P. neglectus*. As a result of this research showed for us, both species of chickpea, (*C. echinospermum* and *C. reticulatum*) have similar responses and were sensitive to *P. thornei*. However, there was considerable genotypic variation for these responses in *C. reticulatum*, such that some genotypes may be more resistant than *C. echinospermum* to *P. thornei* and *C. reticulatum* was more resistant than *C. echinospermum* to *P. thornei*.



**Pathogenicity of *Meloidogyne incognita* and two fungal pathogens: *Fusarium oxysporum* and *Pythium aphanidermatum* on soybean (*Glycine max* L. Merr.)**

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Soybean (*Glycine max* L. Merr.) with its nutritional attributes and applications in family delicacies like soymilk, soyogi, moin-moin, puff puff, akara etc., makes it a very healthy nutritional grain. Useful phytochemicals in soybeans provide immunity against cardio-oxidative reactions, and act as anti-clotting agents and antioxidants while exerting anti-inflammatory effects in the body. Unfortunately, optimised local production is threatened by virus, nematodes and fungi attacks. The root-knot nematode *Meloidogyne incognita*, *Fusarium oxysporum* f.sp. *vasinfectum*, *Pythium aphanidermatum* in single and combined infections, constitute some of the foremost limiting influences on the growth and yield of the crop. This work investigated effects of single and combined infections of each pathogen on growth and yield components including *Bradyrhizobium* root nodules in screen house and field experiments. The most significant single reduction was by the nematode; while *P. aphanidermatum* exerted the most significant growth suppressions in comparison with that caused by *F. oxysporum* only. Significantly higher growth and yield suppressions occurred with *P. aphanidermatum* than with *F. oxysporum* in simultaneous or successive infections with the nematode and each of the fungi pathogens. Reductions by combination of the nematode and respective fungus, were not statistically different in the screen house experiment but were for most parameters in the field trial. In both experiments, number of galls and second-stage juveniles of the nematode were more significantly reduced in combined than in single infection with only nematode. Reductions in the above parameters were significantly higher for the nematode and *P. aphanidermatum* complex than with the nematode and *F. oxysporum*. These outcomes established the threat status and dynamics of these pathogens to the optimisation and sustainability of soybean cultivation and propagation in a country like Nigeria. This scenario, further compounds the challenge of attaining food sufficiency in protein nutrition in Nigeria.

# POSTER SESSIONS



MONDAY, 10<sup>TH</sup> SEPTEMBER

Posters Systematics 1

Posters Ecology 1

Posters Management 1

Posters Biological Control 1

Posters Plant-Nematode Interaction 1

Poster Animal Parasites and other Subjects 1

## Posters Systematics 1

### **S01\_Phylogeny of entomoparasitic nematodes of the genus *Heterorhabditis***

Manoj Dhakal<sup>1</sup>, Khuoung B. Nguyen<sup>2</sup>, David Hunt<sup>3</sup>, Ralf-Udo Ehlers<sup>4</sup>, Sergei E. Spiridonov<sup>5</sup> and Sergei. A. Subbotin<sup>6</sup>

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The genus *Heterorhabditis* contains 16 valid species. In our study we used samples from eleven species: *H. amazonensis*, *H. bacteriophora*, *H. baujardi*, *H. becherriana*, *H. downesi*, *H. floridensis*, *H. georgiana*, *H. indica*, *H. megidis*, *H. noenieputensis* and *H. zealandica* to amplify and sequence five gene fragments: the D2-D3 expansion segments of 28S rRNA, ITS rRNA, *COI*, thin filament (F-actin) associated protein (*unc-87*) and calmodulin (*cmd-1*) genes. Sequences of the D2-D3 expansion fragments of 28S rRNA, ITS rRNA and *COI* genes differentiated all species from each other. Phylogenetic analysis of these genes using Bayesian inference, maximum likelihood and statistical parsimony confirmed a division of the genus into three clades (groups): '*Indica*', '*Bacteriophora*' and '*Megidis*'. The analysis of several hundred ITS rRNA and D2-D3 of 28S rRNA gene sequences downloaded from GenBank and identified as *Heterorhabditis* revealed several cases of species misidentifications and presence of reading mistakes in some published sequences. Molecular analysis did not reveal nucleotide differences in the ITS rRNA gene sequences of *H. somsookae* Maneesakorn *et al.*, 2015 with those of *H. baujardi*, *H. sonorensis* Stock *et al.*, 2009 with *H. taysearae*, *H. pakistanense* Shahina *et al.*, 2017 with *H. indica*. Application of molecular data and some phylogenetic methods for differentiation of sibling species for some genes are discussed.

### **S03\_New and known nordiids (Dorylaimida, Nordiidae) from Iran**

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Three species of the family Nordiidae were recovered from natural or agricultural regions of Iran. The first species, *Longidorella (Saevadorella)* sp., represents an unknown species and was recovered from the rhizospheric soil of grasses in Mazandaran province. It is characterised by 788-874 µm long females, lip region having prominent papillae and separated from the rest body by constriction, 32-33 µm long odontostyle, vulva at 52.5-59.0%, and 33-38 µm long tail having a rounded tip and males with 32-35 µm long dorylaimoid spicules and 5-7 ventral supplements ending at 48-55 µm distance from cloacal pair. The new species was morphologically compared with four species: *L. (S.) saadi*, *L. (S.) magna*, *L. (S.) cuspidata* and *L.(S.) arenicola*. The second species, *Pungentus* sp., belongs to an unknown species, characterised by 2082-2365 µm long females having angular lip region separated from the rest body by a constriction, 33-34 µm long odontostyle, vulva at 43.46-50.8%, and 29-31 µm long tail and was morphologically compared with four species: *P. minnsi*, *P. angulosus*, *P. thornei* and *P. crassus*. The Third species, *P. engadinensis* was also recovered in three different regions of the country (Mazandaran province, city of Damghan and Arasbaran forests). It was morphologically compared with other populations reported all over the world. Besides morphological studies, molecular phylogenetic studies using partial sequences of 28S rDNA D2/D3 fragments were performed and the phylogenetic relations of them with other species and genera were discussed.

**S06\_New morphological, ultra-structural and molecular insights of native Sheathoid nematodes (Nematoda: Criconematidae) from China**

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Sheathoid nematodes of the genus *Hemicriconemoides* are migratory root ectoparasites of many crops but the damage is documented for only a few species. Species of *Hemicriconemoides* are widely distributed in China. To date, 19 species have been reported from different provinces. In the present study, two known and a new species of *Hemicriconemoides* have been characterised morphologically and molecularly. The SEM observation revealed that *H. parachamelliae* n. sp. and *H. parasinensis* have lip patterns of type 1 while *H. fujianensis* has lip pattern of type 2. *Hemicriconemoides parachamelliae* can be characterised by the lip region continuous and possess two annuli, the first lip annulus expanded and slightly wider than the second one. The stylet is 83.0 (80.0-85.0) µm long and the excretory pore is located 5-6 annuli posterior to the base of the pharyngeal bulb. Vulva a distinct slit without vulval flaps, anus located 5-8 annuli posterior to vulva. Tail conoid, terminal annuli elongated and ending into a finely rounded tip. This is the fifth described species from China indicating that the known diversity of *Hemicriconemoides* species has increased. In addition, the molecular information for these species is also updated. Our new descriptions based on molecular sequencing data, light and scanning electron micrographs will assist in future identifications and such an approach is imperative for the reliable identification of sheathoid nematodes.

**S07\_Proposal for two new species of the genus *Labrys* Qing and Bert, 2018 (Rhabditida, Tylenchidae)**

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The genus *Labrys* Qing & Bert, 2018 is apparently one of the rarest nematode genera, currently monotypic, and the type species is only known from its type locality and Iran. The genus is distinguished from other tylenchid genera by possession of a unique offset labial plate, which is constricted dorso-ventrally, tapering towards both tips and detached from the adjacent cuticle with amphidial apertures that appear as short longitudinal slits in scanning electron microscopy (SEM) images. Its typological identification mainly relies on detailed characterisation of this labial plate. Molecular data is, however, available for the type species, and molecular phylogenetic affinities of putative *Labrys* species could corroborate their generic status. During extensive studies on the tylenchid fauna of Iran, two populations of the genus, representing two new species, were recovered from northern (Gilan province) and southern (Khuzestan province) Iran. Detailed morphological studies using SEM confirmed their congeneric status. The Gilan population has females with 425-463  $\mu\text{m}$  long body, *c* value range of 3.5-4.0, *c'* value range of 13-16 and *V* value range of 58-62 (n=8). The Khuzestan population has 590-675  $\mu\text{m}$  long females with *c* value range of 2.6-3, *c'* value range of 23.5-33 and *V* value range of 48-54 (n=11).

**S09\_ *Heterodera sojae*, a new cyst-forming nematode record in China**

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In a survey for cyst nematodes in Jiangxi Province, China, a cyst nematode population was collected and extracted from soybean roots and rhizosphere soil. The morphological characteristics of this population are different from that of the soybean cyst nematode (*Heterodera glycines*). Morphological and molecular characterisation of this population were conducted. For light microscopic observations, specimens were mounted in permanent slides. For molecular studies, single cyst from soybean roots was picked and then ground to extract the genomic DNA. The ribosomal RNA-ITS region and the D2-D3 expansion segments of 28S large subunit rRNA gene were amplified with universal primers AB28, TW81 and D2A, D3B respectively. The sequencing results were deposited in the GenBank database and compared with nematode sequences using the BLAST homology search program. Morphological characteristics of cysts, second-stage juveniles and males of this study population agreed with *H. sojae* reported in Korea. Sequences from the ITS-rDNA (MG859982) was 99% and 98% identical to those of *H. sojae* from Korea (KU160510 and KU160512) and 81% identical to the soybean cyst nematode (*H. glycines*) (KY794762.1). The D2-D3 (MG859981) was 99% identical to *H. sojae* from Korea (KU160511). In addition, analysis of phylogenetic trees constructed by the ITS and LUS D2-D3 both showed that the Jiangxi population and *H. sojae* were grouped in the same clade with a reliability of 100%. The cyst nematode population from Jiangxi was identified as *H. sojae* - a new record species in China.



### **S11\_A study of the carrot cyst nematode, *Heterodera carotae*, in South Africa**

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Carrot (*Daucus carotae*) is one of the four major root and tuber vegetables consumed in South Africa. The carrot crop therefore contributes much to the gross value of agricultural production in the country. In 2008 *Heterodera carotae* (carrot cyst nematode) was first reported from the Tarlton area, with concomitant crop damage. This nematode is generally considered a European species and no previous studies had been conducted on *H. carotae* under South African or African conditions. This presentation deals with a morphological, morphometrical and molecular study of population of *H. carotae* in the Tarlton area, and results from this study are compared with those of European and Canadian populations. The Tarlton population is considered to be conspecific with *H. carotae*. We consider the observed differences as lying within the range of intraspecific variation of *H. carotae*. Of interest is the fact that we were able to take SEM photo micrographs of the females, males, second-stage juveniles and cysts, which, to our knowledge, have never been published before. We therefore consider the SEM study a good contribution to the morphology of *H. carotae*. To determine the number of generations that has been completed by *H. carotae* during a carrot-growing season in South Africa, the life cycle was investigated under field conditions. This involved weekly collections of root and soil samples, from seedling emergence to harvesting (about 18 weeks), extraction of all nematode life stages, and calculation of the abundance of each developmental stage. The soil temperature was recorded throughout the experiment. The time periods and accumulated day-degrees above the basal developmental temperature (10.0°C) required by *H. carotae* to reach different life cycle stages are graphically presented.

**S12\_Genetic diversity analysis of *Heterodera avenae* populations from China based on microsatellite markers and mitochondrial DNA**

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The cereal cyst nematode *Heterodera avenae* causes severe damage to wheat and oat production and is considered to be a major pathogen to agriculture worldwide. *Heterodera avenae* was identified for the first time in Hubei province of China in 1989 and it is now widely distributed in almost all wheat growing regions. To elucidate the genetic differentiation, genetic structure, and demographic history of this parasite, nine microsatellite markers developed from EST database and the mitochondrial gene COI were used to investigate genetic diversity of *H. avenae* populations that were collected from 13 provinces in China. SSR data revealed a very low level of polymorphism in each of the 13 populations, most of the genetic variations was observed within the populations. The POPTREE, STRUCTURE and principal coordinate analysis (PCoA) revealed three genetic clusters: northwest China, northern China and central China group. BayesAss analysis showed very limited gene flow existed between each two of the tree groups. Gene flows from northwest as well as central China to northern China were greater than in the opposite directions, while lower and symmetric gene flow existed between northwest and central China. Meanwhile, a total of nine haplotypes were detected in the examined COI gene sequences, and high genetic diversity was found among the different populations. The haplotype H1 was the most common haplotype and shared by 172 individuals and present in 11 of the 13 populations, indicating it is most likely to be the ancestral haplotype. Neutrality testing and mismatch distribution analysis provided strong evidence for a recent rapid expansion in central China group, while a relatively stable geographical distribution in other two groups. In addition, the Mantel test for mitochondrial and microsatellite data both showed a significant relationship between the genetic distance and geographical distance. The present work can help us to deploy strategies for the control of *H. avenae* in China.

### **S13\_A new quarantine root-knot nematode - a potential threat to Portugal and Europe**

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In 2017, during a survey conducted in Portugal on backyards and public gardens, an unusual esterase phenotype was detected in four root-knot nematode (RKN) isolates obtained from infected roots of *Cereus hildmannianus* (Cactaceae), *Lampranthus* sp. (Aizoaceae), *Physalis peruviana* (Solanaceae) and *Callistemon* sp. (Myrtaceae) collected near Coimbra, Portugal. The isolates were maintained on tomato, 'Coração-de-Boi', and characterised biochemically and molecularly. The esterase phenotypes had a unique pattern of four bands (two major and two minor and fainter bands) (Rm: 0.31; 0.33; 0.39; 0.42) corresponding to the phenotype M4. Although the esterase phenotype allowed its differentiation from other *Meloidogyne* species and was similar to that of *M. enterolobii* (= *M. mayaguensis*), species-specific primers (MK7-F/R) were used to confirm the identification. Amplification yielded the expected fragment of ≈520 bp. Complementary studies are being conducted to validate the identification. *Meloidogyne enterolobii* is an important obligate biotrophic plant-parasitic nematode, included in the EPPO A2 List of pests recommended, in 2008, for regulation as quarantine pests. This RKN species was discovered in China, in 1983, and has been spread and established in several countries of Africa, Asia and North, Central and South America. In EPPO region, *M. enterolobii* was reported in France (no longer present) and Switzerland (associated with severe damage on tomato/cucumber) and intercepted in The Netherlands, Germany and UK several times associated with plant material from Asia, South America and Africa. After the validation of the presence of *M. enterolobii* in Portugal and taking into account its distribution, there is a high probability of spread not only in the Mediterranean region but also in Europe and becoming a threat to the agricultural economy, when, actually, there are no effective strategies for its control.

### **S14\_New record of the root-knot nematode *Meloidogyne luci* in Portugal**

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Several species of the genus *Meloidogyne* have been reported in Portugal: *M. arenaria*, *M. chitwoodi*, *M. hapla*, *M. hispanica*, *M. incognita*, *M. javanica* and *M. lusitanica*. In 2013, the tropical root knot nematode *M. luci* was also found in a potato field near Coimbra, Portugal. This was the first record of this species parasitising potato worldwide. In 2017, *M. luci* was added to the European Plant Protection Organization Alert List. In Slovenia, this species was detected in tomato roots first in 2003 and then in 2015. *Meloidogyne luci* was also found parasitising maize and kiwi in Greece and tomato in Italy. In Brazil, Iran, Chile, Guatemala and Turkey, *M. luci* has been found associated with several important vegetable plants and fruit tree species. New *M. luci* Portuguese isolates were found in *Cordyline australis*, and *Oxalis corniculata* and *Solanum lycopersicum* roots, in Coimbra district (Figueira da Foz and Montemor-o-Velho, respectively). These isolates were maintained on tomato and characterised biochemically and molecularly. The esterase phenotype from young egg-laying female protein extracts exhibited three bands correspondent to the *M. luci* L3 phenotype. Concerning the molecular analysis, cytochrome oxidase subunits I and II regions from mitochondrial DNA are being amplified by PCR, cloned, sequenced and compared with available *Meloidogyne* species sequences in databases. A survey for evaluation of *M. luci* distribution in Portugal is needed to decrease the risk of spread.

**S15\_ Description of a new cyst nematode, *Heterodera* sp. (Nematoda: Heteroderidae), from turfgrass in Korea**

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During a survey of plant-parasitic nematodes associated with turfgrass in Korea, a new grass cyst forming nematode, *Heterodera* sp., belonging to the *avenae* group and to the *H. avenae* complex was intercepted from Kentucky blue grass and rye grass samples. Cysts appeared lemon shaped, infrequently more round, dark brown, turning almost black with age. The species closely resembles *H. avenae*, *H. mani* and *H. pratensis*. Morphologically, the new *Heterodera* sp. differs from *H. mani* by the dark colour of cysts, long vulval slit (10.4-12.5  $\mu\text{m}$  vs 4-10  $\mu\text{m}$ ) and more flattened knobs, which occasionally appear slightly concave anteriorly compared to deeply concave knobs for *H. mani*. It differs from *H. avenae* by shorter cyst length (397.9-682  $\mu\text{m}$  vs 580-975  $\mu\text{m}$ ), shorter stylet of infective juveniles (20.1-22.4  $\mu\text{m}$  vs 24-28  $\mu\text{m}$ ) and a gradually tapering tail with a rounded tail terminus compared to sharply pointed tail terminus in *H. avenae*. It equally differs from *H. pratensis* by cyst length (397.9-682  $\mu\text{m}$  vs 530-800  $\mu\text{m}$ ), fenestral length (45.6 –49.6  $\mu\text{m}$  vs 18-24  $\mu\text{m}$ ), and stylet length of infective juveniles (20.1-22.4  $\mu\text{m}$  vs 24-25  $\mu\text{m}$ ). Molecular analysis of  $\beta$ -tubulin, D2/D3 segments and ITS gene sequence shows that the new species is closest to the grass cyst forming nematode, *H. mani*. The unknown species was intercepted at two sampling sites, each from a different golf course.

**S16\_First record, and morphological and molecular characterisation of *Longidorus crassus* and *Xiphinema* sp. associated with turfgrass in Korea**

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A survey in 2017, on the occurrence of plant-parasitic nematodes associated with turf-grass on Korean golf courses uncovered seven populations of *Longidorus crassus*, a nematode species known to be indigenous to North America, and *Xiphinema* sp. This study thus provided a detailed morphological, morphometric and molecular description of the newly recorded *L. crassus* and *Xiphinema* sp. populations in Korea. Females of *L. crassus* were characterised by the continuous rounded lip region, measuring 16.1-18.9  $\mu\text{m}$  in width; body length of 4275.1-5845.2  $\mu\text{m}$ , total stylet length of 162.9-174.6  $\mu\text{m}$ , V value of 49.1-52.2  $\mu\text{m}$ , vagina measuring approximately half of the body width, and a bluntly rounded, often hemispherical tail. No males were intercepted. Females of *Xiphinema* sp. were characterised by a C shaped body when gently heat killed, slightly set off lip region measuring 12.3-13.4  $\mu\text{m}$  in width and 7.2-8.3  $\mu\text{m}$  in height, a monodelphic reproductive system with a V value of 33.6-36.8  $\mu\text{m}$ , and an elongate tail, conoid and terminating in a bluntly rounded tail tip. Body length and total stylet length were 2768.4-3012.7  $\mu\text{m}$ , and 160.3-172.4  $\mu\text{m}$ , respectively. No males were intercepted in the samples. Species identification was molecularly confirmed by amplification of the D2/D3 expansion and ITS1 regions. Generated sequences were compared with the sequences available in the NCBI database and phylogenetic analysis was done to infer the phylogenetic positions of the reported species. The presence of *L. crassus* in Korea is reported here for the first time.

### **S17\_Confirmation of *Meloidogyne hispanica* on oriental melon in Korea using molecular and morphological diagnostics**

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Root-knot nematodes are widespread and cause much damage to oriental melon (*Cucumis melo* var. *makuwa*) in Korea. When crop rotation is used as a control measure, accurate identification of species of *Meloidogyne* is crucial. *Meloidogyne* species are quite similar in their morphological and biological data and are easily confused. During a survey on distribution of root-knot nematode species in Korea, PCR-RFLP analysis was used to identify 47 *Meloidogyne* populations collected from oriental melon in Korea. The region between *COII* and 16S rRNA of the mitochondrial DNA was amplified by polymerase chain reaction (PCR) using a single female. Production of a single fragment at 1,700bp was digested with *Hinf*1. Thirty-four populations producing 1300 bp and 400 bp fragments were identified as *M. incognita*. Thirteen populations were not digested with *Hinf*1. We further analysed the diagnostic restriction site between tRNA-his and l-rRNA region. This result accorded with the haplotype of *M. hispanica*, which has two restriction sites of *Mnl*1 and one restriction sites of *Hinf*1. The first indication was that the 13 populations might be Korean populations of *M. arenaria* because host differential test results and PCR amplification results of the *COII* region indicated *M. arenaria* race 2. However, tomato was a good host and tobacco, pepper and watermelon were poor hosts. All things considered, our results suggest that the 13 population might be *M. hispanica*.

**S18\_Description of new soybean cyst nematode, *Heterodera sojae***

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During a survey of plant-parasitic nematodes on soybean in Korea, a new species of cyst nematode was found from soybean root. Cysts of *Heterodera sojae* appeared more round, shining, and darker than that of *H. glycines*. Morphologically, *H. sojae* differed from *H. glycines* by fenestra length (23.5–54.2 mm vs 30–70 mm), vulval silt length (9.0–24.4 mm vs 43–60 mm), tail length of J2 (54.3–74.8 mm vs 40–61mm), and hyaline part of J2 (32.6–46.3 mm vs 20–30 mm). It is distinguished from *H. elachista* by larger cyst (513.4–778.3 mm<sup>3</sup> 343.4–567.1 mm vs 350–560 mm<sup>3</sup> 250–450 mm) and longer stylet length of second-stage juvenile (23.8–25.3 mm vs 17–19 mm). Molecular analysis of rRNA large subunit (LSU) D2–D3 segments and ITS gene sequence shows that *H. sojae* is closer to rice cyst nematode *H. elachista* than to *H. glycines*. *Heterodera sojae* was widely distributed in Korea. In 2016, 270 soil samples were collected from soybean fields and examined for the presence of *H. glycines* and *H. sojae*. A total of 111 samples contained cysts (41.1%). Among them 77% were *H. glycines* and 23% were *H. sojae*. *Heterodera sojae* is widely distributed in soybean fields and future studies are necessary.



**S19\_Morphological and molecular characterisation of a population of *Chitwoodius* Furstenberg & Heyns, 1966 (Dorylaimida, Tylencholaimidae) from Vietnam**

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A population of the genus *Chitwoodius* Furstenberg & Heyns, 1966, collected in coffee plantations from Central Highlands of Vietnam, is characterised. Observations with light microscopy show that, in their general morphology and morphometrics, the nematodes examined are very similar to *C. brevidens* Siddiqi, 2006, from which it can be distinguished by minor but apparently significant differences in body slenderness, lip region height, S<sub>1</sub>N position and tail length. SEM pictures, available for the first time for a representative of the genus, show a cap-like lip region with inner labial papillae located halfway between oral aperture and outer labial papillae, and a hexagonal oral field divided into six sectors (or hardly protruding liplets surrounding the oral aperture) by the existence of six deep radial incisures. Sequences of D2-D3 expansion segment of the rRNA large subunit are also presented for the first time for a representative of the genus. Their corresponding analysis results in a strong evolutionary relationship among *Chitwoodius* and *Tylencholaimus*, and supports the monophyly of the family Tylencholaimidae. This is the first record of *Chitwoodius* in Far East territories.

**S20\_A very rare malformation affecting the female genital system of one specimen of *Labronema* sp. (Dorylaimida, Dorylaimidae)**

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Several kinds of abnormalities or malformations affecting the female genital system of dorylaimid nematodes have been repeatedly reported in longidorid forms, but more occasionally in free-living taxa: totally duplicated female genital system, partial duplication of one genital branch in didelphic species, bivulval condition, total or partial reduction of one genital branch, etc. One female of the genus *Labronema*, tentatively identified as *L. carusoi*, presented one of the rarest anomalies as it lacks vulva and vagina. Apart from the absence of these elements of the system, the development of both genital branches is apparently normal and perfectly comparable to that observed in other females found in the same sample. Nonetheless, sperm cells —always abundant in normal females as the species is bisexual with both females and males nearly equally present— were not found within the genital tracts of the abnormal female. This is described and illustrated, and its morphology and morphometry compared to those of other (normal) females of the same population. A rather similar case has been reported in *Xiphinema dentatum*, but also with a significant reduction of both uteri. Vulvaless or Vul mutants were already described in the 1980s in *Caenorhabditis elegans*, an indication that the anomaly herein noted might have a genetic base. The female in question was collected from a grassy soil sample at 1800 m a.s.l. in Sierra de la Pandera, province of Jaén, south-eastern Iberian Peninsula, where its species is represented with a high number of individuals.

## S21\_First report of potato cyst nematode *Globodera pallida* infecting potato (*Solanum tuberosum*) in Kenya

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*Globodera pallida*, the pale potato cyst nematode (PCN), was detected in Nyandarua County (Kenya) in 2016 during a nationwide survey of potato fields. Morphometric characters were measured: Granek's ratio ( $n = 33$ ) ranged from 1.53 – 4.52  $\mu\text{m}$ , ( $\bar{x} = 2.78 \pm 0.78 \mu\text{m}$ ), and the distance from anus – vulval basin was 34.03 – 91.45  $\mu\text{m}$  ( $\bar{x} = 52.75 \pm 13.73 \mu\text{m}$ ). The stylet length of second-stage juveniles (J2s) ( $n = 97$ ) ranged from 15.87 – 25.18  $\mu\text{m}$  ( $\bar{x} = 21.87 \pm 1.43$ ), stylet knobs displayed a robust tulip/anchor-shape. The hyaline tail (HT) and true tail (TT) lengths ranged from 15.54 – 50.44  $\mu\text{m}$  ( $\bar{x} = 23.94 \pm 4.23$ ) and 31.02 – 79.59  $\mu\text{m}$  ( $\bar{x} = 50.64 \pm 5.71 \mu\text{m}$ ), respectively. Body length ( $n = 40$ ) fluctuated from 338.41 – 468.34  $\mu\text{m}$  ( $\bar{x} = 432.23 \pm 24.95$ ). Ten viable cysts were each inoculated onto potato (*Solanum tuberosum*) plants 'Shangi' in pots with five replications, using sterile soil: sand (1:1). Pots were maintained in a screenhouse for 3 months from May - July 2017. The multiplication rate at harvest was  $\bar{x} = 3.6$ , with PCN recovered from potato roots and soil. DNA amplification was performed from 14 cysts and 25 second-stage juveniles using ITS5/PITSp4 and AB28/TW81 primers. The Kenyan ITS5/PITSp4 sequences (NCBI accession no. MG309873) presented 100% similarity to the *G. pallida* isolates KJ409623.1 and AF016869 (Score = 481; E value = 5.02e-132), while the Kenyan AB28/TW81 sequence (NCBI accession no. MG309920) showed 95 and 94% similarity to the *G. pallida* isolates HF583248.1 and HQ670272.1 (Score = 1218 and 1221; E value = 0), respectively. This first report of *G. pallida* in sub-Saharan Africa has paramount phytosanitary and regulatory implications for regional stakeholders in Kenya and the surrounding region.

**S24\_ Reduced genetic diversity among *Meloidogyne graminicola* isolates suggests a recent worldwide expansion**

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*Meloidogyne graminicola* is a plant-parasitic nematode that seriously attacks rice. It was reported firstly in many countries in Asia and America, and was recently reported in Madagascar and Southern Europe. The worldwide distribution of the species raised questions of its population's dynamic, structure, and evolutionary history. In this research, a comparative genomic approach was conducted to investigate the intraspecific diversity among mitochondrial and nuclear genomic sequences of 13 geographically widespread *M. graminicola* populations. The mitochondrial genome (mtDNA) and nuclear ribosomal cluster (nrDNA) were assembled from HiSeq shotgun sequencing data. Only 26 mtDNA polymorphisms were detected (on *ca* 17 kb), including 14 single nucleotide polymorphisms, four indels and eight heteroplasmic sites. Each of these variable sites was unique to an accession (*i.e.*, autapomorphy), hampering phylogeographic reconstructions. When excluding heteroplasmic sites, eight haplotypes were detected among the 13 isolates. A haplotype network was reconstructed showing that the most common haplotype has a central position and is shared by Asian and South American isolates. In addition, two distinct nrDNA sequences (*ca* 8 kb) were reconstructed for each accession, but no polymorphism was detected among the 13 isolates. The low intraspecific diversity in both mitochondrial and nuclear genomes suggests a recent worldwide expansion of *M. graminicola*. To refine our research, we are currently studying the variability within different nuclear loci, including coding and non-coding regions, and exploring the rate of evolution of this species.

[Posters Ecology 1](#)

**E02\_Specific basal metabolic oxygen consumption rate measurement for the infective juveniles of entomopathogenic nematodes, using fibre-optic sensors**

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Entomopathogenic nematodes (EPN) are aerobic organisms and exposing them to low oxygen jeopardises their survival given that infective juveniles (IJ) are constantly exposed to micro aerobic or anaerobic conditions in the soil. Available information on the oxygen consumption among nematodes mainly relates to terrestrial, freshwater and marine nematodes, rather than EPN. This paucity in literature on the respiratory physiology of EPN is a hindrance, given the commercial interest they hold in terms of crop protection. Therefore, gathering information on specific oxygen demands of the nematode/bacterium complex of EPN, during production, storage and formulation maximises their utilisation. Nematode culturing in bioreactors has led to an awareness of their oxygen requirements, leading to a research focus on bioreactor designs and the nematode-bacterium complex. In the present study, fibre-optic sensors were used to determine, by means of basal measurement, the specific oxygen consumption rate (OCR) of the IJ of three locally isolated EPN species: *Steinernema yirgalemense*, *S. jeffreyense* and *Heterorhabditis bacteriophora*. Our results showed that nematode size inversely influences its OCR, whereby smaller nematodes with a higher surface area to volume ratio than larger nematodes have a higher OCR. *Steinernema jeffreyense* and *S. yirgalemense* did not significantly differ from each other in terms of the results, probably due to their proximity in size, with the former being slightly larger than the latter, but they did significantly differ from *H. bacteriophora*. However, the result could not be reflected in all batches for *H. bacteriophora* and *S. yirgalemense*, due to the variation encountered. The results provide baseline screening for comparing respiratory and metabolic physiology among EPN species, using the latest available technology (e.g., FOS).

**E05\_Are synthetic VOCs, typically emitted by barley roots, navigation signals for entomopathogenic nematodes?**

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We tested the chemotactic response of infective juveniles (IJ) of the entomopathogenic nematodes (EPN) *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *S. feltiae* to the synthetic volatile compounds (VOCs) (dimethyl sulfide, hexanal, 2-pentylfuran, and (E)-non-2-enal) typically emitted by barley roots. For the purpose of our investigation we used single VOCs and their blends. We hypothesised that attraction behaviour exhibited by the EPN toward the tested VOCs could be related to the species/strains and would vary with foraging strategy and VOCs. *Heterorhabditis bacteriophora* was the most mobile species in our assay. We confirmed differences among commercial and soil isolated strains of EPN. The movement of EPN toward different VOCs was influenced by the species/strain of EPN. Our investigation showed synergistic effect of dimethyl sulfide as an attractant for EPN. The data showed that chemosensation is more a species/strain-specific trait than a host searching strategy. All compounds tested in our assay influenced the movement of IJ, suggesting that synthetic VOCs, typically emitted by barley roots could play an important role in EPN navigation.

## **E06\_Assessing the incidence of plant-parasitic nematodes on potatoes, vegetables, strawberries and cereals in Southern and Central Norway**

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Plant-parasitic nematodes (PPN) appear to be an increasing problem in Norwegian agriculture and their efficient management is required. Methods of successful management have been sought by personnel involved in farmers' advisory service and among farmers. In order to clarify the nematode situation in different agricultural crops a project for assessing the incidence of PPN was carried out during the growing seasons 2016 and 2017. The Norwegian Agricultural Extension Service was responsible for selecting major crops and sites for sampling. A requirement was that the samples should be collected from crops showing symptoms of nematode damage (fields with patches of poor growth). In total, 100 samples (soil and plant roots) were collected from 14 counties and 46 municipalities. The crops sampled were potato (n=12), vegetables (n=37), strawberry (n=14) and cereals (n=37). The samples were brought to the nematode laboratory of NIBIO, and nematodes were extracted by the Seinhorst elutriator and mounted on microscope slides for morphological identification. Many species of free-living PPN were detected in association with damage across a number of crops including potato, vegetables, strawberry and cereals. Certain nematode groups (stunt-, spiral-, root lesion-, pin nematodes) were present in all crops, while *Longidorids* and *Trichodorids* were present in potato, vegetables and strawberry. The sheath nematode *Hemicycliophora* occurred only in carrot. Root-knot nematodes, *Meloidogyne hapla*, were found in carrots and *M. naasi* in wheat. Potato cyst nematode (*Globodera rostochiensis*) were found on one sample, carrot cyst nematode (*Heterodera carotae*) was found on one sample, cereal cyst nematodes (*H. filipjevi* and *H. avenae*) occurred in 13 samples. This is first record of *H. carotae*, in Norway. Although, there were large differences in the number of samples taken from different localities and crops, the higher nematode diversity in vegetables and potato compared to strawberry and cereals was notable.

**E07\_A survey of nematodes associated with declining pine trees wood in Freiburg (Southwest Germany)**

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A survey was conducted in Southwest Germany to determine the nematodes associated with declining pine trees wood. Wood samples were collected monthly between April 2014 and September 2017 from pine trees *Pinus nigra* and *P. sylvestris* standing in different locations in Freiburg. Nematodes were extracted with Baermann funnel method, quantified and classified according to their trophic structure. The nematodes were identified to genus and species level by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region. More than 15 nematode species were frequently extracted from the wood samples. The fungal feeder nematode *Laimaphelenchus penardi* was the most frequent in wood samples. The bacterivorous species: *Panagrolaimus facetus*, *P. rigidus*, *P. detritophagus*, *Panagrobelus stammeri*, *Plectus andrassyi*, *Pelodera* sp., *Acrobeloides* sp., *Acrobeles* sp., *Eucephalobus elogatus* and *Heterocephalobus elongates* were more abundant than other nematode feeding groups. The fungivores *Aphelenchoides* spp. and *Ektaphelenchus* were observed in many samples. The predator nematode *Micoletzkyia buetschlii* was associated with bark beetle infected trees. The entomopathogenic/fungivorous nematode *Deladenus siricidicola* was extracted from pine trees showing advanced declining symptoms.



## **E08\_Taxonomic structure of plant-parasitic and free-living nematode communities in agroecosystems of Dmanisi (Eastern Georgia)**

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Nematode communities are an integral part of the soil ecosystems and play an essential role in many biological processes. Traditionally, the vast majority of nematological research is devoted to plant-parasitic species, which cause significant damage to crop production all over the world. Agroecosystems are generally characterised by periodic perturbations in the soil, such as the use of pesticides and fertilisers, which hinder the natural succession. Each of these disorders has a specific effect, which can result in decreased diversity. In 6 different agroecosystems of Dmanisi (Eastern Georgia) a total of 141 species of nematodes belonging to 47 genera, 31 families and 8 orders were found. 101 forms were identified up to species. The largest variety of nematodes species was observed in Shindlari (N41.332697; E44.237023), 71 species, and the smallest was in Orozmani (N41.295694, E44.210649), 14 species. The richest by the number of species were from the order *Dorylaimida* represented by 57 species (40.4 % of species composition). One species *Prionchulus fistulosus*, Susulovsky & Winiszewska, 2002 was recorded for the first time in Georgia. As the quantitative composition of nematode communities does not give comprehensive information about their similarity, in different agroecosystems a symmetric matrix of values of the coefficient of Jaccard index was compiled. All the nematodes found can be divided into five groups according to their feeding habits: plant-parasitic, fungivorous, saprobiotic, predatory and omnivores. Plant-parasitic nematodes were represented by 21 species (14 % of species composition). The most common species were *Ditylenchus destructor* Thorne, 1945 and *Tylenchorhynchus brevidens*, Allen, 1955. The group of omnivore nematodes, which was represented by 61 species (43%), appeared to be the richest by the number of species. This group constituted the core of nematode communities. The represented ecological structure of nematodes is typical for both natural ecosystems and certain agroecosystems.

**E09\_Effect of piscivorous and omnivorous colonial birds' activity on soil biota structure, abundance and diversity in the Mediterranean region**

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The soil biota, including soil microorganisms and free-living nematodes, was investigated in the nesting and roosting habitats of the following piscivorous and omnivorous colonial birds: black kite (*Milvus migrans*), great cormorant (*Phalacrocorax carbo*), black-crowned night heron (*Nycticorax nycticorax*) and little egret (*Egretta garzetta*), in Israel's Mediterranean region. Abiotic variables, abundance, trophic structure, sex ratio and genus diversity of soil free-living nematodes and total abundance of bacteria and fungi were measured during the hottest period of 2016. The impact of the birds' activity on the soil biota was most notable in the upper soil layer and weaker in the lower soil layer. Soil properties such as alkalinity (pH) and conductivity were found to be among the main drivers influencing prey–predator relationships in the observed soil habitat by altering the predation success of soil free-living nematodes. The observed nematode species were affected directly or indirectly by the birds' nesting and roosting activity, as reflected by an increasing proportion of common nematodes and the disappearance of rare species in the colonial birds' habitats. Among them, the bacterial- and fungal-feeding nematodes were more sensitive to bird activity, with 67% and 42% susceptible genera, respectively. By contrast, the plant-parasitic nematodes (33%) and omnivore-predator nematodes (36%) were the least sensitive to the disturbance. The applied ecological indices indicated that the different species of colonial birds can have different (stimulatory or inhibitory) impacts on abundance and diversity of the soil biota, affecting the structure of soil free-living nematodes at the generic, trophic and sexual levels.

## **E10\_Evaluating the incidence of plant-parasitic nematodes in main potato and cereal production areas in Ain Defla Province of Algeria**

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In Algeria, potato and cereals are important food sources. In 2016, potatoes were cultivated on 156 196 ha yielding 4.5 million ton with an average of 306 198 kg ha<sup>-1</sup> and cereals were produced on 3 380 300 ha yielding 3.4 million ton with an average of 1 561 kg ha<sup>-1</sup>. Ain Defla province is a major potato and cereal producing area for Algeria, and is located in Midwest, having fertile soils, with semi-arid Mediterranean climate. Factors limiting the production are climatic conditions mostly irregularity of rains. Moreover, nematodes damaging roots generate considerable losses. Surveys in Ain Defla recorded the occurrence of potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, and cereal cyst nematodes (CCN) *Heterodera* spp. PCN species occurs separately or in mixed populations; pure populations of *G. pallida* occur commonly. Variations in nematode densities between localities and fields were noted. Ain Defla PCN populations showed intraspecific variation. The high divergence between both PCN species indicated the possibility of several introductions. In cereals, the species recognised are *H. avenae*, *H. latipons* and *H. hordecalis*, frequently found as mixed populations. *Heterodera avenae* is the dominant species and studies showed that hatching is characterised by three ecotypes. Some populations are adapted to low temperatures. Increase yields, farmer prosperity, and knowledge of nematode management is needed. The collaboration between the University Djilali Bounaama of Khemis Miliana and NIBIO has as priority: *i*) to evaluate the incidence and increase knowledge on nematodes affecting potatoes and cereals; *ii*) to conduct a survey to increase the current information in relation to distribution, population densities, and damage potential in different ecological conditions; and *iii*) to instigate studies on biology, occurrence of pathotypes, and cataloguing varieties with PCN and CCN resistance/susceptibility. This knowledge on nematodes in Algerian conditions is currently required to recommend appropriate management to farmers.

**E11\_Plant-parasitic nematodes on monumental trees at Belmonte arboretum, Wageningen, The Netherlands**

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Monumental trees are registered trees ([monumentaltrees.com](http://monumentaltrees.com)) of extraordinary size or beauty or at least 80 years old. In The Netherlands we have 5.958 registered trees (April, 2018), none of them has been studied so far for the presence of plant-parasitic nematodes. In 2017 and 2018 soil and root samples were taken on 87 monumental trees at the Belmonte arboretum, Wageningen, The Netherlands. The sampled monumental tree composition, at the arboretum, is a mixture of native trees, beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.), and exotic trees. We detected 16 different plant-parasitic nematode genera, with a total species number of 24. The most abundant species on beech and oak is *Hoplotylus femina* s'Jacob, 1960, a species described from Belmonte arboretum. Other common species are *Xenocriconemella macrodora* (Taylor, 1936), *Rotylenchus uniformis* (Thorne, 1949) and *Paratrichodorus pachydermus* (Seinhorst, 1954). New findings for The Netherlands are *Xiphinema pseudocoxi* Sturhan, 1985 on American beech (*Fagus grandifolia* Ehrh.) and *X. taylori* Lamberti *et al.*, 1992 on tree of heaven (*Ailanthus altissima* Mill.).

### **E13\_Structure of plant-parasitic nematode communities found in French production areas of root crops according to agricultural and environmental variables**

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Plant-parasitic nematodes are very abundant microorganisms that occur in every agrosystems and can have major impact on crop yields. With the current context of chemical pesticides reduction, it is important to find alternative ways to deal with those pests. To develop management strategies, knowledge about community structure and functioning is needed. Here we investigated 37 crop fields including at least one root crop in the rotation in nine French regions during two consecutive years to assess the potential effect of anthropic (such as crop rotations, soil works, pesticides) and environmental (climate, soil characteristics, such as pH or heavy metals) variables on plant-parasitic nematode communities. Those fields were chosen as they were sampled by French sanitary services for control of potential *Meloidogyne chitwoodi* occurrence because of risky crop rotations. We identified and counted 11 nematode taxa (10 genera and 1 family) in the different fields. Despite no significant abundance variations occurred between both sampling years, structures of communities varied among the French regions. We also collected 6 years' backgrounds of the fields from farmers and soil variables from a public database to assess their impacts on the whole communities (using redundancy analyses) and each taxon separately (using generalised linear mixed models). Our results suggest that, at large scale, environmental variables, more than cultural practices, seem to explain a great part of the significant differences between communities. We mainly highlighted significant effect of soil condition variables (heavy metals, acidity and carbon/nitrogen ratio) and climatic variables (rainfalls and temperatures). However, those variables had differential effect on the different taxa. We discussed how they can affect plant-parasitic nematodes either directly (effect on physiology for instance) or indirectly (by modifying food quality or food webs).

## **E18\_Monitoring and pathotypes distribution of potato cyst nematodes in Poland**

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Potato cyst nematode (PCN), *Globodera rostochiensis* and *G. pallida*, belong to quarantine pests attacking potato. Both species are subject to strict regulations and quarantine in many countries, even where they have not already occurred. Now *G. rostochiensis* is widely distributed in Europe. The first report of its occurrence in Poland comes from 19<sup>th</sup> century. In 1987 it was found in the territory of northern Poland and the pest spread towards the south-west. At present the pathotype Ro1 of *G. rostochiensis* is most common in Poland but the possibility of emergence of other pathotypes of *Globodera* spp. on Polish potato fields significantly increases due to easy transfer of potato tubers across countries. The nematodes pose a real threat for potato production, because of fast distribution in a new territory and long-term survival of cysts in soil. The eradication of cysts is troublesome and ineffective and planting of resistant potato varieties is the best way to limit the pest population in fields. The most frequently used source of resistance against nematodes is *Solanum tuberosum* ssp. *andigena*, the source of the *H1* gene, which confers resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis*. The multiresistant varieties are rare and the use of different resistant varieties in the succession may prevent the further replacement of species or pathotypes of *Globodera*. Therefore, the cultivation of potato varieties with multiple resistance for many pathotypes of *Globodera* spp. may influence the growth of entire PCN population. The main goal of our study was to assess the distribution of PCN and evaluation of resistances to nematodes in currently available set of cultivars in Poland.

**E20\_Horizontal distribution patterns of nematode assemblage in surface sediments of a seasonally hypoxic bay**

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Hypoxia in bottom water directly and adversely affects benthic life in marine ecosystem. In this study, we examined distribution pattern of free-living nematode assemblages in surface sediment of four sampling stations (St.5, 18, 20 and 21) in a seasonally hypoxic enclosed bay (Omura Bay, Japan) to reveal the influence of habitat heterogeneity and low oxygen conditions on the community diversity and functions. The bay experiences severe hypoxia every summer from the centre (St.21) and the south west area (St.20). The northern area (St.5) is less affected by hypoxia due to its proximity to the bay mouth. By contrast, the south-east area (St.18) represents a closed-off part of the bay that is heavily affected by anthropogenic inputs. Higher nematode abundance and diversity were found in St.5 than the other stations. Nematode communities were clustered into three groups by sampling station (St.5, 18, and 20 & 21) and there were significant differences in composition (one-way ANOSIM,  $r = 0.684$ ,  $P < 0.05$ ) and in feeding types (one-way ANOSIM,  $r = 0.323$ ,  $P < 0.05$ ) among the groups. These findings strongly suggest that diversity and functions of nematode assemblages are markedly affected by horizontal habitat heterogeneity and seasonal hypoxia in Omura Bay.

**E21\_Habitat filtering influences community structures of nematodes at *Cryptomeria japonica* forests in Japan**

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Japanese cedar (*Cryptomeria japonica*) is the most commonly planted species in Japan. Monocultural forests can lead to poor biodiversity, and thus decline in functional redundancy in forest ecosystems is a concern. Since some nematodes are affected by subtle environmental changes, the characterisation of their communities can serve as bio-indicators in certain ecosystems. The purpose of this study was to clarify the litter and soil nematode communities of Japanese cedar forests. For this aim, we collected both litter and soil samples at nine positions within 1 ha plot at seven cedar forests in central Japan. Free-living nematodes of the samples were extracted by the Baermann funnel and a tray technique for 48 h at 25°C. The extracted nematodes were counted under a stereo microscope to estimate density per 100 g of dried soil. Among the nematodes counted, 80–120 individuals were randomly selected for detailed morphological identification under a light microscope. Nematodes were divided into five trophic groups on the basis of the characteristics of their oral parts: bacterivorous, fungivorous, herbivorous, predaceous and omnivorous. A total of 47 and 62 nematode genera were identified from litter and soil samples whose abundances were  $159 \pm 21$  (50 ml litter)<sup>-1</sup> for litter samples and  $575 \pm 77$  (100 g dry soil)<sup>-1</sup> for soil samples. Bacterivorous and fungivorous nematodes were consistently detected in abundance, and were especially predominant at the litter sample accounting for  $46.9 \pm 2.9\%$  and  $30.8 \pm 2.8\%$  of relative abundances, respectively. For some soil samples, herbivorous nematodes dominated accounting for  $21.1 \pm 3.9\%$ . The NMDS scatter-plot showed that nematode communities were clustered significantly into those from either litter or soil samples. These results suggest that nematode trophic composition and community structures were filtered by habitat types.



**E23\_Potato crop infected with *Meloidogyne incognita* in AL-Qassim fields, Saudi Arabia**

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A potato (*Solanum tuberosum* var. 'Hermes') crop exhibiting extensive galling of tubers was observed in potato field near Buryidah City in AL-Qassim area, Saudi Arabia at the end of the growing season in 2017. Potatoes of different varieties ('Spunta', 'Lady Rosetta' and 'Hermes') have been grown for the last 8 years in a 33 ha field using a rotation with barley or corn crops. In 2017, yield (tubers) loss reached approximately 30 ton ha<sup>-1</sup> due to blemishing of tubers, caused by a species of root-knot nematode. More tuber blemishing and greater yield loss were observed in a 3 ha site of the field where the purple nutsedge weed (*Cyperus rotundus*) was present. Morphological identification of the species based on perineal patterns of freshly isolated females indicated the nematode was *Meloidogyne incognita*. Species identification will be further confirmed by molecular methods.

**E26\_First record of the root-lesion nematode *Pratylenchus thornei* (Tylenchida: Pratylenchidae) on strawberries in Serbia**

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*Pratylenchus thornei* Sher & Allen is considered to be important pest of cereals and can cause wheat yield loss up to 70%. This nematode was also recognised as one of the main parasitic nematodes on wheat in Serbia 50 years ago. Less is known about its impact on other crops. In 2014, the soil samples together with strawberry plants originating from Belasica, the central part of Serbia, were investigated in the Nematology laboratory of the Institute of Plant Protection and Environment. The strawberry plants were wilted and the root system was poorly developed and mostly necrotised. The samples revealed, beside other plant-parasitic species, the root-lesion nematode population of *Pratylenchus thornei*. The morphological characteristics of adult nematodes fitted well with the original description. Genomic DNA was amplified with TW81 and AB28. The obtained sequence of the ITS regions of the population (GenBank Accession No. KR002683) confirmed species identification with 99% homology with the *P. thornei* available in the database. This is the first record of *P. thornei* on strawberries in Serbia. Presumably, large scale damage was not caused by this nematode solely, since *Rotylenchus fragaricus* and *Meloidogyne* sp. were also observed and could create a synergistic disease complex.

## **E29\_Occurrence and distribution of plant-parasitic nematodes associated with bananas in Kenya**

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Ranked as the fourth most valuable crop globally, bananas (*Musa* spp.) are herbaceous plants grown by almost every household in Kenya on a subsistence basis, as well as in commercial plantations. Both dessert and cooking bananas are popular and grown in Kenya. Among various constraints, plant-parasitic nematodes (PPN) are important threats to banana production. To determine the occurrence and distribution of PPN on cooking bananas (East African Highland (EAH)) and dessert bananas (Cavendish), a nationwide survey was undertaken in major banana producing areas of Kenya; root samples were collected from over 300 banana farms. PPN were recovered from 100% of the farms with higher abundance of total nematodes observed in the western region, where altitudes are relatively high (>1600 m) compared with the other sampled areas. Overall, 'EAH' bananas appeared to be more affected by the nematodes, and at higher altitudes, when compared to 'Cavendish'. Across banana types *Pratylenchus* spp. were the most frequently occurring PPN (79%), followed by *Helicotylenchus* spp. (8%) and *Meloidogyne* spp. (4%). *Radopholus similis* was recovered only very occasionally. Although tail shapes of *Pratylenchus* spp. differ, indicating different species, preliminary molecular analyses show that *Pratylenchus goodeyi* is the most prevalent species affecting bananas, across altitudes. The study is ongoing, while results to date warrant efficacy studies to compare the effect of altitude (temperature) on the pathogenicity of *P. goodeyi* populations. Findings from this study can be used to develop effective management techniques against PPN on *Musa* spp. to enable farmers across various environments to best manage PPN, towards increased food security.

### **E30\_Free-living nematodes as affected by organic and conventional farming systems in Kenya**

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In Africa, while nematology remains understudied, most attention is focused on plant-parasitic nematodes. Often overlooked, however, are the free-living nematodes (FLN), which are of considerable importance in terms of nutrient mineralisation and as indicators of soil health. In a study conducted in Kenya, East Africa, the effect of long-term organic and conventional farming systems was assessed for their impact on the FLN communities. An organic system, which received compost, *Tithonia diversifolia* and neem cake (*Azadirachta indica*), was compared with conventional (fertiliser and nematicide), farmer practice (manure, *T. diversifolia* and wood ash) and a non-amended control on a maize/bean intercrop (first season) followed by a bean only crop (second season). Nematode diversity (genera) was highest in the organic system, for both seasons, with 21 genera recovered; most genera were bacterivores, followed by omnivores, predators and fungivores. Nematode abundance under organic farming was three times greater than in the other farming systems, with bacterivores most abundant in both seasons. Ecological and diversity indices varied significantly among the farming systems, whereby the highest maturity index, Shannon index, enrichment index and structural index were observed in the organic system, followed by conventional system and farmer practice. Principle response curves applied revealed that over time, organic farming was most effective in facilitating the buildup of FLN, particularly *Cephalobus* (bacterivores) and *Aphelenchoides* (fungivores), compared to conventional farming. In conclusion, organic farming supports much higher FLN densities and community diversity than conventional farming systems. This information provides evidence on the unfavourable effects of conventional farming using chemical inputs, which erode an important biological resource base. This information is useful to extension services to create awareness to farmers on such benefits.

**E31\_Unravelling the interaction between plant rhizosphere bacterial community and the occurrence of root-knot nematode**

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Root-knot nematodes (RKN, *Meloidogyne* spp.) are the most threatening pathogens of numerous crops worldwide. Soil microbiome is crucial for plant health. Understanding the roles plant rhizosphere soil microbiome plays during root knot nematode infection could provide insight into crop protection management strategies. In this study, the 16S rRNA genes of the bacterial communities of nematode infested and non-infested rhizosphere soils from tomato, eggplant, bitter melon and cucumber plants were sequenced by using the Illumina Hi-Seq platform. The non-infested soils had more microbial diversity than the infested soils from all the plant rhizospheres, and both soil types had exclusive microbial communities. Specific microbes from the phyla of Proteobacteria, Chloroflexi, Nitrospirae, and Gemmatimonadetes were uniquely present in non-infested soils. Moreover, the inoculation of the microbiomes from eggplant and cucumber non-infested soils to the rhizosphere of tomato plants significantly reduced the galls of root knot nematodes, while the microbiome from infested soil showed an increase of galls. Two bacterial strains belonging to *Pseudomonas* sp. and *Bacillus* sp. were screened out from the microbiome of non-infested eggplant soil with the potential for biological control of RKN infection on tomato. These results suggest that microbes may be regulating RKN infection in host plants.

## Posters Management 1

### **M01\_Bio-control strategies: in vitro evaluation of biodegraded bark of *Ficus mucoso* on *Meloidogyne incognita* eggs and juveniles**

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Agriculture is saddled with the menace of plant-parasitic nematodes; they infect all plant parts and cause reduction in yield. Synthetic nematicides have been used in the management of nematodes with impressive results but pesticide residues have been detected in fruits and vegetables, especially in developing economies. This disadvantage in pesticide usage has brought about interest in alternative methods of managing plant-parasitic nematodes. In this regard *Ficus mucoso* stem bark was collected, air dried and divided into two equal parts. One part was subjected to microbial action below the ground at 20cm deep for a month, after which it was exhumed and extracted in n-butanol, while the second part was extracted fresh in n-butanol. The crude extracts were chromatographed and the fractions were purified and characterised using FTIR and NMR spectroscopy. Crude extract and isolates were tested *in vitro* for nematicidal activity against *Meloidogyne incognita* eggs and juveniles. Significant ( $P=0.05$ ) differences were observed in the activity of the extracts and isolates. Strong nematicidal activity was exhibited by the isolates from the decayed extract (FCMO/DCD/CMP), with significant ( $P=0.05$ ) reduction in hatch and high juvenile mortality (77 and 86%, respectively). Substantial activity was observed in isolates from the (FCMO/FRS/CMP) fresh extract (59 and 64%), while the crude extract demonstrated moderate activity (31 and 40%). Methanamine and methyl-2 phenylindole was established as the constituents of the isolated fractions from the decayed extract, while cyclopentanone, hexadecanoic acid and cyclopropaneoctanal are confirmed in the isolated fractions from fresh extract. Spectroscopic results affirmed that microbial action on the buried stem bark produced a different compound which exhibited strong nematicidal action.

**M02\_Effect of biochar soil amendment on fenamiphos and cadusafos nematicides behaviour in sandy soil.**

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The effect of two types of biochar amendments, date palm (PBS) or eucalyptus leaves (EBS), on fate of fenamiphos and cadusafos applications to sandy soil were examined in laboratory and glasshouse conditions. Measurements included uptake by tomato plants, dispersion, and absorption onto soil particles. In a second experiment, the effect of fenamiphos on *Meloidogyne incognita* second-stage juveniles (J2) was tested in PBS. Addition of biochar decreased the degradation of both nematicides. Specifically, the half-life ( $T_{1/2}$ ) of cadusafos increased from 28.56 days in sandy soil to 163.86 and 151.47 days in 1% PBS and 1% EBS amended soil, respectively, under laboratory conditions. Fenamiphos  $T_{1/2}$  values increased about 200% by addition of biochar to soil in the laboratory, and 600% in glasshouse conditions. Sorption capacity as  $K_f$  was increased from 1.22; 0.39 ( $\mu\text{g}^{1-\text{Nf}} \text{g}^{-1} \text{ml}^{\text{Nf}}$ ) in sandy soil to 4.49; 6.84 in 1% PBS and 3.49; 4.62 in 1% EBS in cadusafos and fenamiphos, respectively. Addition of both biochar types slowed the downward mobility of both nematicides in soil columns. Both compounds leached from soil columns but were not detected in the initial leachate. Uptake of both nematicides by tomato plants was reduced by 97 and 85% for cadusafos and fenamiphos, respectively. The percentage of mortality counts of J2s was less in PBS (18.31%) than in SS (43.14 %) at 50% the recommended dose rate of fenamiphos. Mortality counts remained unchanged at the full dose rate of fenamiphos.

### **M05\_Post-infectious nematode resistance in sweet potato cultivars in South Africa**

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Worldwide, *Meloidogyne incognita* and *M. javanica* are widely distributed in tropical and subtropical regions, with huge yield losses reported in various crops, including sweet potato (*Ipomoea batatas*) in rural communities of South Africa. Following the withdrawal of synthetic chemical nematicides, nematode resistance was viewed as the most preferred strategy for managing high nematode population densities. Identification of post-infectious nematode resistance is important in cultivars intended for use in nematode management since it can be introgressed in plant breeding programmes. Thirty-seven sweet potato cultivars/lines were screened against *M. incognita* and *M. javanica* at the Agricultural Research Council in collaboration with local universities, with empirical-based evidence of non-host status among at least four cultivars/lines. Subsequent host-status and host-sensitivity studies confirmed that the cultivars/lines were resistant to the test *Meloidogyne* species, with introgressible post-infectious nematode resistance.



### **M07\_Exploring new class of chemical nematicides : Finding hits and its optimisation**

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The resulting record of less-than-perfect environmental or human health safety has resulted in the widespread deregistration of several agronomically important nematicides. The organophosphates, carbamates and fumigants, which have been traditionally used, are increasingly restricted due to environmental and toxic problems. Therefore, there is a constant demand for the development of a novel nematicide that exhibits a broad nematode control spectrum at low application rates as a solution to problems such as impact on the human health and global environment. But the development of chemical controls for plant-parasitic nematodes is a formidable challenge. Because most phytoparasitic nematodes spend their lives confined to the soil or within plant roots, delivery of a chemical to the immediate surroundings of a nematode is difficult. The outer surface of nematodes is a poor biochemical target and is impermeable to many organic molecules. Delivery of a toxic compound by an oral route is nearly impossible because most phytoparasitic species ingest material only when feeding on plant roots. Therefore, nematicides have tended to be broad-spectrum toxicants possessing high volatility or other properties promoting migration through the soil. Recently, we have discovered several hits with nematicidal activity against *Meloidogyne incognita* through assay of the chemical library (~8000 cpds) from Korea Chemical Bank in KRICT. Among them, the K-64302 compound gave a more than 90% mortality even at 5 ppm level (in vitro test) and it has novel structure that has not been researched. At this presentation, the process of selecting hits and the activities of K-64302 derivatives will be discussed briefly.

**M13\_Development and yield of potato 'Maris Peer' in soil infested with *Pratylenchus penetrans***

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The potato, *Solanum tuberosum*, represents one of the most important crops in Great Britain with 'Maris Peer' being the third most commonly grown cultivar. Fifteen species of root-lesion nematodes (RLN) are known to infect potatoes, although *Pratylenchus penetrans* is generally considered to be the most damaging species. The first controlled conditions experiment investigated the impact of populations of mixed juveniles and adults of *P. penetrans*, ranging from 0.125 to 4 nematodes g<sup>-1</sup> soil, on growth and yield of 'Maris Peer'. Different proportions of coarse sand and compost (John Innes No. 2) were mixed to form three different sandy based soils: ST1 (10% compost and 90% coarse sand), ST2 (20% compost and 80% coarse sand) and ST3 (40% compost and 60% coarse sand). Six weeks after inoculation and planting, the yield of potato was significantly affected by soil type with ST1 giving the lowest yield, but not driven by nematode densities. However, nematodes were detected within the roots of potatoes grown in each soil type, revealing that *P. penetrans* was able to invade this cultivar but without affecting the yield at these population densities. A second controlled experiment using a broader range of nematode densities, from 2 to 32 *P. penetrans* g<sup>-1</sup> soil is underway, to provide a better understanding of the pathogenicity and damage thresholds of *P. penetrans* with the 'Maris Peer' cultivar. The evidence gained from these studies should provide potato growers with indications of the threat from root lesion nematodes on potatoes.

**M14\_Nitrogen and sulphur inputs affect the performance of biofumigation with Indian mustard (*Brassica juncea*) in the suppression of potato cyst nematodes (*Globodera pallida*)**

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Potatoes are an important crop in Great Britain with 121,000 ha planted in 2017 achieving 6.04 Mt. The most important pest affecting this crop are the potato cyst nematodes (PCN) (*Globodera pallida* and *G. rostochiensis*), which occur in 48 and 35% of potato growing land (ware) in England & Wales and Scotland respectively. Uncertainty over the future availability of nematicides has resulted in research on alternative crop protection measures such as biofumigation. Biofumigation involves the use of brassicaceous inter-crops with a high glucosinolate (GSL) content. When macerated and incorporated into moist soil, an endogenous enzyme known as myrosinase (thioglucoside glucohydrolase, E.C.3.2.1.147) hydrolyses foliar aliphatic GSLs into an array of biocidal and volatile compounds including isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidine-2-thiones. As part of a 3-year Agriculture & Horticulture Development Board (AHDB) project investigating biofumigation for PCN management, the effect of variable rates of nitrogen and sulphur on the biomass of Indian mustard 'Caliente 99' (*Brassica juncea*), stem and leaf GSL accumulation and suppression of PCN were investigated under field conditions at a site in Crudgington, Shropshire, UK. Both nitrogen and sulphur application to *B. juncea* resulted in significant reduction in the viability of encysted eggs of PCN, 6 weeks after the brassica residues had been incorporated ( $P < 0.01$ ). Additionally, nitrogen significantly increased the fresh and dry weight of *B. juncea* stems and leaves ( $P < 0.01$ ). Based on the findings of this field experiment, we would recommend applying nitrogen at a rate of 100 kg ha<sup>-1</sup> with sulphur at a rate of 25-50 kg ha<sup>-1</sup> to summer sown Indian mustard for the reduction of PCN. Further analysis of stem/leaf GSLs associated with treatments in this experiment are in progress.

## M17\_Effects of Salibro™ on the nematode soil community structure

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The soil nematode community consists of different trophic and functional groups whose relative proportions might be modified by the activity of nematicides. A field trial was carried out to determine changes in the *Meloidogyne incognita* soil populations and the resident soil nematode community after the application of Salibro™ at three dosages: 0 (untreated control), 1000 and 2000 g a.s. ha<sup>-1</sup> and at harvest of a subsequent zucchini crop. The feeding type composition, trophic structure, and c-p classes before and after Salibro™ application, showed a dominance of bacterivore nematodes within the cp1 and cp2 classes, corresponding to disturbed and N-enriched soils, which concurs with that expected for an intensive agricultural conventional system with high inputs of fertilisers. Salibro™ at 1000 and 2000 g a.s. ha<sup>-1</sup> reduced root galling caused by *M. incognita* by 31-42%, their population densities in soil by 70-90% and those in zucchini roots by 39-47% at harvest. A 2-fold increase in the nematicide dosage did not increase its efficacy, when compared to untreated controls. The application of Salibro™ at 1000 and 2000 g a.s. ha<sup>-1</sup> had little effect on other soil nematodes under the experimental conditions, as assessed 4 days post-nematicide treatment. At harvest, 3 months after nematicide application and zucchini planting, the nematode community structure had changed by increasing the percentages of herbivores and decreasing those of bacterivores on the nematode assemblage in all treatments. Soils treated with Salibro™ at 1000 g a.s. ha<sup>-1</sup> showed an increase in the fungal energy channel in comparison with the untreated soil or that receiving 2000 g a.s. ha<sup>-1</sup>. Overall, Salibro™ showed good safety to non-plant-parasitic nematodes.

## M22\_The need for a strategic perspective in crop rotation

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Endoparasitic plant-parasitic nematodes (PPN), including migratory endoparasites such as *Pratylenchus* spp., cause yield reduction in agriculture and horticulture world-wide. In Norway, nematicides are banned due to their adverse effect on human health and the environment. Thus, management of PPN relies on cultural practices, such as crop rotation. Endoparasitic PPN tend to have broad host-ranges, which complicates the design of effective crop rotations. Information on the reproductive rate and damage potential of nematode species on different crops is of crucial importance when designing a successful crop rotation. Results from several experiments indicate that in order to reduce the numbers of PPN, the sequence of crops is more important than the length of the rotation. The crop rotation should aim at protecting the most economically valuable crop. An oat (*Avena sativa*) field in Norway was heavily infested with *Tylenchorhynchus dubius* (1200 ind. (250 ml soil)<sup>-1</sup>) and a low population of *Pratylenchus crenatus* (10 ind. 250 ml soil)<sup>-1</sup>). The primary goal was to reduce *T. dubius* by growing turnip rape (*Brassica rapa* ssp. *oleifera*), with carrot as the following crop. *Tylenchorhynchus dubius* was reduced by 77-85% after turnip rape. By contrast, the population of *P. crenatus* increased by more than tenfold. The increased numbers of *P. crenatus* could be damaging to the carrot crop. This illustrates that crop rotation should be a long-term strategy, with carefully designed rotations to protect the most economically valuable crop (e.g., carrot). This also illustrates the challenges of designing a crop rotation that effectively reduces multiple nematode populations. In a started project, we will use photography with Unmanned Aerial Vehicles and transects to monitor nematode populations and damage in several fields throughout the growing season, and over several seasons. These fields will serve as naturally occurring experiments. We want to develop decision-making tools for nematode management in Norway.

**M23\_A new technique for protection of packaging wood and timber against the pine wood nematode *Bursaphelenchus xylophilus***

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The Pinewood Nematode (PWN), *Bursaphelenchus xylophilus*, the etiological agent of the Pine Wilt Disease (PWD), is one of the worst plant pests of pines and coniferous trees in general. Over a short distance, beetles from the family *Cerambycidae* ensure PWN transmission; however, long distance transfer occurs between states and continents through the transport of packing wood and timber. It is therefore very important to treat all wood material from affected areas. Wood treatment primarily uses thermotherapy, which has recently proven to be less effective. The use of fumigants such as methyl bromide is ecologically unacceptable. This is why scientists around the world are evaluating other wood treatment methods. In our study, the effect of ethanedinitrile fumigation on the mortality of *B. xylophilus* was investigated. After the first trials in *Pinus sylvestris* sawdust in 50 ml polyethylene tubes, 100% mortality was achieved at 2 g for 4 h or 4.5 g for 2 h. At higher concentrations, efficacy was 100% after 0.5 h of action. This method is very promising from the point of view of ecology because the fumigant is decomposed to non-toxic compounds. This study was funded by the Czech Republic's Technology Agency TH02030329 New fumigation technology for the eradication of invasive and quarantine pests distributed in raw materials in the Czech Republic and the EU.

**M24\_Ethandinitrile as a methyl bromide alternative for root-knot nematode (*Meloidogyne* sp.) management**

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The Northern root-knot nematode, *Meloidogyne hapla*, is a key pest of root vegetable in the Czech Republic, especially in major production areas along the Elbe river. With restricted possibilities of chemical management after the methyl bromide ban, research efforts for alternative management techniques emerged. Ethandinitrile (EDN) is one of promising substances; its small molecule readily penetrates the soil, it possesses high and fast biological activity and rapidly decomposes into non-toxic compounds. Efficacy of EDN treatment was tested in pot experiments. Soil naturally infected with *M. hapla* was obtained from a field in central Bohemia and homogenised in a mixer and 200 g was placed into a plastic pot, transferred into fumigation chamber and treated by EDN at concentrations of 7, 12, 20 and 36 g m<sup>-3</sup>, for five exposure periods, 4, 12, 24, 36 and 48 h. Seven days after treatment carrot plants were planted into treated soil, grown until five true leaves had developed and the effect of EDN treatment was evaluated. Results show clear decreasing of *M. hapla* gall numbers with increasing of EDN concentration and exposure period. Increase of leaf weight was also detected in varieties treated by EDN at concentrations of 12 and 20 g m<sup>-3</sup> and 12 h, 24 h and 36 h exposure periods. Research was supported by Technology Agency of the Czech Republic, project number TH02030328.

**M25\_Plant-parasitic and non-parasitic nematode assemblages associated with commercial maize cultivars grown under conservation agriculture in South Africa**

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Maize (*Zea mays* L.) is the most important grain crop being produced in South Africa under diverse environments and practice systems. The omnipresence of plant-parasitic nematodes in local agricultural soils, however, poses a threat to the sustainable production of maize, soybean and other rotation crops. The aim of this research was to determine the nematode assemblages associated with 28 cultivars that were cultivated under conservation agriculture practices at Ottosdal in the North-West Province of South Africa. Root and rhizosphere samples of the 28 cultivars were obtained during three sampling intervals throughout the 2018 growing season. Eggs and second-stage juveniles of *Meloidogyne* and *Rotylenchulus* were extracted from 50 g roots using an adapted NaOCl method. Individuals of other nematode pest genera were extracted and identified from 5 g roots using the sugar-flotation method, while both plant-parasitic and non-parasitic nematodes were extracted from rhizosphere samples using the decanting and sieving, followed by the sugar-flotation methods. Substantial variation existed among the 28 cultivars in terms of population densities of *Meloidogyne* and *Rotylenchulus* per 50 g roots. A similar scenario was evidenced for other plant-parasitic nematode genera identified from 5 g root and 200 g rhizosphere samples of the 28 cultivars. The trophic groups of beneficial nematodes also varied substantially across the 28 cultivars and the three sampling intervals. The present study gave new insights regarding the host status of maize cultivars to *Meloidogyne* and *Rotylenchulus* spp. Furthermore, population densities of some nematode pest genera were exceptionally high in soil samples from this trial site, indicating that practising conservation agriculture can lead to a build-up in population densities of damaging nematodes. Results obtained from this study will enable nematologists to make informed decisions regarding cultivar use to combat nematode problems in maize-based cropping systems.



## M26\_A potential natural-based nematicide delivery system for root-knot nematode management

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The management of the plant-parasitic nematodes of the genus *Meloidogyne* (root-knot nematodes; RKN) is still being done by applying nematicides. Considering that these are being phased out, due to their harmful effects on human health and the environment, the first challenge of research on RKN is to find efficient and sustainable biomanagement strategies. The main goal of this study is to assess the effects of a developed natural-based nematicide delivery system (NDS) on the mortality of *M. hispanica* second-stage juveniles (J2). 1,4-Naphthoquinone, a bioactive compound from walnut husks residues with nematicidal activity, was impregnated in light weight expanded clay (LECA) pellets. Taking into account the release kinetics of the bionematicide soaked pellets and the in vitro effect of 1,4-naphthoquinone on *M. hispanica* J2 mortality, impregnated pellets were added to autoclaved soil (50 g dry matter per replicate) adjusted at 50% of soil water holding capacity, in glass vessels. Water and non-impregnated LECA were used as controls and each treatment was replicated five times. Soil was inoculated with  $\approx 170$  *M. hispanica* J2. After 5 days of incubation, in the dark at  $25 \pm 2^\circ\text{C}$ , the nematodes were extracted from soil using the Tray method and quantified. The J2 recovery rate in the controls was  $\approx 40\%$  of the initial population. A high impact of NDS on *M. hispanica* J2 was observed, causing 99% J2 mortality. The developed NDS potentially protects crops from nematode attack and damage and seems to be an efficient alternative for RKN management.

**M27\_BioNem Mulch - A new sustainable and integrated system for root-knot nematodes management in tomato crops**

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Root-knot nematodes (RKN), *Meloidogyne* spp., are important plant-parasitic nematodes in most agricultural areas, affecting productivity and quality of value crops, such as tomato. The use of synthetic nematicides is a frequent and efficient control strategy but there is an increasing concern on the risks associated to these chemicals, prompting the development of novel bionematicides, safer to humans and the environment than conventional pesticides. Recent studies have demonstrated that naphthoquinones have nematocidal activity but their applicability on RKN management should be further explored. Currently, mulching tomato plants is a common practice; nevertheless, the waste disposal of non-biodegradable mulches raises several environmental problems. Thus, the search for new biodegradable plastic mulches has been increased and new alternatives for enhancing sustainable and environmental friendly agricultural activities are strongly desired. The project BioNem Mulch focus on the development of a new integrated and sustainable strategy for RKN management on tomato crops, through the use of fully degradable-in-soil bionematicide delivery systems based on film-coated biodegradable plastic mulches, with phytochemicals. Project supported by POCI COMPETE 2020 – FEDER and “Fundação para a Ciência e Tecnologia” (Project nº 029392).

## **M28\_Gene expression analysis of *Meloidogyne luci* after exposure to 1,4-naphthoquinone**

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The increasing concern of producers and consumers about the risks posed by nematicides has stimulated research to the development of 'natural' nematicides, derived from plant extracts. 1,4-Naphthoquinone (1,4-NTQ), from *Juglans nigra* walnut husks, was found to have a strong nematocidal activity against root-knot nematodes, *Meloidogyne* spp., causing >40% mortality at 50 ppm. Two effector genes, manganese superoxide dismutase (*mnsod*) and glutathione-S-transferase (*gst*), belong to the defence against local ROS production and host defence suppression categories, respectively, were selected to evaluate *the impact imposed by 1,4-NTQ in M. luci* *gst* and *mnsod* gene expression. Primers used to amplify the *gst* and *mnsod* genes in *M. luci* were the same as used previously with *M. hispanica*. Partial gene sequences were obtained and their phylogenetic relationship analysed. Second-stage juveniles (J2) were exposed to Triton X-100 100 ppm (used as 1,4-NTQ solvent) or 1,4-NTQ 2.5 and 5.0 ppm concentrations, during 6 h. Water was used as control. After exposure, J2 total RNA extraction, quantification, reverse transcription into cDNA and semi-quantitative PCR experiments were performed. Actin gene was used as endogenous control. No amplification products were observed from cDNA of all treatments for *mnsod* gene. Amplification products of ≈100-bp were observed from cDNA for the *gst* gene. However, this gene exhibited weak amplification bands in Triton X-100, while, apparently, being equally amplified in cDNA from J2 exposed to water and 1,4-NTQ 2.5 and 5 ppm. New genes were selected and are being studied in order to understand the regulation mechanisms of RKN in response to environmental stressors, as 1,4-NTQ, and the effects of this compound on RKN life cycle completion.

## **M29\_FOCUS, improving detection of pinewood nematode infected trees through remote sensing methods**

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The negative ecological and economic impact of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, over Portuguese Maritime Pine, *Pinus pinaster*, stands requires implementation of decisive management actions. However, such actions are hampered by important logistic limitations to the systematic detection and mapping of PWN infected trees. Remote sensing technologies have been studied in recent years as a potential source of critical and actionable information to direct management efforts in an efficient way. Nonetheless, the challenges associated with the detection of infected trees are significant and require the use of multiple data sources, efficiently integrated into accurate data products. As a result, to this date, no operational products are available to forest managers despite several steps taken towards that goal. Project FOCUS (Forest Operational Monitoring Using Copernicus and UAV data) addresses these challenges by incorporating satellite (Sentinel-2, Landsat-8, and commercial Very High Resolution), Unmanned Aerial Vehicle (UAV), and airborne (APEX) data into an integrated detection methodology. New algorithms are being developed in the project, supported by the acquisition of multi- and hyperspectral data, and an intensive set of field surveys at multiple test sites in Portugal. Ancillary field data is also being collected, enabling the characterisation of baseline conditions and in situ host tree evolution through the decline process. Ultimately, the wealth of data collected and the products generated may shed light into dispersion patterns and the development of vulnerability maps for pro-active forest management. The project is funded by the European Union's Horizon 2020 program through Grant Agreement 776026.

### **M30\_Characterization of root-knot nematodes infecting tomato plants in Nepal**

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Root-knot nematodes (RKN), one of the economically most important plant-parasitic nematodes, cause severe crop losses all over the world. In order to develop and apply effective management systems, it is essential to be aware of the problem, to identify the nematode species, and to determine the impact on the host. Here we performed a survey through nine different districts of Nepal, with more than 60 farmers producing tomato questioned about their knowledge of RKN. More than 65% of farmers were aware of RKN, whereas 45% had less or no knowledge of them. Root samples including galls from several local varieties of tomato plants, such as 'Srijana', 'Samjhana', 'Dalila', 'Sarita' and 'Surya 111', were collected for identification of species. Currently, the samples are being analysed through both morphological and molecular techniques *i.e.*, perineal patterns and NAD5 sequences, respectively. Preliminary results reveal *Meloidogyne incognita* as the predominant RKN species. In parallel, we analyse the host response of three local tomato varieties, 'Srijana', 'Samjhana', 'Dalila' and 'Moneymaker' as control in glasshouse infection assays.

**M33\_Chemical mutagenesis and screening of new soybean mutants potential for identification of novel SCN-resistant genes**

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The soybean cyst nematode (SCN; *Heterodera glycines*) is a devastating pathogen in soybean production and causes a large quantity of annual yield loss worldwide. As the resistant soybean can shift during long-term interaction and domestication, it is vital to identify more new resistance genetic sources for identification of novel genes underlying resistance to SCN for management of this pathogen. We first developed two ethane methylsulfonate-mutagenesis populations from soybean 'PI 437654', which has broad resistance to almost all SCN races, and 'Zhonghuang 13', which confers strong resistance to lodging in China. Many types of morphological phenotypes such as four- and five-leaflet leaves were observed from these two mutagenised populations. Second, 13 mutants were forward genetically screened and confirmed from 400 mutant lines of the PI 437654 population, exhibiting alteration of resistance to SCN race 4, the mutant rate is 3.25%. Third, these identified mutants were further verified that there were no changes in the genomic sequences of the three known SCN-resistant genes, *GmSHMT08*, *GmSNAP18* and *GmSANP11* and that all of them were still resistant to SCN race 3, similar to the wild-type soybean. Taken together, we can conclude that the 13 mutants identified and confirmed in this work carry the mutations of the new gene(s) which contribute(s) to the resistance to SCN race 4 in PI 437654 and can be potentially used as the genetic soybean sources to further identify the novel SCN-resistant gene(s).

**M34\_The use of terpenes for the control of *Meloidogyne javanica***

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are a major problem reducing vegetable production in Greece. Since the withdrawal of methyl bromide, and in the absence of any replacement substance, the continuous use of chemical nematicides has raised environmental and health issues. Currently, there are only few nematicides left in use, and their repeated applications interfere with the mechanisms of enhanced degradation in soil, expressed in the field as inconsistent efficacy. Therefore, there is an urgent need for development of alternative nematode control methods, such as plant-natural products of secondary metabolism. Among these are terpenes, found as bioactive constituents in several plant essential oils which have been shown to have a broad range of biological activities such as antimicrobial, antioxidant, antifungal, insecticidal and nematicidal. In this study, we have evaluated the nematicidal activity of four terpenes: carvacrol, eugenol, geraniol and thymol. The optimisation of Phospholipid Fatty Acid (PLFA) analysis has been also evaluated in order to attain a picture of the size and the structure of the soil microbial community after applying the four terpenes to soil samples at different doses. Specifically, the nematicidal activity of terpenes has been tested in paralysis trials, hatch arrest and egg-development inhibition bioassays as well as in pot experiments after incorporation of terpenes in nematode infested soil at concentrations of 62.5 to 1000 ppm. Also, this study aimed to assess the sublethal doses (ED50) of terpenes on nematodes infecting tomato plants. It has been observed that the nematicidal activity could be correlated with the level of doses and the duration of immersion and incorporation of terpenes. Terpenes are a promising tool in plant protection and their use has shown an effect on soil microbial communities as recorded using the PLFA analysis.

### **M38\_Potential value of *Solanum linnaeanum* and *S. sisymbriifolium* for sustainable agriculture**

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*Plant-parasitic nematodes (PPN)* represent an important constraint for global food security, as they contribute to severe losses in crop production worldwide, reducing productivity and crop quality. PPN control is mainly achieved by cultural practices, crop rotation and resistant cultivars, combined with some available chemical pesticides that are still authorised. The need for discovering less toxic and environmentally acceptable substitutes for commercial pesticides is greater than before, generating a significant market opportunity for the development of alternative and biorational products such as botanical pesticides by screening naturally occurring compounds in plants. The Solanaceae are economically important and many species contain powerful alkaloids, highly toxic and known for their pesticide effect. The project deals with the improvement of potato crop based on *Solanum linnaeanum* and *S. sisymbriifolium* extracts for controlling PPN. The potato cyst nematodes (PCN), *Globodera* spp., and root-knot nematodes (RKN), *Meloidogyne* spp., are the most aggressive and widely disseminated PPN. Finding alternative methods to management those organisms is crucial. *Globodera pallida* is the most difficult PCN species to control and *Meloidogyne chitwoodi* is a quarantine species. In intensive agriculture, specialisation and economic impositions cause heavy disease pressure and intensive use of pesticides, leading to high production costs and environmental unfriendly production methods. This study will evaluate the use of plant extracts to control PPN leading to an alternative use of pesticides, in connection with the EU requirements for drastic reduction of the use of non-environmental friendly agro-chemicals. Selected Solanaceae extracts/fractions/compounds will be further analysed to discover whether they have any toxic effect in non-target organisms (animals/plants). The main goal of the proposed study is to evaluate the potential value of these *Solanum* species in controlling agriculture enemies, anticipating that their economic exploitation will be putatively profitable. These studies will contribute to find a promising naturally occurring phytochemical(s) for PPN management.

#### **Acknowledgements**

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### **M39\_Effects of dry and wet biogas digestate application on root knot nematodes**

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Biogas production from organic matter has increased in recent years and, thus, the application of digestate, a byproduct of anaerobic digestion, to soil as a biofertiliser has become more common. Digestate are of two types: dry biogas digestate and wet digestate. Dry digestion fermentation processes are operated for materials with total solid concentrations between 15% and 35% and, wet digestion processes are operated for materials with total solid concentrations below 10%. Organic amendments are known to have positive effects on soil structure community and microbial activity; the diversity of soil microbial communities can be the key to the capacity of soils to suppress soil-borne plant pathogen. The objectives of our study were to determine the effect of biogas digestate application on suppression of root-knot nematode (RKN) population densities, and to evaluate changes in microbial communities after digestate amendment. Results of the RKN densities enumerated with a real-time PCR assay showed that dry and wet biogas digestate treatments significantly reduced the number of RKN by 85% and 47% compared with that of control treatment after 60 days incubation period. Dry digestate mixed with rice straw treatment showed remarkable decrease. Dry digestate mixed with rice straw amendment successfully reduced the nitrate leaching compared with that of control treatment. Community level physiological profiles with Biolog showed considerable differences between dry and wet digestate treatment. The present study provides a new insight for digestate application effects on soil borne plant pathogen and nitrate leaching.

**M45\_Optimisation of hatching assay to understand the elusive phenomenon of hatching in potato cyst nematode, *Globodera rostochiensis***

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The potato cyst nematodes (PCN), *Globodera rostochiensis* and *Globodera pallida*, are two economically important potato pests in potato growing regions worldwide. Integrated pest management is under threat, with effective nematicides being withdrawn on environmental and health grounds, lack of multi-trait quantitative resistant potato cultivars and ineffectiveness of short-term crop rotations. Consequently, alternative crop protection strategies are urgently needed to ensure a sustainable production of potato. The dependence of PCN hatching on root exudate can be exploited in developing a novel strategy to control PCN by its application causing their 'suicidal hatch' when there are no host plants grown in the field or rotation crops are grown. PCN population can be greatly reduced through 'suicidal hatch' as the hatched second-stage juveniles starve to death within 2 weeks without the host. The main objective of this project is to characterise the profile of hatching compounds using different chromatographic methods, to analyse the phyto-chemistry of different potato germplasms and correlate the levels with hatching, with the potential to use novel bioassay techniques. In this study, the hatching device and hatching assays were optimised to understand the elusive phenomenon of hatching in *G. rostochiensis*. Experiments were conducted in 24 well cell culture plates with translucent inserts made up of polystyrene materials and adapted to have 38- $\mu$ m pore size mesh, allowing the comparison of hatching of *G. rostochiensis* juveniles from individual cysts and isolated eggs. Each treatment had six replicates, test solutions were changed every 6<sup>th</sup> day, and the entire experiment was repeated four times. Juvenile emergence was monitored on every other day to identify the period when the peak of hatching takes place. Furthermore, optimisation for the preparation of pure egg inoculum was achieved.

## **M46\_Control of potato cyst nematodes, *Globodera* spp.: a sustainable approach**

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Cyst nematodes are sedentary endoparasites that have unique interactions with their host plants. *Globodera rostochiensis* and *G. pallida* are the most economically important cyst nematodes, being a serious problem for potato crops. Both species of potato cyst nematodes (PCN) are A2 quarantine pests for European and Mediterranean Plant Protection Organization (EPPO) and several measures are available for their control. However, these measures are still insufficient. In the Solanaceae family, *Solanum sisymbriifolium* combines the capability to produce hatching agents and ensure resistance to PCN, being used as a trap crop for PCN control in several countries. A greater understanding of the molecular basis under the resistance mechanism of this species will contribute to develop new methods and strategies to control PCN. This project addresses the identification of the molecular mechanisms that lie behind *S. sisymbriifolium* natural resistance. Total RNA from root tissue of *S. sisymbriifolium* and *S. tuberosum*, infected and non-infected with PCN, will be used to generate libraries for transcriptome sequencing. Knowledge from this work will be applied to recommend new strategies to control PCN, like using RNAi to silence the genes responsible for nematode infection. Raquel Varandas grant SFRH/BD/130172/2017 supported by national funds through *Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior* and the European Social Fund through the *Programa Operacional do Capital Humano* of the National Strategic Reference Framework.

### **M47\_The potential of SALIBRO™ on *Globodera pallida* reproduction**

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Potato cyst nematodes (PCN), *Globodera* spp., cause damage to potatoes and *G. pallida* is the most difficult species to control. Several control measures are available. Nevertheless, none of the methods is 100% effective and many nematicides are being banned due to their toxic effects. The discovery of new classes of nematicides, more effective and less toxic to the environment is important. Dupont™ owned, discovered and pre-commercialised Salibro™, a nematicide based on the active ingredient fluazaindolizine. This study was undertaken to evaluate the effects of Salibro™ (5 and 50 mg kg<sup>-1</sup>) on *G. pallida* reproduction, to improve crop protection systems based on more environmental friendly chemical products. *Globodera pallida* reproduction was evaluated on potato plants ('Désirée') inoculated with second-stage juveniles (J2) or cysts pre-exposed (1 or 7 days) to Salibro™. A concentration of 50 mg kg<sup>-1</sup> negatively affected the reproduction of *G. pallida*. However, when cysts were exposed, instead of J2, the Reproduction Factor was only affected after longer exposures to the nematicide. Our results show that when the J2 are inside the cyst, the efficacy of Salibro™ is reduced and a higher concentration of the nematicide and a longer time of exposure may be needed to affect the J2. Therefore, the effects need to be validated in field conditions. The specificity of Salibro™ against plant-parasitic nematodes, coupled with its lower mammalian toxicity and environmental safer profiles, make Salibro™ a useful and friendly tool for nematode management in the future. This research was funded by Corteva Agriscience™, Agriculture Division of DowDuPont™, France; by Portuguese Foundation for Science and Technology (FCT), PT2020 Partnership Agreement and COMPETE 2020 under the project UID/BIA/04004/2013 and from Instituto do Ambiente, Tecnologia e Vida. Ivânia Esteves (SFRH/BPD/68856/2010) is funded by a post-doctoral fellowship financed by FCT QREN-POPH-Typology 4.1-co-financed by MES national funding and The European Social Fund.

## Posters Biocontrol 1

### **B01\_FIMTrack: behaviour of *Drosophila melanogaster* during infection by entomopathogenic nematodes**

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*Drosophila melanogaster* is one of the most widespread model organisms. It is used in various fields of research including genetics, immunology, developmental studies, behaviour and others. For behaviour research a new method called FIMTrack was developed to simplify the collection and processing of locomotor data of *Drosophila* larvae. The method can be also used for other insect species e.g., *Galleria mellonella* and *Caenorhabditis elegans*. It is based on frustrated total internal reflection (FTIR) and enables observation of groups of *Drosophila* larvae, which are crawling on the surface of a translucent agar gel. Thanks to this method we can easily collect data (movement distance, velocity, area of the larvae, etc.) necessary for evaluation of *Drosophila* response to various stimuli (heat, light, parasites, etc.). FIMTrack was used to evaluate the behaviour of third instar *Drosophila* larvae during infection with entomopathogenic nematodes (EPN). We used two different EPN species (*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*), because different hunting approaches of these EPN have been described. After application of EPN we observed some differences in *Drosophila* behaviour, they moved faster trying to avoid contact with nematodes. They also bent, rolled and twisted more frequently trying to get rid of EPN on their cuticle. When the larvae could choose between a path covered by EPN or control they preferred the safer route without EPN implying they can sense the presence of the pathogen. Larvae can also distinguish between EPN and non-pathogenic nematodes (*C. elegans*). Thanks to FIMTrack we were able to observe the behaviour patterns of *Drosophila* larvae with great resolution in real time enabling us to have a very close look at the interaction between larvae as host and their nematode parasites. This study was supported by grant No. 17-03253S from the Czech Science Foundation, FIMTrack table was purchased with funding from Carl Tryggers Foundation CST 16:474.

**B06\_Toward conservation biological control in vineyards: how the implementation of different cover crops can affect the activity of native entomopathogenic nematodes?**

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Cover crops are increasing as sustainable agronomic measurements to provide various ecosystem services, for example, protection against soil erosion, release of nutrients and support aboveground and belowground biodiversity, including soil beneficial organisms such as entomopathogenic nematodes (EPN). We hypothesise that the presence of cover crops, associated with the vineyard and its stable rhizosphere as perennial agroecosystem, might enhance the prevalence of EPN. In addition, we speculate that the type of cover will modulate the EPN soil food web assemblage. Here we evaluated the presence and activity of native EPN associated to four types of covers (n = 3 per treatment): tillage, spontaneous, flower-driven, and seeded with *Bromus perenne* (Poaceae). Composite soil samples (12 cores, 2.5 cm diam. × 20 cm depth) were taken in the ongoing experiment site in the vineyard, *Vitis vinifera* var Tempranillo (clon RJ-26, rootstock '110-Richterin') during late spring and early autumn 2017 in La Grajera (Logroño, Spain). To ensure balanced spatial distribution, four samples were taken per plot, comprising two pairs of vineyard-cover crop. By using qPCR approaches, each sample (n = 48 per sampling) was tested for the presence and abundance of nine EPN and other related organisms: six nematophagous fungi (NF), five free-living nematodes (FLN), and two ectoparasitic bacteria. In addition, we assessed the EPN activity by the traditional insect-bait. In spring 2017, nematode activity was higher ( $P = 0.068$ ) in covers than in vineyard, while later autumn-time this trend was only observed for the covers tillage and seeded. The species *Steinernema feltiae* and *Heterorhabditis bacteriophora* were isolated from the vineyard (the second one for the first time in La Rioja). EPN suffered low competition with other nematodes for the cadaver, resulting in low EPN-FLN incidence in the isolates. Ongoing studies and surveys in 2018 will provide additional evidence on their natural distribution and assemblage.

**B09\_Description and co-phylogenetic study of *Xenorhabdus indica* from *Steinernema pakistanense* and its prospects in Indian IPM programme**

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India is a mega-diverse country but little entomopathogenic nematode (EPN) diversity (nine species) has been reported so far. In view of this, a survey was done in agricultural fields of the sub-humid region of Meerut district, India and two strains of EPN, labelled CS31 and CS32, were isolated using the *Galleria* baiting technique. Based on morpho-taxometrical studies, and molecular data, the nematodes were identified as *Steinernema pakistanense*, making this discovery the first report of this species from India. We performed a molecular and biochemical characterisation of the symbiotic bacteria of *S. pakistanense* for the first time. Furthermore, a co-phylogenetic analysis of the bacteria from the monophyletic clade containing a symbiont of *S. pakistanense*, together with their nematode hosts, was conducted, to test the degree of nematode–bacteria co-speciation. *Steinernema pakistanense* isolates (CS31 and CS32) were also tested in a laboratory assay for efficacy against two major polyphagous pests, *Helicoverpa armigera* and *Spodoptera litura*. The morphology of the Indian isolates (CS31 and CS32) corresponds mainly to the original description, with the only difference being the absence of a mucron in first-generation females and a missing epiptygma in the second generation females. ITS sequences of *S. pakistanense* of both the Indian strains (CS31 and CS32) differ from the original ITS sequences by 1 bp at position 268, which is T in the present specimens and C in the type population, while they show no nucleotide differences between each other. No variation in the D2D3 sequence was found between the CS31 and CS32 strains, but a variation was seen within *S. pakistanense*. The sequences of bacterial *recA* and *gyrB* genes have shown that the symbiont of *S. pakistanense* is closely related to *Xenorhabdus indica*, which is associated with some other nematodes from the ‘bicornutum’ group. Co-phylogenetic analysis has shown a remarkable congruence between the nematode and bacterial phylogenies, suggesting that, in some lineages within the *Steinernema-Xenorhabdus* complex, the nematodes and bacteria have undergone co-speciation. The biochemical tests of *X. indica* produced mostly negative reactions and showed only positive results with Esculin hydrolysis and Glucose. In the virulence assay, both strains caused a 100% mortality of both tested insects after 48 h, even at the lowest doses of 25 infective juveniles per insect, suggesting that *S. pakistanense* is very virulent towards the insect pests tested, and thus this species could be considered for use in the biocontrol of these organisms in India.

**B10\_The nematode *Parasitylenchus bifurcatus* is parasitising not only Harlequin ladybird *Harmonia axyridis* but also the two spotted lady beetle *Adalia bipunctata***

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In years 2014 to 2016 around 450 specimens of the invasive Harlequin ladybird, *Harmonia axyridis*, and the native two spotted lady beetle, *Adalia bipunctata*, were collected in Denmark and Slovenia. Ladybirds were dissected and checked under a stereomicroscope for the presence of nematodes. Nematodes were discovered in samples of *H. axyridis* collected in different locations in the Copenhagen area in Denmark and at Brdo pri Lukovici and Trboje in Slovenia, and further in *A. bipunctata* from Copenhagen and Gentofte, Denmark. The incidence of parasitised *H. axyridis* was around 30% for the samples from Slovenia as well as Denmark. Morphometrical analysis of nematodes revealed characteristic features of the nematode species *Parasitylenchus bifurcatus* Poinar & Steenberg, including straight stylet lacking basal thickenings, a bursa and a forked tail tip in the vermiform females and juvenile males. A region of 18S rDNA was amplified, cloned, sequenced (acc. nos. LT547719 - LT547726, LT629306 - LT629307), and compared to other sequences in the public domain. High similarity (>99.4 % nucleotide identity) of the determined sequence to *P. bifurcatus* sequences and phylogenetic analysis confirmed the species identification. Nematodes parasitising *H. axyridis* and *A. bipunctata* did not differ in observed morphometrical and molecular characters. With further laboratory tests we were able to show that cross-transmission of *P. bifurcatus* from *H. axyridis* to parasite free culture of *A. bipunctata* is possible. The parasitic nematode *P. bifurcatus* compromises fecundity of its host and was therefore considered as a potential biological control agent to control high abundance of the invasive *H. axyridis* ladybirds. However, our result showed that the nematode is also able to parasitise the native ladybird species *A. bipunctata*, so *P. bifurcatus* is not an optimal candidate for biological control of *H. axyridis*. Financially supported by Slovenian Research Agency (P4-0072) and Ministry of Agriculture, Forestry and Food of the Republic of Slovenia (C2337).



**B13\_Potential of actinomycetes isolated from *Apis andreniformis* in controlling root-knot disease of chili**

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Root-knot disease caused by *Meloidogyne incognita* is a serious problem in chili production. This pest damages the chili root system and causes significant yield losses. *Meloidogyne incognita* species spreads throughout the chili plantations in north, northeastern and central regions of Thailand. Our target is to find the antagonist to control the nematode together with increasing yield of chili. Thirty-three actinomycete isolates obtained from black dwarf honey bee (*Apis andreniformis*) were subjected for primary screening in vitro for their effects on hatching and juvenile mortality of *M. incognita*. Eleven isolates that gave significant control of *M. incognita* were confirmed in reducing root-knot nematode under glasshouse conditions. Strain KPS-A032 and KPS-A031 reduced hatch rate the most by 69.5% and 69.4%, respectively. In pot experiment, strain KPS-A032 presented the highest control efficacy in reducing root gall of chili by 50% and size of giant cell of chili root when compared to the control. In addition, strain KPS-A032 was capable of surviving and proliferating in the soils throughout the entire 35 days of a tillage period. However, applying the strain to plants every 15 days is recommended to achieve better control efficacy of the strain. Besides its control potential, strain KPS-A032 could promote plant growth by increasing shoot weight and yield of chili. Moreover, morphological and molecular analyses of strain KPS-A032 indicated that this strain belonged to genus *Streptomyces*.

**B15\_The presence of earthworm mucus secretion could alter entomopathogenic nematodes' activity as biological control agents**

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Earthworms can promote the dissemination of beneficial soil organisms, including entomopathogenic nematodes (EPN). It is known that EPN displacement along the soil profile can be favoured by the presence of earthworms. However, the impact of other earthworm activities, such as feeding behaviour or mucus production, is still poorly understood. We expected that feeding activity might decrease the number of viable EPN in the soil, reducing their ability to kill insects. However, we speculated that the presence of mucus secretion will not affect the biocontrol skills. We evaluated two earthworms' actions (feeding activity and mucus secretion) in two laboratory experiments. We tested whether the presence of mucus can alter the infectivity of infective juveniles (IJ) against *Galleria mellonella* (Lepidoptera: Pyralidae). Treatments included controls (water and mucus) and two concentrations (1.5 and 10 IJ cm<sup>-2</sup>) per EPN species in the presence and absence of mucus. Second, we evaluated the killing ability of EPN against *G. mellonella* larvae when applied in autoclaved soil alone or in combination with earthworms or mucus. In both experiments, we tested the earthworm *Eisenia fetida* (Haplotaxida: Lumbricidae) in combination with four steinernematids (*Steinernema carpocapsae*, *S. feltiae*, *S. glaseri* and *S. khuongi*) and two heterorhabditids (*Heterorhabditis bacteriophora* and *H. zealandica*). In both experiments, the presence of mucus resulted in a significant reduction of the larval mortality caused by certain steinernematids, while heterorhabditids exposure to mucus did not affect mortality. However, the presence of earthworms did not affect either of the two types of EPN. Our results illustrate how the presence of earthworms' secretions (mucus) can alter the long-term persistence in the soil of certain EPN species as well as its action as biological control agent in a dense-dependent and species-specific manner.

**B16\_How *Trichoderma harzianum* can help the control of *Meloidogyne javanica* in a commercial tomato crop in the South East of Spain**

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A field trial was carried out in a 486 m<sup>2</sup> plot belonging to a 11.600 m<sup>2</sup> tomato commercial glasshouse infested with root-knot plant-parasitic nematodes (*Meloidogyne javanica*), and located in La Cañada (Almería, Spain). The trial was performed over an entire cropping season and the glasshouse was managed under an integrated pest management program (IPM). The trial evaluated the results against root-knot nematodes using two different *Trichoderma harzianum* strains by recording the population of free juvenile in the soil, root damage and reproduction of the nematode. The results demonstrated that the strain T22 of *T. harzianum* was more effective in controlling *M. javanica* populations than the strain 1306.

### **B19\_Ability of *Pochonia chlamydosporia* to colonise the rhizosphere of tomato rootstocks**

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Sustainable management of economically important soilborne diseases such as root-knot nematodes (RKN), *Meloidogyne* spp., calls for the integration of ecologically sound control strategies. The application of biological control agents, activation of plant defence mechanisms and grafting onto resistant cultivars are promising control methods. Nevertheless, nematode control provided by these methods independently is variable and often limited. The COCOoN Project (POCI-01-0145-FEDER-016611, PTDC/AGR-PRO/3438/2014) aims to develop a management strategy against RKN, based on the combined use of the biological control agent, *Pochonia chlamydosporia*, with the activation of natural plant defence mechanisms and partially resistant rootstock cultivars. The facultative egg parasite *P. chlamydosporia* grows endophytically in plant roots but rhizosphere colonisation, essential for nematode control, varies among different plant species and cultivars. The goal of this study was to evaluate the ability of a *P. chlamydosporia* isolate to colonise the rhizosphere of 27 commercial tomato rootstock cultivars and two commercial cultivars ('Moneymaker' and 'Tiny Tim'). Seeds were grown in 150 cm<sup>3</sup> pots with sterilised sandy soil inoculated with 5000 chlamydospores (g soil)<sup>-1</sup>. After 30 days, the number of colony forming units (CFU) cm<sup>-2</sup> of root was assessed in a semi-selective medium. Significant differences were detected and 48% of the cultivars tested were considered as poor hosts including the commercial 'Moneymaker'. Six rootstock cultivars supported a moderate growth of the fungus and eight tomato rootstocks and 'Tiny Tim' were rated as good hosts (>200 CFU cm<sup>-2</sup> of root). Rhizosphere colonisation should be considered in the design of a management programme based on application of the fungus to increase nematode control of grafted tomato.

**B22\_ *Diabrotica v. virgifera* management using a genetically improved strain of *Heterorhabditis bacteriophora***

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The Western Corn Rootworm (*Diabrotica virgifera virgifera*) is one of the most damaging pests of maize. Since the ban of neonicotinoid seed treatments most European farmers rely on the application of less effective granular soil insecticides. As a sustainable non-toxic alternative, the entomopathogenic nematode, *Heterorhabditis bacteriophora*, has been tested for the last 12 years in field trials with plants artificially infested with insect eggs to ensure an even population density. The nematodes were applied into the furrow with 200 l ha<sup>-1</sup> together with the maize seeds, using special injectors mounted on the single-seed drilling machine. At this time of application *Diabrotica* eggs are still in diapause. Nematodes survived and remained virulent until larvae hatched at approximately 4-6 weeks later. With this precise application method, nematodes achieved a mean reduction of the pest population of 65% (ranging from 33-82%) and in 11 of 16 trails outperformed results obtained with the chemical standard. Reduction of the population will in the long-term reduce the problems with this invasive pest. Nevertheless, entomopathogenic nematodes are still more expensive than chemical insecticides and, therefore, we aimed at a reduction of the application density by genetically improving persistence and virulence of *H. bacteriophora*. Results of field trials indicate a better performance of the improved strain, justifying a reduction of the application density from 2 to 1 billion ha<sup>-1</sup> and bringing application costs into the range of synthetic chemicals.

**B26\_BioAct DC (*Purpureocillium lilacinum* strain 251) - A complementary tool for integrated nematode management in vegetable crops**

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Nematopathogenicity of the fungus *Purpureocillium lilacinum* strain 251 has been well documented in the literature. Several commercial products have been introduced but, like many fungal-based biologic products, they have enjoyed limited commercial success due to inconsistent performance under practical conditions. In 2013, Bayer developed BioAct DC, a novel liquid formulation of *P. lilacinum* strain 251. The present study was conducted to profile the biological properties of the new product and to investigate its use for integrated nematode management together with fluopyram (Velum Prime). Results show that *P. lilacinum* strain 251 is able to parasitise eggs of *Meloidogyne*, *Pratylenchus* and *Heterodera* species. In addition, the new liquid formulation was tested to be fully compatible with commercial drip irrigation systems. Drip-application studies at two agricultural sites in Spain revealed an initial spore distribution within the upper 10 cm of the soil close to the emitters. Furthermore, sequential application of *P. lilacinum* strain 251 and fluopyram in large-plot tomato and cucumber trials resulted in improved fruit yield as compared to the respective solo applications.

**B28\_Morphological and molecular characterisation of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) from Croatia and their virulence potential**

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A survey of entomopathogenic nematodes (EPN) was conducted in natural and agricultural soils from the valley region in *southeastern* Croatia. Insect-parasitic nematodes were isolated from 20 of the 100 soil samples (20%). Morphological and molecular characterisation were used for analysis of the diversity and phylogenetic similarity of recovered EPN. Out of 20% of positive samples, three isolates were identified as strains conspecific with *Heterorhabditis bacteriophora* (15%), two strains of *Steinernema feltiae* (10%) and 15 isolates of *Oscheius* spp. (75%). The strains of *H. bacteriophora* were recovered mainly from natural soils, whilst *S. feltiae* were from agricultural soils. The variations in morphometrical characteristics of infective juveniles (IJ) and males were detected among Croatian strains compared with the original description. The analysis of ITS region revealed the greatest similarity of Croatian strains of *H. bacteriophora* with Irish HP88 and *H. georgiana* strain from USA, whilst *S. feltiae* showed the greatest similarity with Slovenian B30 and English A2 strains. The native strain of *H. bacteriophora* was bioassayed against *Lasioptera rubi* (the raspberry gall midge) larvae, and *S. feltiae* was tested for virulence against adults and larvae of *Tribolium castaneum* (the red flour beetle) under laboratory conditions. The virulence of native strains of EPN was affected mainly by nematode concentration, insect life stage and temperature regime. The highest mortality rate of the raspberry gall midge was observed within 8 days after inoculation in treatment with 200 IJ per insect larvae. Larval mortality of *T. castaneum* was up to 82% at 700 IJ per insect, while mortality of adults was lower and reached 58% at the highest nematode concentration. This is the first report of EPN of families *Heterorhabditidae*, *Steinernematidae* and genus *Oscheius* from Croatia, expanding their currently known geographic range.

**B29\_Nematicidal potential of *Heracleum mantegazzianum* against root-knot nematode, *Meloidogyne incognita*, on tomato**

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*In-vitro* and *in-vivo* experiments were conducted to evaluate the nematicidal effects of giant hogweed, *Heracleum mantegazzianum* on the root-knot nematode, *Meloidogyne incognita*. In laboratory assays, the extract from *Heracleum mantegazzianum* was assessed and it killed 80% of nematode juveniles and also inhibited hatching. Hogweed extract was found to be highly effective causing death of 55% of nematodes after 24 h and more than 80% were dead after 72 h exposure, which proved that hogweed efficiently killed the nematodes. In a glasshouse bioassay, hogweed improved the root growth with fewer galls and less nematode infestation. Experiments revealed that the total number of nematodes extracted from roots of tomato plants after treatment with hogweed extract were dramatically reduced as compared to control. Based on our investigations, the hogweed could be used to manage the nematodes in farmers' fields by incorporating into soil before sowing. To best of our knowledge, we are the first to introduce the usage of hogweed against plant-parasitic nematodes.



**Posters Plant Nematode Interaction 1**

**P02\_Phylogenetic analysis of root-knot nematodes species in eggplant growing area of Punjab, Pakistan**

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The aim of current research is to contribute to knowledge of the root-knot nematodes (RKN), associated with eggplant growing area of Pakistan by determining the level of genomic diversity. For this purpose various primer sets (TRNAH/MRH106; MORF/MTHIS; 194/195; C2F31108 and some species specific primers) were used for mitochondrial (mt) DNA extraction; similarity of genome sequence; evolutionary relationship of the sample sequences; pairwise identity and then phylogenetic analysis of RKN was made by using neighbour joining method, aligning the sequences with MUSCLE, Sequence Demarcation Tool (SDT) and Mega7 softwares, respectively. Variation in the presence of the restriction enzyme (RE) was determined by online software (NEBcutter V 2.0 and ORF finder). Haplotype based diagnosis in phylogenetic analyses of mtDNA region revealed that the few Punjab isolate clustered with *M. inornata* a Belgium isolate and *M. haplanaria* clustered with a USA isolate of tomato, while one of the samples clustered with *M. luci*.

**P07\_Analysis of attractant for root-knot nematode, *Meloidogyne incognita***

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Root-knot nematodes are plant-parasitic nematodes that infect many crop species such as sweet potato and tomato. The global damage caused by root-knot nematodes has been estimated to be over several billions of dollars annually. Nematodes initiate the infection by invading the root tip of the host plant. It has been suggested that nematodes recognise the host plant root through attractants secreted by the host; however, the identity of the attractant currently remains unknown. In this presentation, the nematode-host recognition mechanism, and the nematode attractant from the host plant will be discussed.

**P10\_Characterization of the role of the putative effectors chorismate mutase and isochorismatase in the interaction of *Hirschmanniella oryzae* and rice**

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The rice root nematode, *Hirschmanniella oryzae*, is a frequently occurring plant-parasitic nematode in flooded rice fields and is distributed world-wide. It causes severe yield reductions and economic losses in specific environmental conditions. Two potential effectors that could aid the nematode during infection were found in its transcriptome: chorismate mutase and isochorismatase, potentially involved in deregulating the salicylic acid and phenylpropanoid pathways in plants. Although the pathways in which these proteins are involved are not endogenous to nematodes, both proteins were proven to be enzymatically active. Transgenic rice lines overexpressing either one of these genes demonstrated the importance of both effectors in lowering plant defence. Transgenic rice lines showed reduced expression of genes involved in the phenylpropanoid pathway and in diterpenoid phytoalexin biosynthesis. In addition, infection experiments showed an increased susceptibility of these lines to nematode infection, both to migratory (*H. oryzae*) and sedentary (*Meloidogyne graminicola*) species. Taken together, results show that both chorismate mutase and isochorismatase are involved in lowering plant defence, thereby enabling the nematode to colonize the plant.

**P11\_Genotyping of fifty *Meloidogyne incognita* isolates revealed possible polymorphisms related to the infection phenotype against sweet potato cultivars**

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*Meloidogyne incognita* is a major root-knot nematode species causing damage to a wide range of crop production worldwide. Although no strong host specificity is observed, the infection level varies depending on the host plant species, indicating that there exists a type of 'host preference' in the *M. incognita* infection. This kind of preference is also recognised toward different sweet potato (*Ipomoea batatas*) cultivars. Propagation of *M. incognita* species has often been observed in sweet potato fields located throughout Japan. In a previous study by Sano and Iwahori (2005), *M. incognita* was collected from soil in those fields, and single egg masses were isolated and maintained in a laboratory condition. The isolates were then categorised into Sweet Potato (SP) races according to the distinct infection phenotypes against five sweet potato cultivars, 'Norin-1', 'Norin-2', 'Tanegashimamurasaki-7', 'Elegant Summer' and 'J-red'. In this study, with the aim of understanding the genotype relationship to the infection phenotype, we performed whole genome sequencing of 50 *M. incognita* isolates and classified them based on single nucleotide polymorphisms (SNP). We also scored the infection phenotypes using five sweet potato cultivars and categorised the isolates into SP races. Our results indicated that the 50 isolates were classified into seven major phylogenetic groups. Several groups clearly represented the SP races, suggesting a possible genotype-phenotype relationship. We further compared insertion-and-deletion (InDel) polymorphisms among isolates and identified regions that correlated to the different infection phenotype. These results are helpful in future research to identify *M. incognita* genes that have an effector role.

**P13\_ Cloning and RNA interference analysis of a calponin-related protein gene *Dd-unc-87* from the potato rot nematode *Ditylenchus destructor***

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*Ditylenchus destructor* is one of the major threats to the sweet potato production in China. The control of stem nematode of sweet potato is mainly based on the high toxic nematicide such as aldicarb, which leads to pesticide residues, environmental pollution and the emergence of nematode resistance. The alternative methods such as the development of nematode-resistant crops through RNA-mediated interference (RNAi) will be the most promising measures. The *Caenorhabditis elegans* gene *unc-87* is an important component of nematode body wall muscle and plays an important role in the movement of nematodes. The *D. destructor* orthologues of this gene, namely *Dd-unc-87* was cloned and used to test RNAi in this migratory nematode. The *Dd-unc-87* cDNA has a 1134 bp long open reading frame that is putatively translated into a 377 amino acids peptide with an estimated molecular mass of 42 kDa and isoelectric point of 7.08. The UNC-87 protein, which is predicted to be present in the nucleus, contains seven calponin-like domains, and has no signal peptide in its N-terminal. Blastx comparison and phylogenetic analysis based on the amino acid sequence of *Dd-unc-87* showed that *Bursaphelenchus xylophilus* was the closest one to *D. destructor*. RNAi was performed by soaking *D. destructor* in the solution containing dsRNA of *Dd-unc-87*. The levels of *Dd-unc-87* mRNAs were significantly reduced in the nematodes soaked for 24 h. The migration of the treated nematodes in a sand column (height 60mm, diam. 25mm) had no significant difference with the control. But the reproduction of the treated nematodes cultured in the carrot disk was significantly lower than that of the control one, with a decrease of about 49%. It is suggested that the *Dd-unc-87* plays a positive role in the reproduction of nematodes, and may not play a significant role in the migration of nematodes, which was inconsistent with previous reports

**P16\_Cloning and functional characterisation of a putative protein disulfide isomerase (MgPDI) in the root-knot nematode, *Meloidogyne graminicola***

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The rice root-knot nematode, *Meloidogyne graminicola*, is considered as a quarantine pest in most rice-growing countries. It is an obligate sedentary endoparasite of rice. The active oxygen species (ROS) is the first line of defence utilised by host plants against parasites, while nematodes, counteractively, employ antioxidants to facilitate their infection. In the present study, the gene encoding *M. graminicola* protein disulphide isomerase (MgPDI) was identified. It is highly conserved in the putative active-site sequence (CGHC) when aligned to the PDI in other nematodes. *In situ* hybridisation showed that *MgPDI* is specifically localised within pharyngeal glands of pre-parasitic second-stage juveniles (J2). *MgPDI* is significantly up-regulated in the late parasitic J2. Characterisation of the recombinant protein showed that the purified MgPDI had activities of insulin disulfide reductase, and it also protects DNA and living cells from ROS damage. These results indicate that MgPDI functions as an antioxidant that plays an important role in response to oxidative stress. In addition, silencing of *MgPDI* by RNA interference in the J2 lowers their multiplication factor, which reflects the overall ability of the nematode to be a successful parasite. In conclusion, *MgPDI* plays an important role in the interaction between *M. graminicola* and rice, maybe through inducing local changes in the redox status of infected host tissue.

**P24\_ Isolation of *Heterodera schachtii* effectors interacting with the promoter of *Arabidopsis thaliana* PANB1**

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*Heterodera schachtii* is a plant-parasitic nematode with an economically important impact on sugar beet production. The second-stage juveniles invade the root of their host and move intracellularly toward the vascular cylinder, where they induce the formation of a plant-derived, hypertrophic and hypermetabolic syncytium and become sedentary. The syncytium serves as the sole nutritional source for the developing juveniles. Due to this dependency, it is crucial for *H. schachtii* to initiate and maintain the syncytium in order to complete its lifecycle and produce progeny. Vitamin B<sub>5</sub>, the precursor to co-enzyme A, is an essential nutrient for all animals. Plants, in comparison, can synthesise VB5 *de novo* in a three-step pathway. The first committed step is performed by AtPANB1. Transcriptome data has revealed that *AtPANB1* is significantly up-regulated in the syncytium, induced by *H. schachtii* in *Arabidopsis thaliana* roots. The essential role of this enzyme for cyst nematode infection was further confirmed, as *AtPANB1* knock-out mutants were less susceptible to infection by *H. schachtii*. Considering the strong and peculiar expression of *AtPANB1* in the syncytium, we assume that nematodes secrete effector proteins to regulate the expression of *AtPANB1*. Accordingly, a yeast one-hybrid assay was established to screen a *H. schachtii* cDNA library for proteins interacting with the promoter of *AtPANB1*. Potential candidates have yet to be characterised.

## **P26\_Effect of the *Mi* gene on the reproduction of *Meloidogyne luci* on tomato genotypes**

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Root-knot nematode (RKN) resistance in tomato is conferred by a single dominant gene designated as *Mi* that confers resistance to the three most common warm-climate *Meloidogyne* species: *M. arenaria*, *M. incognita* and *M. javanica*. In the 1940s, the *Mi* gene was introgressed into the cultivated tomato from the wild species *Solanum peruvianum*. The objectives of this study were to screen the RKN resistance *Mi* gene in 27 tomato genotypes, by the amplification of marker Mi23, and evaluate the ability of *M. luci*, added to the EPPO Alert List, in 2017, to reproduce on these tomato genotypes. Amplification of Mi23 marker gave a DNA fragment of 380-bp for tomato genotypes without the *Mi* gene (*mimi*), a fragment of 430-bp for homozygous genotypes at the *Mi* locus (MiMi) and two fragments for heterozygous genotypes (Mimi). Thirteen Mimi tomato genotypes, two MiMi and twelve lacking the *Mi* gene for resistance to RKN were identified. Host suitability was assessed 60 days after inoculation with 5000 eggs on the basis of root gall index (GI) and reproduction factor (Rf). Tomato 'Coração-de-Boi' was used as control. Significant differences in reproduction were detected between and within the *Mi* allelic conditions, which reflects a possible dosage effect of the *Mi* gene and an influence of the genetic background of the plants, respectively. Genotypes 'BERMELLO RZ F1' (Mimi) and 'Tisey F1' (*mimi*) were considered hypersensitive, the nematode induced galls (GI > 2), but often could not complete the life cycle (Rf < 1). The remaining *mimi* genotypes were susceptible to *M. luci* ( $2.43 \leq Rf \leq 99.73$ ). Rf on Mimi and *mimi* genotypes varied from 0.0 to 7.2, with nine genotypes being classified as resistant. Resistant genotypes can be used to control the three most common *Meloidogyne* species and *M. luci*. However, they should be included in integrated pest management programmes to prevent the selection of virulent isolates.



**P27\_Key genes for root development are also relevant for galls and giant cell formation induced by root- knot nematodes**

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are a group of phyto-endoparasitic nematodes with a negative impact on plant productivity. The gradual banishment of effective but contaminant chemical nematicides makes necessary the search for biotechnological alternatives. RKN feeding cells, called giant cells (GC), are induced from vascular cells by nematode effector molecules. GC expands and undergoes numerous changes, e.g., a modified cell cycle with repeated mitosis and aborted cytokinesis. Additionally, a knot or gall is formed around the GC by the divisions and hypertrophy of several tissues within the root. The transcriptomes of early-developing *Arabidopsis* GC/galls in *Arabidopsis* (Barcala *et al.*, 2010) were compared to those characteristics of different root cell types (Brady *et al.*, 2007). Results indicated that the transcriptomes of GC/galls were enriched in genes characteristic of undifferentiated root cell types, as those from the quiescent centre (QC), protoxylem and the lateral root initial cells (Cabrera *et al.*, 2014). Hence, we studied the expression and function of key genes for the development of these cell types. Genes with essential roles in the root QC establishment and stem cell maintenance like *SCHIZORIZA* (*SCZ*), *SCARECROW* (*SCR*), *SHORT ROOT* (*SHR*) and *WUSCHEL-RELATED HOMEBOX 5* (*WOX5*) were induced in *Arabidopsis* galls. Additionally, mutant lines for these genes showed a decrease in the number of infections and in some cases in the reproduction parameters as compared to the wild type lines. Moreover, *HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), a protoxylem marker in the root apical meristem, was also induced in galls and the mutant line *ahp6* presented a significant reduction in the number of galls formed by RKNs. Our main conclusion is that genes characteristic of root developmental processes are also relevant during gall and GC development.

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**P28\_An aspartic peptidase secreted by the pinewood nematode *Bursaphelenchus xylophilus***

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Aspartic peptidases are a family of proteinases with catalytic aspartate residues in the active site which have been reported in a large variety of organisms, with different functions. The importance of aspartic peptidases in animal parasitism have been reported; however, only a few studies have been performed on plant-parasitic nematodes aspartic peptidases and their possible role in parasitism. In *Bursaphelenchus xylophilus* secretome, these proteins have been detected upregulated. In the present study, the cDNA coding sequence of one of these aspartic peptidases (BxASP101) was cloned and sequenced and complete amino acid sequence predicted. BxAsp101 consists of 397 amino acid residues including a signal peptide; the conserved pepsin-like domain; a typical domain of aspartic peptidase A1 family; and an aspartic peptidase active site and two catalytic motifs, each one contributing with an Asp catalytic residue. The putative protein has a calculated molecular mass of 44 kDa and a theoretical isoelectric point of 4.8. BxASP101 Blastp analysis showed only 32–39% amino acid sequence identity to available aspartic proteases from parasitic and free-living nematodes. Phylogenetic analysis indicated that BxASP101 clearly differs from other nematode aspartic peptidases, forming a separate branch. BxASP101 in silico three-dimensional structure analysis predicted a monomer with bilobal configuration and an extended active site cleft localised between the two lobes with each N- and C-terminal lobe contributing with one active Asp residue. These results revealed that BxASP101 is probably a functional aspartic peptidase and might be related to *B. xylophilus* pathogenicity.

**P29\_POINTERS -Host tree-pinewood nematode interactions: searching for sustainable approaches for pine wilt disease management**

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, responsible for enormous economic and ecological impacts, is considered a major threat to forestry ecosystems. In recent years, progresses have been made to elucidate pieces of PWN pathogenicity mechanism, but the 'puzzle' is not yet complete and innovative, holistic strategies are needed for full understanding of the infection process. Proteomics is a powerful tool that can help understanding cellular activities and metabolic pathways involved in tree resistance, but its application to study the PWN infection is still in its beginning, with most research targeted to the nematode. It is known that proteins secreted by the PWN are crucial for its pathogenicity, but it is important to investigate the PWN secretome in pine species with contrasting susceptibilities and the proteome profiles of these infected trees. Further, the knowledge on elicitors as activators of chemical defences in plants can also contribute to identify metabolic pathways involved in resistance that could be used to develop practical elicitor-based strategies. In this sense, POINTERS, a multidisciplinary and integrative project, was established: i) to clarify the PWN infection mechanisms; ii) to recognise metabolic pathways involved in host tree resistance/susceptibility; and iii) to identify PWN pathogenicity and tree resistance biomarkers. This project will provide new insights into the nature and function of PWN pathogenicity proteins and host tree response mechanisms to gain a full understanding of the infection process and to advance new control approaches that can be targeted to the development of practical and sustainable disease management strategies. Project funded by POCI COMPETE 2020 -FEDER and Fundação para a Ciência e Tecnologia (FCT) (Project nº 31999).

**P33\_ Identification and functional analysis of two *expansin* genes, *Hg-exp-1* and *Hg-exp-2*, from the soybean cyst nematode, *Heterodera glycines***

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Soybean cyst nematode, *Heterodera glycines*, is a devastating disease on soybean. Expansin protein is secreted through the stylet and plays an important role in the parasitism of *H. glycines*. The full-length cDNAs of two expansin genes, *Hg-exp-1* and *Hg-exp-2*, were successfully cloned from the second-stage juveniles of *H. glycines*, with a length of 905 bp and 951 bp, and the peptides with length of 288 and 2A95 amino acids were encoded. Both of the two predicted proteins have a signal peptide in the N-terminal, without a transmembrane domain, indicating that they are secretory proteins. Blastx search result showed that HG-EXP-1 sequence of *H. glycines* was highly consistent with GR-EXPB1 (CAC83611) and GR-EXPB2 (CAC84564) in *Globodera rostochiensis* and DA-EXPB1 (ADJ57307) in *Ditylenchus africanus*. Southern blot analysis suggested that the expansin genes are probably the multicopy genes or members of a small multigene family. *In situ* hybridisation analyses showed the transcripts of them accumulated exclusively in the subventral pharyngeal gland cells of *H. glycines*. The results of *Hg-exp-1* interference *in vitro* showed that the transcriptional level of the target gene was reduced in nematode treated with dsRNA for 16 h. After the *Hg-exp-1* gene was silenced, the number of second-stage juveniles and females in soybean root were decreased by 38.3% and 43.4%, respectively. The two expansin genes were proved to play an important role in the early parasitism of soybean cyst nematode.

**P35\_Genetics and mapping of grapevine resistance from a muscadine source to the dagger nematode *Xiphinema index***

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The dagger nematode, *Xiphinema index*, vectors, from plant to plant in the soil, *grapevine fanleaf virus* (GFLV), the major grapevine virus worldwide. Resistance to *X. index* in grape rootstock breeding by arresting or delaying viral transmission is a promising GFLV control alternative to highly toxic chemical nematicides, which have been removed from use. In the muscadine, *Muscadinia rotundifolia*, accession NC184-4 is today one of the best sources for resistance (R) to *X. index* and can be used through its F1 resistant individual VRH8771 (= *Vitis vinifera* × NC184-4). Inheritance of resistance to *X. index* (non-viruliferous individuals) has been evaluated using a one-year test under glasshouse conditions for the selection of durable *R* factors. Despite the difficult hybridisation between the genera *Vitis* and *Muscadinia*, a backcross (BC1) progeny of 66 individuals between VRH8771 and the susceptible (S) *V. vinifera* 'Cabernet-Sauvignon' (CS) has been obtained and phenotyped for resistance. Given that BC1 individuals exhibit highly heterogeneous vigours, a Principal Component Analysis (PCA) approach has first shown that the criteria used for resistance phenotyping and for plant development vary independently. A clear segregation has been observed and, out of the 66 individuals, the 58 R : 8 S ratio (~ 7 R : 1 S) suggests the hypothesis of three dominant and independent *R* factors. Using a VRH8771 × CS genetic map in progress based on microsatellite and GBS (genotyping by sequencing) markers, a mapping method derived from BSA (bulked segregant analysis) allowed the detection of markers linked to resistance at three chromosomal locations that fit this hypothesis. Future studies will aim at confirming the segregation of each factor in BC2 progenies obtained by backcrossing appropriate BC1 individuals carrying a single factor with S (recessive) individuals.

**P36\_Combating *Meloidogyne chitwoodi* on potato through a new detection tool and effector discovery**

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In the potato growing regions of northwestern USA (Oregon, Idaho and Washington), the root-knot nematode, *Meloidogyne chitwoodi*, is an important pest because it infects both potato roots and tubers. The tuber infections lead to blemishes that significantly affect the potato crop's value. *Meloidogyne chitwoodi* has limited worldwide distribution and is considered a quarantine pest by several regulatory agencies so early detection and control strategies are important. For nematode detection, the Gleason lab developed a Loop-mediated isothermal AMPlification (LAMP) assay that can easily identify *M. chitwoodi* and its close relative *M. fallax*. A positive reaction results in a visible colour change in the reaction tube. Our data show that the LAMP assay works on soil DNA extracts and is more sensitive than conventional PCR for nematode detection. Although *M. fallax* was reported on turfgrass in California, it is not endemic to the USA, and it is not found in northwestern USA, where a large percentage of potato production occurs. A positive LAMP reaction from these US soils would suggest the presence of *M. chitwoodi*. Because there are no cultivated potatoes with *M. chitwoodi* resistance, the Gleason lab is also focusing on the potato-nematode interaction at a molecular level to develop tools for engineering potato resistance. In this effort, we are working to identify *M. chitwoodi* secreted proteins involved in parasitism (*i.e.*, effectors). We performed a transcriptome analysis on *M. chitwoodi*, looking for genes upregulated early after infection of susceptible potato roots. Our data indicate that several gland-specific genes are up-regulated during potato infection, including the effector Mc265 that is involved in suppression of plant basal defences. Overall, we have utilised molecular tools for *M. chitwoodi* identification and effector research in order to provide new resources for the management of this nematode on potatoes.

**P40\_Morphological and molecular characterisation of burrowing and root lesion nematodes associated with bananas (*Musa* spp.) in Tanzania**

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Plant-parasitic nematodes (PPN), particularly the burrowing (*Radopholus similis*) and root lesion (*Pratylenchus coffeae* and *P. goodeyi*) nematodes, are among the major pests affecting banana in Tanzania. Banana (*Musa* sp.) is an important staple food and cash crop for about 20% of the population in Tanzania. Control of nematodes using nematicides has been difficult because of protection in the roots which are away from the surface and reach of nematicides. Most nematicides are also environmentally hazardous. Thus, alternative strategies for the control of nematodes are a prerequisite for sustainable nematode management. Here nematode species collected from four agro-ecological zones in Tanzania were identified morphologically and morphometrically. PCR and sequence analysis of the ITS1-5.8S rDNA region of these nematodes and phylogenetic analysis were used to confirm the morphological identification. Five species of nematodes (*R. similis*, *P. goodeyi*, *P. coffeae*, *Helicotylenchus multincinctus* and *Melodogyne* sp.) were identified from the collected samples. Of these the burrowing (*R. similis*) and root lesion (*P. coffeae* and *P. goodeyi*) nematodes were identified as the major ones. Sequence and phylogenetic analysis showed high relationship to other burrowing and root lesion nematode sequences from GenBank. Results of this study provide an insight to the presence of harmful plant-parasitic nematodes in banana cultivated in Tanzania and may contribute to further studies aimed at developing efficient nematode management strategies to improve crop production in Tanzania.

**P41\_Enemy as friend? – *Meloidogyne incognita* induced antagonism against white rot (*Sclerotia sclerotium*) on tomato in pot experiment**

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Tomato plants were artificially infected with *Sclerotinia sclerotiorum* mycelial discs and *Meloidogyne incognita* juveniles, and at the same time treated with Symbivit<sup>®</sup>, a mycorrhizal product. Our hypothesis was that in a complex system, where the presence of pests and pathogens is simultaneous, mycorrhiza is able to induce resistance in the plant. During the growing season, health conditions of test plants were recorded. After 18 weeks of cultivation, yield and the weight of fresh shoots and roots, *M. incognita*-damage, and mycorrhizal colonisation were evaluated. The artificial *M. incognita* infestation significantly reduced yield and increased fresh root weight. Tomato yield and the number of live plants were both decreased by *S. sclerotium*. Mortality of plants was observed only the case of plants not infested with root-knot nematodes. The mycorrhizal product had no effect either on the yield, or on the growth of plants. Our experiment concluded that mycorrhiza was found ineffective against both root-knot nematode and white rot. Therefore, our original hypothesis was not confirmed. The finding that plants artificially infested with *M. incognita* did not die off due to the white rot infection suggests that root-knot nematodes may have induced a resistance in those plants against the disease.

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**P42\_FMRFamide-like peptide family members of *Pratylenchus penetrans***

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The root lesion nematode, *Pratylenchus penetrans*, is an economically important migratory endoparasite of a wide diversity of crops. It causes substantial damage to cortical tissues, resulting in decay and development of lesions in the roots. Nematode neuropeptides such as FMRFamide-like peptides (FLP) are known to be involved in various biological processes such as feeding, reproduction and locomotion, making this family of genes an appealing target for parasite control strategies. A set of FLP was identified in the *P. penetrans* transcriptome and found to be significantly expressed during infection of potato, *Solanum tuberosum*. *In silico* analyses of these FLP transcripts were performed, including ORF prediction, C-terminus conserved FMRFamide domain identification, BLAST analysis against WormBase and NCBI databases, and phylogenetic analysis against FLP from other plant-parasitic nematodes and *Caenorhabditis elegans*. From this characterisation a total of thirteen members of the FLP family were identified. *In situ* hybridisation and gene silencing by RNA interference of the highest expressed FLP genes in *planta* are being performed in order to elucidate the localisation and possible roles of these neuropeptides in *P. penetrans*, and ultimately to assess its potential as targets for root lesion nematode control.

### **P43\_ Investigating the role of miRNAs in the interaction between root-knot nematode and rice**

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MicroRNAs (miRNAs) are 20 – 24 nucleotide long RNA molecules that play a regulatory role in a large spectrum of biological processes in plants ranging from development to stress response. They function by regulating gene expression at the transcriptional and posttranscriptional level. Studies have demonstrated that miRNAs are interesting targets for the enhancement of desirable agricultural traits such as yield and stress resistance. In this study the involvement of rice (*Oryza sativa ssp. japonica*) miRNAs during the infection by the root-knot nematode (*Meloidogyne graminicola*) was investigated. *Meloidogyne graminicola*, a major economic threat to worldwide rice yield, is a sedentary plant nematode that infects rice by penetrating near the root tip and establishes a feeding site in the vascular cylinder by creating multinucleate cells known as giant cells. These giant cells become metabolically overactive and deprive the rice plants of nutrients. This process leads to gall structures in the roots. By performing small and total RNA (with rRNA depletion) sequencing on healthy roots and galls at 3 days post infection, we identified 15 differentially expressed miRNAs likely to play an important role in the infection process. Putative targets for the miRNAs were predicted and cross referenced with the total RNA dataset. The expression profile of differentially expressed targets was validated using RT-qPCR. Among the targets is a gene encoding an ascorbate oxidase, which could play a role in the defence response of rice against nematodes. Furthermore, three miRNAs are putatively involved in a miRNA-decoy-target system whereby the miRNAs are sequestered by decoy lncRNAs, enabling expression of otherwise repressed target miRNAs. This research will add to a deeper understanding of miRNA involvement in rice immunity, which, in turn, may contribute to new nematode control strategies.

**Poster Animal Parasites and other Subjects 1**

**AP01\_ Resequencing of the rat lungworm *Angiostrongylus cantonensis* genome**

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The nematode *Angiostrongylus cantonensis* is a zoonotic pathogen in rats and the etiological agent of human rat lungworm disease. Despite its public health importance, relative little is known about this parasite at molecular and genomic level until the genome of *A. cantonensis* was sequenced by Yong *et al.* and the Sanger Institute recently. Unfortunately, these published genomes are not sequenced deep enough for reliable genome annotation. Here we described the whole-genome resequencing of the *A. cantonensis* genome (Taipei isolate) to ~278-fold coverage. The sequencing reads are assembled into 1,147 scaffolds (N50 = 1.02 Mb, Min = 895 bp, Max = 4.47 Mb, Mean = 235.1 kb). Compared with the previous release from Sanger Institute, this study represents a major improvement on the completeness of the *A. cantonensis* genome. The 269.74 Mb draft assembly is slightly larger than the size reported previously (253~260 Mb). The completeness of the sequenced genome is estimated to be 82.26% (complete) and 99.60% (partial) using the Core Eukaryotic Genes Mapping Approach. About 21% of the genome consists of LINE repeated sequences. By using ultra-deep RNA sequencing datasets (28 Gb) from different developing stages, we annotated 16,687 genes in the genome. The gene density is approximately 46 genes/Mb. Over 92% of the predicted genes are supported by RNAseq data. Introns are present in 91% of the genes with an average intron length of 956 bp. About 51% of the introns are less than 300bp. The maximum number of exons that a single gene contained is 101. More than half of the annotated genes (9,456) can be mapped to the gene ontology terms (8263) and Pfam domains (8178). Among the predicted proteins, 1,534 of them possessed signal peptide sequences but without trans-membrane domain. These putative excretory-secretory products will be valuable targets for the development of new diagnostic kits.

#### AP04\_Gastropod-associated nematodes of Western Caucasus

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A systematic survey on the diversity and distribution of nematodes associated with terrestrial gastropods in Republic Adygea, Russian Federation was carried out. The only gastropod species found infected was the snail *Oxychilus difficilis*. All *O. difficilis* examined were infected by one to three nematode species, *Alloionema* sp., *Phasmarhabditis* sp. and *Angiostoma kimmeriense* Korol & Spiridonov, 1991. In the 28S rDNA analysis, *Alloionema* sp. and *Phasmarhabditis* sp. differed from all known species of these genera with the degree of genetic divergence suggesting that each represented the new species. The new strain of *Phasmarhabditis* sp. is maintained on freeze-killed *Helix* sp. in the laboratory. It is characterised by dauer juveniles 730 (exsheathed) to 859 (ensheathed)  $\mu\text{m}$  long, females with a dome-shaped spiked tail and males possessing spicules with a hole at a distal tip. The 28S rDNA analysis has shown it being closest to *P. bohémica* with its remarkably shorter dauer juveniles. Attempts to rear *Alloionema* failed and a new search for it is to be undertaken. *Angiostoma kimmeriense* was originally described from the related snail species *Oxychilus deilus* endemic for Crimean Peninsula. Discovery of this species enabled molecular characteristics to be obtained for the species for the first time. Surprisingly, the 28S rDNA analysis has placed it among the members of *Phasmarhabditis* and close to *Phasmarhabditis* sp. EU 195967. Although the affinity of *Phasmarhabditis* and *Angiostoma* was supported by 18S rDNA analysis by Ross *et al.* (2010), such traits as the large size, limited number and the site of infection points at parasitic rather than pathogenic way of life is that of *A. kimmeriense*, yet it retains the morphological features of *Phasmarhabditis* of a cylindrical stoma and characteristic arrangement of male genital papillae. Whether *A. kimmeriense* represents the case of emergence of parasitism within a necromenic/pathogenic genus requires further research.

**O03\_Development of the novel TaqMan assay for identification and quantification of *Aphelenchoides besseyi* from rice**

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Rice is one of the most essential food sources for human beings. Rice white tip nematode, *Aphelenchoides besseyi*, is one of the most destructive plant-parasitic nematodes causing yield losses in rice. Therefore, *A. besseyi* has been on the quarantine list since 1981, according to the European Plant Protection Organization (EPPO/OEPP). In order to prevent the spread of *A. besseyi*, accurate diagnostic protocols are needed. In this study, we developed TaqMan probe assay based on small subunit ribosomal DNA (SSU rDNA) of *A. besseyi*. A dilution series of plasmid DNA of *A. besseyi* showed a high correlation ( $R^2 = 0.99$ , efficiency 95%) with Ct values. The different stages, such as egg, juvenile, male and female were successfully identified with real-time PCR assay. In addition, single live juveniles were detected without DNA extraction. Since the novel assay is specific and sensitive for detection of *A. besseyi*, it can be used for detection and quantification of rice white tip nematode.

**O04\_Multiplex real-time PCR assay for simultaneous detection and identification of *Bursaphelenchus xylophilus*, *B. mucronatus* and *B. fraudulentus* – three closely related species from the *xylophilus* group**

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The quarantine nematode, *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease in Asia (Japan, China, Korea, Taiwan) and Europe (Portugal and Spain). Distinction between *B. xylophilus* and other closely related, but non-pathogenic, species from the *xylophilus* group can be ambiguous when based exclusively on morphological characters. In Europe, the morphology of two native species, *i.e.*, *B. mucronatus* and *B. fraudulentus* closely resembles that of *B. xylophilus*. Moreover, all of these nematodes can colonise various species of pine. Therefore, for phytosanitary purposes it is necessary to have a reliable tool for both precise and rapid distinction among the three nematode species. Here, we report results of a multiplex real-time PCR analysis that employed a set of newly designed universal primers (2) and specific probes (3) for simultaneous identification and discrimination of the three *Bursaphelenchus* species. The species-specific real-time PCR gave specific products for *B. xylophilus*, *B. mucronatus* and *B. fraudulentus* templates. The designed primer combination produced reliable results in multiplex PCR assays with 25 geographically distant populations of the listed species, and no cross-reactions were observed with other tested species (*i.e.*, *B. pinophilus*, *B. piniperdae*, *B. populi* and *Parasitaphelenchus papillatus*). All species-specific real-time PCR reactions performed on DNA extracted from individual nematodes consistently produced specific amplicons, confirming the reproducibility of the method. The lowest concentration of DNA detected in the multiplex real-time PCR analysis was 30 fg  $\mu\text{l}^{-1}$ . The described molecular method is simple, rapid and reliable. It can be used for simultaneous identification of all the three tested species from the *xylophilus* group.

TUESDAY, 11<sup>TH</sup> SEPTEMBER

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## Posters Systematics 2

### **S25\_Diversity of anisakid species (Rhabditida, Ascaridomorpha, Anisakidae) in fish commercialised in the province of Jaén, Spain**

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The family Anisakidae (Rhabditida, Ascaridomorpha) is a very important nematode group according to its interactions with humans, especially the third-stage larvae, which can produce lesions in the intestine tract and allergic reactions. In the present study five species of fish captured from Mediterranean and Atlantic coasts and commercialised in the province of Jaén (Southern Iberian Peninsula) have been examined: blue whiting (*Micromesistius poutassou*), European pilchard (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*), European hake (*Merluccius merluccius*) and Atlantic salmon (*Salmo salar*). After the examination of 250 specimens, three anisakid species were found: *Anisakis pegreffii*, *A. simplex* and *Contracaecum* sp. Of these species, *A. simplex* was shown as the most abundant species, followed by *A. pegreffii* and, in very low amount, *Contracaecum* sp. was found. The study showed that the fish coming from Atlantic waters were more infected by anisakid larvae than those coming from Mediterranean waters. This study included morphological (light and scanning electron microscopy), morphometric and molecular (ITS) characterisation of the anisakid species.



**S26\_Morphological characterisation of four species of the genus *Macrolaimus* Maupas, 1900 (Rhabditida, Chambersiellidae) from Eurasia**

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*Macrolaimus* (Rhabditida, Chambersiellidae) is a rare nematode genus. With nine valid species and four *species inquirendae*, it is characterised by its lip region with six triangular liplets covering the oral aperture, inner labial sensilla absent, outer labial sensillae seta-like, cephalic sensilla papilliform, large cheilo-gymnostom, and female reproductive system monodelphic. Three European (*M. canadensis*, *M. crucis*, *M. ruehmi*) and one Asian (*M. arboreus*) species have been studied with both light (LM) and scanning electron microscopy (SEM). The results show a rather uniform pattern in key features, but also some differences in other characters. Lip region is very similar in the four species, but *M. ruehmi* displays slightly higher liplets. Lateral field consists of two longitudinal alae (three longitudinal incisures), being visibly narrower in *M. ruehmi*. Stoma shows appreciable variation in the relative length of gymnorhabdia and cheilorhabdia: gymnorhabdia representing one-third of cheilorhabdia in *M. canadensis*, one-half in *M. arboreus*, and equal *M. crucis* and *M. ruehmi*. Postvulval uterine sac appears hardly longer than the corresponding body diameter in *M. canadensis* and *M. ruehmi*, very short in *M. canadensis*, and absent in *M. crucis*. Female tail always conoid, with short spinneret-like mucro in *M. ruehmi*, long acute mucro in *M. arboricola* and *M. crucis*, and short dorsad bent mucro in *M. canadensis*. Finally, male pre-cloacal genital papillae are six in *M. crucis*, four in *M. canadensis* and *M. ruehmi*, being unknown in *M. arboricola*.

## **S27\_Diversity of plectids (Nematoda, Plectida) in caves from the Southern Iberian Peninsula**

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Plectids (order Plectida) are a nematode group associated with aquatic environments, both marine and freshwater, and wet soils. In underground cavities as caves and chasms they appear in sandy soils with a variable grade of organic matter. In the Southern Iberian Peninsula there are numerous cavities where plectids, although not the largest group, appear with some frequency. In this study, five cavities have been examined: El Jabalí cave (Santiago de la Espada-Pontones), Curva del Espino chasm (Siles), La Colada chasm, El Campamento chasm and La Murcielaguina system (the three in Hornos). This study reveals the existence of four species all of them belonging to the family Plectidae: *Ceratoplectus armatus*, *Plectus aquatilis*, *P. exinocaudatus* and *P. pseudelongatus*). Furthermore, *P. aquatilis* is the most frequent species, been found in three of the five cavities examined. Regarding the cavities, La Colada chasm, with three species, has the most diversity.

### **S31\_NeMys/WoRMS – Database with taxonomic and ecological information on nematodes**

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Since 1998 nematologists from many countries worldwide use the NeMys online tool to verify checklists of valid nematode species, its authority and distribution, to look into the available literature and also to identify some genera and species. From 2014 on, NeMys is integrated into the World register of Marine Species (WoRMS) hosted at the Flanders Marine Institute (VLIZ), operated by an international team of taxonomists editors and the WoRMS data management team. To date NeMys has been more directed to marine and brackish nematodes but as an evolving tool, NeMys is starting to focus also on continental nematodes, comprising freshwater and terrestrial records. With 8.666 checked nematode species, NeMys has become an indispensable tool for taxonomical and ecological research and for educational purposes, displaying information based on the latest classification. This year, LifeWatch is promoting a workshop with the editors where new tasks will be indicated/created to address the gaps, the non-marine nematodes will get extra attention and more species will be aggregated to the system. New identification keys will also be added, including a key to genera. The poster presents the main features and tools provided by NeMys. One of the major advantages is that registered users have full access to the available literature, helping to have a correct identification up to species. In 2017 the number of visitors reached 31,321 and this year (2018) until April it already reached 13,013. This increasing use of NeMys confirms the importance of sharing taxonomic and ecological information, improving the efficiency of our nematologists' network.

### **S32\_Morphological and molecular characterication of *Coomansus cf. zschokkei***

Aleksandar Mladenov, Milka Elshishka, Stela Lazarova, Lyudmila Lozanova, Georgi Radoslavov and Vlada Peneva

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A cosmopolitan mononchid genus, *Coomansus*, comprises more than 30 species occurring in different habitat types, including polar deserts and high mountains. *Coomansus zschokkei* has been reported from many European countries, Asia (Far East, Korea), Northern America (Costa Rica and USA) predominantly from subalpine habitats and forests, but also in freshwater lakes. Surprisingly, the records containing morphological descriptions are comparatively few. Here we present additional data on the morphology and occurrence of the species from Bulgaria along with molecular data (populations from Rila and Vitosha Mts; the population from Pirin Mt was characterised only morphologically). Further, we discuss the species identity reviewing the literature records. Bulgarian specimens of different origin differ slightly in number of metric characters and some qualitative characters. The studied material also differ considerably in several features (shorter buccal capsule and tail, higher V values, dorsal tooth position, shorter spicules *etc.*) from the topotype population. Populations from Spain and Romania had larger body, buccal capsule, tail and spicules compared to the type population and Bulgarian specimens. So far, both Bulgarian populations have been characterised by using D2-D3 expansion regions of 28S rRNA and their phylogenetic relationships with the closest mononchid genera were analysed by using Bayesian inference. The pairwise comparison of D2-D3 sequences revealed 1.3 % distance between Vitosha and Rila populations. We hypothesise that *C. zschokkei* might represent a species complex. Collecting additional materials and studying different molecular markers are needed to clarify the taxonomic status and variability of the species. The present study was supported by the Program for Support of Young Researchers and Ph.D. Students at the Bulgarian Academy of Sciences (Grant no.17-6/2017).

**S33\_ *Labronema magnum* (Dorylaimida), a new record from Arctic islands**

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*Labronema magnum* was described from wet sandy soil near a river in Sweden, north of the Arctic Circle by Altherr in 1972. In the original description, made on the basis of two female specimens, the author provides morphometric data and drawings. Here, we present additional morphological and morphometric data of *L. magnum* from Hooker Island, Franz Josef Land Archipelago (Russian Arctic) and *L. cf. magnum* recovered from Devon Island, Canadian Arctic Archipelago. The most important characters of *L. magnum* from Hooker Island are observed for the first time using SEM. *Labronema cf. magnum* differs from the type specimens by having a shorter body, well visible excretory pore, and presence of cuticular irregularities around vulva. Further, the specimens from this population are very similar to that from Hooker Island; however, it has a shorter body, different body habitus and shape of amphids, lower index 'a', well visible excretory pore, and cuticular irregularities around vulva. This is the second report of the species and represents a new geographical record for polar deserts. The present study was supported by the project ANIDIV 3.

**S34\_Analysis of population diversity of *Ditylenchus destructor* from different hosts in Gansu, China**

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The potato rot nematode, *Ditylenchus destructor* Thorne, 1945, is a pathogenetic nematode of great economic importance causing significant loss in production of potato, sweet potato and several Chinese herb medicines, including *Angelica sinensis*. It is a plant-parasitic nematode causing a high degree of damage. It is widely distributed in Asia, Europe, North America, Australia and Africa. The potato rot nematode is a quarantine organism in many countries and regions and infects more than 100 species of plants in a wide variety of families. The results of the analysis by many researchers of ITS-rRNA gene sequences revealed significant variations between different isolates in length and base composition. Moreover, several short and long repeated DNA motifs were found in the ITS1 rRNA gene fragment of this species. It was proposed to distinguish seven ITS rRNA haplotypes within potato rot nematode populations. In our study, rDNA-ITS gene sequences of nine populations collected from potato, *Angelica sinensis* and *Codonopsis pilosula* were compared and analysed. Our results showed that ITS-rRNA gene sequences of seven populations were clustered to A, C, D and F haplotypes. However, two ITS-rRNA sequences from *C. pilosula* were different to all kinds of haplotypes and it might be helpful to distinguish more haplotypes of this nematode in future.

### **S35\_Taxonomy and barcoding of plant-parasitic nematodes**

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<sup>1</sup>*Nematology Research Unit, Department of Biology, Ghent University, KL Ledeganckstraat 35, 9000 Ghent, Belgium;* <sup>2</sup>*National Plant Protection Organization, Wageningen, The Netherlands.*  
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For efficient management of plant-parasitic nematodes (PPN), their accurate identification to species level is crucial. Recently, the molecular diagnostics of nematodes based on DNA markers, mostly SSU and LSU of rRNA genes, have been gaining enormous interest despite their limited taxonomic resolution for certain nematode taxa. The mitochondrial *COI* gene, on the other hand, is the designated marker for many animals, as it evolves relatively quickly and carries sufficient 'signal' to allow species-level delimitation. Consequently, more studies refer to the importance of mitochondrial genes for nematode diagnostic purposes. However, GenBank search indicated a clear relative lack of nematode mitochondrial sequence information compared to rRNA gene markers. Therefore, in this study, mitochondrial sequence data for PPN especially the *COI* gene, in addition to rRNA gene sequences, have been generated to use them as more effective molecular barcodes. Most importantly, each new sequence started from a comprehensive nematode morphological and taxonomical analyses to secure a strong link between the morphology and the DNA sequences. Despite the importance of plant-parasitic nematodes, our study revealed a remarkable amount of uncovered diversity, both in the temperate and the tropical regions. Relevant (new) plant-parasitic species are (re)described based on a combination of light microscopy, scanning electron microscopy, ribosomal and mitochondrial DNA sequences.

### **S37\_Plant-parasitic nematodes associated with rice (*Oryza sativa* L.) in Central Java (Indonesia)**

Prabowo Lestari<sup>1,2</sup>, Nurul Dwi Handayani<sup>1,2</sup>, Marjolein Couvreur<sup>1</sup>, Antarjo Dikin<sup>2</sup>, Johannes Helder<sup>3</sup> and Wim Bert<sup>1</sup>

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The diversity and distribution of plant-parasitic nematodes associated with rice on organic and conventional farms in Central Java were determined. Soil bulk samples were randomly collected from nine locations of the organic and conventional farms in three regencies of Central Java, and nematodes were extracted using the modified Baermann method. The overall goal of this research was to provide a comprehensive overview of plant-parasitic nematode biodiversity from Central Java rice crops, and this inventory is based on the molecular, morphological and plant-pathological information. Nematodes were identified microscopically and on the basis of taxon-specific DNA motifs, and a comparison between both approaches was made. Several species have been found for the first time in Indonesia. For species without an associated molecular barcode, a comprehensive morphological and taxonomical analysis was made to secure a direct link between morphology and DNA sequence. The five most important genera of plant-parasitic nematodes found to be associated with rice were *Helicotylenchus*, *Hirschmanniella*, *Meloidogyne*, *Mesocriconema*, and *Tylenchorhynchus*. *Helicotylenchus crenacauda*, *Tylenchorhynchus zeae* and *Hirschmanniella oryzae*, one of the major pathogens in paddy rice production, were shown to be present in both organic and conventional rice farms.



### **S38\_ Molecular variation and distribution of potato cyst nematodes in Indonesia**

Nurul Dwi Handayani<sup>1,2</sup>, Prabowo Lestari<sup>1,2</sup>, Marjolein Couvreur<sup>1</sup>, Antarjo Dikin<sup>2</sup>, Johannes Helder<sup>3</sup> and Wim Bert<sup>1</sup>

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World-wide trading of plant materials in combination with agricultural practices has facilitated the spreading of cyst nematodes outside their centres of origin, and this phenomenon causes problems in numerous agricultural crops. The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are of great economic importance; despite all phytosanitary measures these pathogens cause losses of potato production worldwide. Although it is known that potato cyst nematodes are also present in Indonesia, their distribution and diversity is hardly known. This study will present the first comprehensive data on Indonesian potato cyst nematode populations. The samples were collected from 29 locations of potato crops in the Indonesian archipelago including Sumatera, Java, Sulawesi and West Nusa Tenggara. Morphological analyses will be combined with ITS rDNA and COI mtDNA sequencing and analyses of the molecular diversity allows to visualise phylogeographical patterns. These first data on species identification, population genetics and distribution of potato cyst nematode over the Indonesian archipelago is needed to define appropriate control methods.

**S39\_ Characterisation of a putative new dagger nematode species, prevalent in Golestan province, north of Iran**

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Several populations of a dagger nematode species were recovered from different natural regions of Golestan province, north of Iran. The species belongs to the non-*americanum* group and is characterised by having 3.8-5.4 mm long females having 155-173  $\mu\text{m}$  long odontostyle and 96-107  $\mu\text{m}$  long odontophore. By having two equally developed female genital branches, short rounded tail and spines in the tubular part of the uterus clearly visible in fresh females on temporary slides and also in permanently slides, it belongs to the group 6 of traditional intragenus grouping of *Xiphinema*, and is farther characterised by having four juvenile developmental stages. The tail shape of the third juvenile developmental stage is more distinctive, and helpful for its separating from closely similar species *viz.* *X. adeno-hystherum*, *X. cohni*, *X. iranicum*, *X. pyrenaicum*, *X. robbinsi* and *X. sphaerocephalum*. The BLAST search using sequences of genomic 18S, 28S D2/D3 and ITS1 rDNA as well as non-genomic *COI* sequences revealed there were no currently available sequences exactly matching them. Molecular phylogenetic analyses were performed using the aforementioned sequences of several populations, and a haplotype network was drawn of the species.

**S40\_Self-pressurised rapid freezing (SPRF): an easy-to-use and low-cost alternative cryo-fixation method for nematodes, also for low specimen numbers?**

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Self-pressurised rapid freezing (SPRF) has been applied to study two species of free-living nematodes, *Acrobeles complexus* and *Caenorhabditis elegans*. The method employs plunge freezing of a sufficient amount of specimens (in water) contained in a sealed capillary tube into the cryogen, a semi-solid form of nitrogen (nitrogen slush). Water inside the tube expands upon cooling (due to hexagonal ice formation) thereby generating pressure intrinsically supporting vitrification of the samples. The preservation of the ultrastructure of both analysed nematodes is evidenced by the study of spermatozoa structure. Transmission electron microscopy reveals clear details in mitochondria, membranous organelles and microfibers in the pseudopods. Our results indicate that the preservation after SPRF of unprotected specimens is comparable to that achieved with high pressure freezing (HPF) in the presence of a cryoprotectant. Furthermore, immunogold localisation of a cytoskeletal protein, *i.e.*, the nematode-unique major sperm protein (MSP), demonstrates for the first time that SPRF fixation preserves antigenicity. This high quality preservation method is limited only by the inner diameter of the capillary copper tubes (0.35 mm) and by the need for multiple individuals. To extend the suitability of SPRF to lower specimen numbers we have investigated how to achieve compartmentalised heterogeneous ice nucleation spatially separated from the biospecimens.

**S41\_The bipartite mitochondrial genome of *Ruizia karukerae* (Rhigonematomorpha, Nematoda)**

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Mitochondrial genes and whole mitochondrial genome sequences are widely used as molecular markers in studying population genetics and resolving both deep and shallow nodes in phylogenetics. In animals the mitochondrial genome is generally composed of a single chromosome, but mystifying exceptions sometimes occur. We determined the complete mitochondrial genome of the millipede-parasitic nematode *Ruizia karukerae* and found its mitochondrial genome consists of two circular chromosomes, which is highly unusual in bilateral animals. Chromosome I is 7,659 bp and includes six protein-coding genes, two rRNA genes and nine tRNA genes. Chromosome II comprises 7,647 bp, with seven protein-coding genes and 16 tRNA genes. Interestingly, both chromosomes share a 1,010 bp sequence containing duplicate copies of *cox2* and three tRNA genes (*trnD*, *trnG* and *trnH*), and the nucleotide sequences between the duplicated homologous gene copies are nearly identical, suggesting a possible recent genesis for this bipartite mitochondrial genome. Given that little is known about the formation, maintenance or evolution of abnormal mitochondrial genome structures, *R. karukerae* mtDNA may provide an important early glimpse into this process.

## **S42\_The Siddiqi Nematode Slide Collection**

Bex Lawson<sup>1</sup>, Thomas Prior<sup>1</sup>, Safia Siddiqi<sup>2</sup> and Gerrit Karssen<sup>3</sup>

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The Siddiqi nematode slide collection is now housed at Fera for long term restoration and curation. The collection is estimated to comprise of 25,000 slides, the majority of which contain type specimens. The full catalogue will be included as part of the Nematode Collection Europe (NCE).

## **S45\_Characterisation of nematode pests of Enset (*Ensete ventricosum*) and their management**

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Enset (*Ensete ventricosum* Welw. Cheesman) is an important starch staple crop, cultivated primarily in southern and south west Ethiopia. Enset is the main crop of a sustainable indigenous African system that ensures food security in a country that is food deficient. Related to the banana family, enset is similarly infected by plant-parasitic nematodes. From previous survey studies *Pratylenchus goodeyi* appears to be the dominant nematode pest, which is believed to contribute to reduced productivity of enset. However, while surveys have demonstrated high *P. goodeyi* infection levels, there is relatively scant information on how damaging the nematode is to enset production. There is also little information on the variability of the nematode pest in terms of levels of pathogenicity on enset and if so, how this may relate to variability in climate and temperature zones under which enset is grown. The objective of our study is to identify nematode pests of enset, assess the possible damage of nematode pests, with emphasis on *P. goodeyi*, and in relation to the presence of other diseases, how climate and agro ecology may affect this, assess cultivation and management options and to screen the resistance of enset.

## **S46\_Molecular identification of *Pratylenchus* species on wheat in Huanghuai Region of China**

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*Pratylenchus* species are migratory endoparasites of plant roots, causing root lesion of many crops and causing extensive damage to agricultural production all over the world. In order to clarify the species of *Pratylenchus* that co-infected with *Heterodera avenae* on wheat from Huanghuai region of China, twenty eight soil samples were collected from wheat field from four provinces in the region. Nematodes were isolated and five suspected species of genus *Pratylenchus* were randomly picked up from each sample, and the DNA of individual nematode was extracted as the template for PCR amplification. The fragments of rDNA 28S D2-D3 and mtDNA-COI were amplified and the PCR products were sequenced. Blast search and phylogenetic analyses both indicated that three *Pratylenchus* species, *P. coffeae*, *P. neglectus* and *P. scribneri*, are present in test samples. All DNA templates were further amplified using species-specific SCAR primers. The results showed that a single band of 140 bp was amplified from most of samples collected from Jiangsu, Anhui and Shandong provinces using primer pairs PNEG-F1/D3B5 for *P. neglectus*, a single band of 630 bp was mainly amplified from Henan province using primer PC1/PC2 for *P. coffeae*, a single band of 130 bp was amplified from a sample from Jiangsu province using primer PsF7/PsR7 for *P. scribneri*. The results of SCAR detection confirmed the species identification according to sequence analysis. Our result demonstrated that *P. neglectus* is the dominant species, and its co-infection either with *P. coffeae* or *P. scribneri* occurred quiet commonly in the region. Using mtDNA-COI gene as molecular marker, enables the close related *Pratylenchus* species to be distinguished efficiently; therefore, it could be more suitable to identify *Pratylenchus* species rather than the 28S D2-D3 marker.

## Posters Ecology 2

### **E32\_Outbreak of rice root-knot nematode (*Meloidogyne graminicola*) on direct-seeded rice in Hunan Province, China**

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In the recent decade, increasing costs and growing scarcity of labour led to a change in rice cropping pattern, and to a substantial rise in the cultivation area of direct-seeded rice in Hunan province. Changes in agricultural practices have led to a dramatic increase in *Meloidogyne graminicola* populations. During May and June from 2013 to 2018, nine root-knot nematode populations were found in direct-seeded rice fields, especially early direct-seeded rice fields, in nine counties (Hanshou, Pingjiang, Hengdong, Miluo, Yuanjiang, Yiyang, Ningxiang, Zhuzhou and Changsha) of Hunan Province, China. In both cases, the main symptoms are chlorosis, stunting and hook-shaped gall formation on the roots of rice plants. Females and egg masses were collected from rice roots from the nine populations. Morphological measurements of 20 females and 20 second-stage juveniles (J2) from each isolate all matched the original description of *M. graminicola*. The perineal patterns of the Hunan isolates were dorso-ventral, oval to almost circular in shape, moderate in height of arc, and lacking obvious lateral lines. Tail tip was prominent. The J2 average body length and stylet length were 438 (378–487)  $\mu\text{m}$  and 13.8 (12.8–15.3)  $\mu\text{m}$ , respectively. DNA of single J2 from nine isolates was extracted, the ITS1-5.8S-ITS2 rDNA region was amplified with V5367/26S (TTGATTACGTCCCTGC - CCTTT/TTCACTCGCCGTTACTAAGG) and then sequenced. The sequences were subjected to a database search using BLAST to verify the identity. Sequences from the ITS region were 790 bp and shared 99% to 100% identity with the analogous sequences of *M. graminicola* available in GenBank. The ITS sequences of the analysed Hunan *Meloidogyne* spp. thus all belong to *M. graminicola*. Due to the risk of *M. graminicola* on rice, further investigation and development of control measures are in progress.



### **E33\_ Terrestrial, free-living nematodes in the rhizosphere of watermelon plants in south-west Nigeria**

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Watermelon is a highly profitable crop consumed in Nigeria and across Africa. Although literature abound about plant-parasitic nematodes on major tropical crops in Nigeria, a paucity of information exists on nematodes associated with watermelon production in Nigeria. Most studies have focused largely on the plant-parasitic nematode group with little attention given to the other groups of free-living or beneficial nematode, referred to as terrestrial non-parasitic nematodes below. This study was conducted to record the abundance and prevalence of non-parasitic nematodes obtained from watermelon fields across south-west Nigeria. Thirty free-living nematode genera were identified from soil samples obtained from 50 watermelon fields during 2016 and 2017 in the 25 Agricultural Development Zones across south-west Nigeria. *Cephalobus*, followed by *Aphelenchus*, *Aporcelaimus* and *Rhabditis* were the predominant genera identified from this study. Significant differences were observed in the faunal analysis in terms of mean Maturity Indices metabolic footprints, feeding type composition and coloniser-persister structure of nematode assemblages obtained from this study. Principal Component Analysis revealed significant correlations between soil properties and nematode assemblages identified across the zones. Results that emanated from this study provided base-line information on the abundance and prevalence of free-living nematode genera present in watermelon fields across south-west Nigeria.

### **E35\_Seasonal changes in nematode populations in mangroves of the Red River (North Vietnam)**

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Seasonal changes in nematode community were studied on the station situated in the mangrove forest of *Kandelia candel* in the Red River delta. Quantitative samples were collected seasonally in muddy sediments between mangrove trees (in October, January, April and August). In total 69 species from 44 genera and 21 families were identified in samples. Total nematodes abundance was rather stable with no prominent spikes, the mean values fluctuate around 600 ind. 10 cm<sup>-2</sup>, seasonal differences were not significant. Species composition is stable during the year and changes appear in relative proportion of the group of dominated species. Two seasonal aspects in community structure are evident: in autumn and winter *Sabatieria* + *Terschellingia* are most common, whilst *Ptycholaimellus* + *Metachromadoroides* predominate in spring and in summer. Seasonality in community composition mainly reflects the combination of different life cycles of the most abundant species. Age structure was described for 11 most common species by seasons. Seasonal changes in abundance were found for all the species to different extents; five species reveal strong seasonal changes with maximum populations in different seasons. The relative proportions of juveniles in populations was used to evaluate differences in life strategies of common species. Life cycles varied from rather smooth with continuous reproduction along the year to prominent seasonality in reproduction.

**E36\_Characterising soil food webs: efficient extraction of soil nematodes, microarthropods and microorganisms.**

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In a metagenomic survey to characterise communities of native entomopathogenic nematodes (EPN) in Greek citrus orchards, we sought evidence that soil properties affected EPN spatial patterns directly or indirectly by affecting predatory microarthropods. Because samples of nematodes extracted from soil by sucrose centrifugation (SC) also contain soil mites, collembola, protozoans and fungal spores, we asked whether SC might be an efficient method to recover both EPN and microarthropods. Recently, the common conventional method to recover soil microarthropods was modified to employ two flotation steps in combination with the use of Berlese funnels (FBF) to obtain organisms for a clean DNA extraction from a large sample of soil. We compared FBF to SC for the ability to recover soil microarthropods. Two, 2 l soil samples were taken from soil beneath each of 12 citrus trees. Each pair of samples was extracted by the SC or FBF methods. The numbers of soil mites and collembolans recovered by SC were 55% and 177% greater, respectively, than those recovered by FBF ( $P < 0.005$ ). qPCR of DNA from nematode samples extracted by SC was previously shown to measure bacterial and fungal antagonism of nematodes more accurately than conventional soil dilution/cultivation methods. The results of this study suggest that SC is among the best current soil extraction methods to characterise food web guilds that modulate EPN services.

### E38\_Longidorid nematodes at mountain Tara in Serbia - diversity, distribution and habitats

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The Tara Mountain is a part of the European biome of coniferous mountain boreal woodlands. Prevailing higher altitudes are 1000 to 1100 m. Winter is snowy, not very cold, summer mild, not very dry. Tara is a repository of wildlife, with numerous relict and endemic species, protected within the National Park 'Tara'. Several reserves harbour endemo-relict Serbian spruce, *Picea omorika*. This preliminary report focuses on longidorid nematodes (Longidoridae: Dorylaimida). Samples were collected systematically within a grid of 2 × 2 km tetrads. Longidorids were found in 59% (40/68) of samples/localities. The dominant habitat type was mixed forest of spruce, fir and beech (*Piceo-Abieti-Fagetum*) on limestone, occupying mainly the central and western highlands; the occurrence of longidorids was highest there, in 16/19 (84%) of samples. In other major habitat types (broad leaved forests, coniferous forests, grasslands and/or shrubs, and transitional terrains), occurrence in samples was 40% to 50%. Identification of some species is not yet completed. Nine  $\alpha$ -taxonomic entities have been identified, five pertaining to *Longidorus* spp. and four to *Xiphinema* spp. *Longidorus piceicola* was frequent in mixed forests of the central highland, but not found in the western highland. *Longidorus silvae* was not found on serpentinite terrains. *Longidorus* sp.1, possibly a new species (code A45,B45,C3,D3,E1,F3,G1,H1), was infrequently found in forests on limestone. *Longidorus* sp.2, (code A12,B12,C2,D3,E1,F3,G1,H12), with slender body and short odontostyle, was rare. The most frequently found species (complex?) was *Xiphinema dentatum* s.l., exhibiting considerable morphological variation between field populations. *Xiphinema parudentatum*, recently described from Tara, was found in a few localities of the central highland. *Xiphinema densispinatum* was infrequently found in various habitats on limestone. Only one finding of a *Xiphinema americanum*-group species, pertaining to *X. taylori* s.l., is possibly a recent introduction. The purpose of this work is to contribute to understanding the ecology and diversity of longidorids.

### **E39\_Plant-parasitic nematodes in Serbia - the results of a five years occurrence survey**

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The survey of the occurrence of economically important plant-parasitic nematodes was conducted in the frame of Serbian Plant Protection Forecasting Service in the period of 2013-2017. Special attention was paid to three species of plant-parasitic nematodes, *Heterodera schachtii*, *Ditylenchus dipsaci* and *Anguina tritici*. Monitoring of population densities and testing propagation material were the basic strategies in predicting these species in order to offer the farmers the proper recommendation before planting. Among these species, *H. schachtii* was the most frequent. It was detected in 32 localities in the sugar beet growing areas of Serbian Northern province Vojvodina as a result of narrow crop rotation. Many sugar beet producers reported yellowing and wilting, especially in 2014 caused by weather conditions with rainy spring and dry summer. The most effective measure against sugar beet cyst nematode in these areas was to keep the nematode population below the economic threshold using crop rotation and tolerant varieties. *Ditylenchus dipsaci* was found in 12 localities in garlic propagation production in Vojvodina. In 2017 heavy infestations were noticed with stunting and chlorosis influenced by cold weather conditions in the spring. Planting of propagation material free from this pest and crop rotation with non-host were the main measures used to prevent stem nematode spreading. *Anguina tritici* was the least frequent species, practically eliminated from the main grain field areas in northern Serbia by using high quality seeds and crop rotation. In 2014, it was detected only in three locations of central Serbia with the symptoms of wrinkling and swelling as a result of monoculture and uncleaned seeds. The sanitary measures with seed certification were implemented in order to eradicate this pest in these areas.

### E43\_Plant-parasitic nematodes in raspberry in Norway

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Raspberry, *Rubus idaeus*, is an important crop in Norway, with recent annual net sales of 2 120 – 2 526 tons. This does not include self-picking and direct sales to customers. Raspberry ‘Glenn Ample’ fields should stay productive for 12-15 years, but it happens that some plants deteriorate, produce few canes, wilt gradually and finally die. Since plant-parasitic nematodes (PPN) could be involved in this condition, a survey of PPN in raspberry fields was carried out in 2017. Samples from 47 fields with growth problems were collected by the Norwegian Agricultural Extension Service in the counties Sogn og Fjordane (n=26), Agder (n=8), Møre og Romsdal (n=4), Akershus (n=3), Buskerud (n=2), Vestfold (n=2), Telemark (n=1) and Nordland (n=1). Root lesion nematodes (*Pratylenchus*) and spiral nematodes (Hoplolaimidae), occurred in 83% of the samples, *Tylenchus sensu lato* in 63%, pin- and ring nematodes in 52%, stunt nematodes and stubby root nematodes in 50%, needle nematodes and cyst nematodes in 2% of the samples. The highest densities (ind. (250 ml soil)<sup>-1</sup>) recorded were 545 spiral-, 295 root-lesion- and 225 stubby root nematodes. Some differences were noted in the relative abundance of PPN between the county Sogn og Fjordane in Mid-Norway and Agder in the South. Spiral nematodes, root-lesion nematodes, and pin- and ring nematodes were more frequent in Sogn og Fjordane, while stubby root nematodes and Longidorids were more frequent in Agder. Species identified were *Tylenchus davainei*, *Cephalenchus leptus*, *Tylenchorhynchus dubius*, *Pratylenchus crenatus*, *P. penetrans*, *P. fallax*, *Helicotylenchus canadiensis*, *H. pseudorobustus*, *H. variocaudatus*, *Rotylenchus fallorobustus*, *Paratrichodorus pachydermus* and *Longidorus elongatus*. Compared to other studies our material is different with regard to the high frequencies of spiral nematodes and Trichodorids. Frequently, spiral nematodes, root-lesion nematodes, and pin- and ring nematodes were more abundant in root soil compared to bulk soil.

**E46\_Distribution of the nematological community in rivers of the High Guadalquivir Basin (SE Iberian Peninsula) and the influence of abiotic and vegetational variables**

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A nematological study was carried out identifying the species of the different orders in five rivers of the high basin of the Guadalquivir River in Andalusia, three on the left bank that may present alterations of urban and agricultural or industrial use, and two on the right bank included in protected natural areas. After analysing biotic (vegetational) and abiotic variables of the collected samples in each sampling point – by following a pre-established design - we have verified that the quality of water suffers from a serious decrease from the headwaters (usually much cleaner) to certain areas of their course. An important impact on the change of nematode communities can be observed, as they are markedly different at an order level. The frequencies of each order in the five rivers are shown, either in the upper, middle-lower or mouth sections. The Dorylaimida order appears to be the most frequent in the headwaters (40-50%) in most of these water courses, as only in one of them does it share a similar frequency with the orders Monhysterida and Plectida. However, when analysing the nematological community in the medium-low sections, a radical change occurs in three of the five rivers, which coincide with those with contaminated water and in the left bank of the basin, in which the Rhabditida order is the most frequent, 40%, quadrupling the percentage of dorylaimids, scarcely represented, as occurs with the plectids. Therefore, we can consider both Dorylaimida and Rhabditida orders as ideal taxa for a limnological study, because of their diversity, abundance and wide distribution within the nematodes group.

#### **E49\_ First report of three plant parasitic nematode species on tomato in Cape Verde**

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Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetables on irrigated land in Cape Verde. In June 2015 tomato plants ('Savana') with symptoms of stunting, leaf wilting and poor fruiting, were recorded in a field in Achada Colaço, Santiago Island. Roots of these plants were galled, indicating the occurrence of root-knot nematodes. In this study we provide morphological and molecular characteristics of three plant-parasitic nematode species on tomato in Cape Verde. Eight tomato plants with soil surrounded root systems were harvested. Nematodes from roots and soil samples were isolated and nematode DNA was extracted. Our morphological and molecular studies revealed the presence of *Pratylenchus brachyurus*, *P. delattrei* and *Meloidogyne incognita* in root systems and root zones of tomato plants. Morphological characteristics of these three nematodes were in agreement with the previous descriptions. For molecular analyses, 18S rDNA and 28S rDNA were amplified and sequenced. The obtained 28S rDNA sequences from *P. brachyurus* and *P. delattrei* were used for phylogenetic analyses. *Meloidogyne incognita* was identified using species-specific primers Mi2F4/Mi2R1. Molecular data confirmed the results of morphological observations. The presented study gives the first record of the occurrence of the three plant-parasitic nematode species on tomato in Cape Verde. Representatives of *P. brachyurus*, *P. delattrei* and *M. incognita* species are considered economically important plant-parasitic nematodes that are widespread mainly in tropical and subtropical regions. The obtained results broaden our knowledge on plant-parasitic nematode biodiversity of Cape Verde as well on the morphometry and molecular characteristics of this serious pest of several important plants.



**E52\_Survey on nematodes in caves in the Bohemian Karst (The Czech Republic)**

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Except for the paper by Du Preez *et al.* (2017) there is a lack of comprehensive information on nematofauna in caves. In The Bohemian Karst (a Protected Landscape Area situated between Prague and south-western part of the Central Bohemian Region) more than 600 registered caves of various size are located. The goal of the present survey was to find out whether nematodes (and subsequently which kind of) are present in these caves. During the years 2016-2018 a preliminary nematological survey was conducted. Eight different caves were sampled and by using a centrifuge extraction method more than two hundred specimens of nematodes from a different trophic groups (except predators) belonging to 15 genera were found and diagnosed.

Du Preez, G. *et al.* (2017). Nematodes in caves: a historical perspective on their occurrence, distribution and ecological relevance. *Nematology* 19, 627-644.

**E55\_Assessment of the presence and abundance of endoparasitic nematodes in roots of apple trees from selected orchards in South Tyrol by using a mobile adaptation of a Seinhorst spray mist-chamber**

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Apple production plays an important economic role in South Tyrol (Northern Italy). The high-density orchards are mainly located in the major river valleys and their side slopes. Most of the orchard sites have been planted with apple trees for many decades. Consequently, apple replant disease (ARD), which is manifested by stunted growth, delayed cropping and mortality of trees, may represent a major challenge for the renewal of apple orchards. Apart from abiotic soil factors, biotic interactions have been particularly implicated in the complex etiology of ARD. Several recent studies have addressed the soil and rhizosphere bacterial and fungal communities in ARD-affected orchards in South Tyrol. Less attention has been given to plant-parasitic nematodes, which were associated with root destruction and growth reduction in other geographic areas dealing with replant problems. The present study represents a first step to a systematic survey on the occurrence and abundance of root-lesion nematodes in roots of apple trees. The samples were collected from selected orchards in South Tyrol affected to different degrees by ARD. In addition, the construction of a mobile adaptation of a Seinhorst spray mist-chamber for the extraction of endoparasitic nematodes from root samples is described.

## E56\_Detection of root-knot nematodes in kiwifruit orchards in Turkey

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The Black Sea Region is one of the primary cultivation areas for kiwifruit production in Turkey. In recent years, interest in the kiwi production in Samsun province has increased as an alternative to hazelnut in this region. However, root-knot nematodes are the important potential threat to limit the production of kiwifruit in the world. This study aimed to determine the occurrence and distribution of root-knot nematode species in kiwifruit orchards from Samsun province. Surveys were conducted in twenty-five kiwifruit orchards from six districts in this province during the period of September-November 2017. The roots of 10-20 plants from each kiwifruit orchards were checked for gall symptoms caused by root-knot nematode. When kiwifruit roots with galls were detected, root and rhizosphere soils were collected. Esterase enzyme phenotypes of females were used to identify *Meloidogyne* isolates. Survey results indicated *Meloidogyne* were detected in 23 (92%) of 25 kiwifruit orchards. While *Meloidogyne luci* and *M. hapla* were present in 13 and 12 kiwifruit orchards, *M. arenaria* and *M. incognita* were found in five and one, respectively, kiwifruit orchards surveyed. However, mixed population containing *M. hapla* was detected in six kiwifruit orchards and of these orchards, three were mixed with *M. luci*, one was mixed with *M. arenaria* and two contained all three species. These results showed that *M. luci* was the most prevalent species and this is the first record of *M. luci* parasitising kiwi plants in Turkey.

**E58\_Due to drought, could irrigation increase plant-parasitic nematode populations in grape vines?**

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Grape vines have been cultivated in Cyprus since the 2<sup>nd</sup> millennium B.C. with more than fifteen varieties considered as indigenous. Nowadays, grape vines are mainly cultivated in the South-South West part of the island and most of the vineyards are grown as anhydrous (rainfed). Since the middle of the last century, Cyprus is facing long droughts with warmer summers and dry winter. As a response, vine growers began to install irrigation systems in their vineyards, affecting the soil properties and, as a result, affecting the soil biota and specifically plant-parasitic nematode populations (PPN). Under the LIFE GREEN GRAPE PROJECT *LIFE16 ENV/IT/000566*, we have conducted surveys in irrigated and non-irrigated vineyards in order to estimate the population levels of PPN in the soil, using the Baermann funnels method and root examinations. Nematodes were identified using molecular techniques (PCR and NGS). Preliminary results have shown the presence of *Meloidogyne* species in irrigated vineyards, plus a large variety of non-parasitic, free-living nematodes. Furthermore, we are investigating the presence of native antagonistic microorganisms (free-living nematodes, protozoa, bacteria, and fungi) in the soil, that can be used as potential biocontrol agents. The present study will reveal the vine-root biota, the potential increase of PPN in vineyards and assist policy maker to promote early management actions on the control of PPN and other vine diseases such as Trunk Diseases, due to the need of irrigating the Cyprus vineyards under drought conditions.

**E59\_Comparative whole-genome analysis reveals a dominant invasive population in China and molecular insight into invasiveness and adaptation of *Bursaphelenchus xylophilus***

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is an important invasive species, which killed millions of *Pinus* trees in Asian and European introduced regions. To trace the migration paths and understand genetic underpinning of invasiveness of PWN, we have sequenced and analysed whole genomes of large cohort of 55 PWN strains isolated from both the native region (15 strains) and introduced regions, including 35 strains isolated from different geographic regions in China. Whole-genome comparative analysis showed that most of single nucleotide polymorphism sites (SNPs) harboured in the native population. Phylogenetic and principle component analysis indicated a dominant invasive population (DIP) existing in China. Targeted analysis of rDNA sequences enabled us to trace the origin and migration paths of DIP at a global scale. A progressive loss of genetic diversity was observed along the invasion path, indicative of genetic drifts and multiple genetic bottlenecks. We identified genes affected by variation sites with base frequency 100% in DIP (fixation) but less than 50% in the native population, and found that protein kinases, major facilitator superfamily and ABC transporters, chemoreceptors, peptidases, cytochrome P450 and other detoxification enzymes, are rich, which are involved in signal transduction, transport and catabolism, amino acid metabolism, xenobiotics biodegradation, etc. We suppose these genes might be associated with adaptation of the nematode on pine hosts and vector beetles, so that be related to PWN invasiveness. We also identified DIP-specific genomic variations and found most of those variations clustered in a few scaffolds, indicating the effect of selective sweeps. Based on our results, we suppose that genetic drifts, mutations and selective sweeps are evolutionary driving factors in shaping the genomic variations and genetic differences between the DIP and others, which enable the invasive PWN to achieve the ability to respond to new environmental conditions.

## E61\_Plant-parasitic nematodes in tree nurseries in Flanders

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Tree nurseries are an important economic activity for Flanders with a strong focus on export. In recent years, more nematode problems have been noticed. The long field period, ranging between one to several years, allows initially low populations to build up to damaging levels. Due to limited availability of land, the intensity of planting is high, resulting in severe losses when seedlings or young plants are planted in highly infested fields. We expect that nematode problems will increase and become difficult to deal with in the near future due to restrictions in chemical soil disinfestation. Growers are often not aware of nematodes and try to counter growth reduction with extra fertilisation, irrigation or application of fungicides. Green manures are receiving a lot of attention as a measure for nematode control, but their efficacy depends on many factors and applying them as a control measure should be carefully investigated before recommendations can be made. One important factor is knowing which plant-parasitic nematodes are present. Therefore, a survey was organised in which more than 140 samples were taken all over Flanders. *Pratylenchus* spp. were the most prevalent; several species were found in 70% of the samples. They were followed by *Tylenchorhynchus* spp. (59%), *Paratylenchus* spp. (59%), *Trichodorus* spp. (58%) and *Rotylenchus* spp. (44%). The highest densities of *Pratylenchus* spp. and *Tylenchorhynchus* spp. were found in sandy and sandy loam soils. The abundance of *Paratylenchus* spp. was highest in loamy soils. It is clear from our survey that several important groups of plant-parasitic nematodes are present in tree nurseries. Increasing growers' awareness of nematodes is needed, together with the development of an integrated nematode management programme for tree nurseries.

### **E63\_Effects of mercury (Hg) on soil nematodes: a microcosm approach**

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Mercury (Hg), one of the most toxic heavy metals, is commonly used in the gold extraction process in many countries. Our previous field work on the impact of Hg on a small-scale mining area in Sibutad revealed no significant negative effects on nematode-based indices despite Hg concentrations up to 127-fold higher than the permissible level set by UNEP (2013). Using a microcosm approach, we now applied similar Hg concentrations as commonly found in these field sites (2.5, 5 and 10 ppm Hg) and determined their impact on nematode communities from a different soil with different physico-chemical soil attributes under controlled conditions. Our results showed (a) limited 'bottling' effects (incubation effects) after a 45-day incubation period: a nematode abundance decrease of up to 37%, but absence of significant differences in diversity and nematode assemblage composition; (b) Hg concentrations of 2.5 ppm significantly impacted total nematode abundance but not the other nematode assemblage descriptors, which were, however, significantly impacted from Hg levels of 5 ppm onwards. Our results demonstrate that total nematode abundance was the most sensitive descriptor to Hg pollution, whereas diversity and assemblage composition were impacted only at higher Hg concentrations. The discrepancy between our microcosm and previous field-based results are probably related to differences in physico-chemical soil attributes.

**E64\_Studies on nematodes from Longidoridae and Trichodoridae in Northwestern Marmara region of Turkey**

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The dagger nematodes, needle nematodes and stubby root nematodes, *Trichodorus* spp., are a large group infesting several host plants. Some species from these genera have the ability to transmit virus diseases to host plants. An extensive nematode study was carried out in Northwestern Marmara Region of Turkey. Within a 5 year period *Xiphinema index* Thorne et Allen, 1950; *X. italiae* Meyl, 1953; *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951; *X. turcicum* Luc & Dalmasso, 1963; *X. ingens* Luc & Dalmasso, 1964; *X. pyrenaicum*, Dalmasso, 1964; *X. opisthohysterum* Siddiqi, *X. diversicaudatum* (Micoletzky, 1927), Thorne, 1939, *Longidorus elongatus* de Man, *L. attenuatus*, Hoper, 1961 and *Trichodorus similis* were found associated with different hosts plants such as vineyards, apple, pear, cherry, walnut, fig, almond, quince, apricot, kiwi, olive, pine, plum, pomegranate, wheat, sunflower, tomato, onion, cypress and spruce. This study includes all survey results, gives information about geographical distributions and host ranges of *Xiphinema* spp., *Longidorus* spp. and *Trichodorus* spp. in Northwestern Marmara Region of Turkey.



**E65\_ *Mesocriconema xenoplax* (Nematoda: Criconeematidae) parasitising turfgrass in Portugal**

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Turf-grasses are among the most widely used ornamental plants in the world, serving important functions in soil stabilisation and providing safe surfaces for recreational activities. In different public gardens surveyed during 2016 in Caxias, a region near Lisbon, Portugal, root and soil samples were collected from the soil rhizosphere of turfgrass. The grass showed yellow patches, stunting and poor growth and the roots presented discolouration. Specimens were recovered from soil in large numbers, following the sieving and decanting technique from cores taken across the yellowing patches. The nematodes collected were identified as *Mesocriconema xenoplax* (peach ring nematode) based on morphological characters, both males and females with all the observed features being in agreement with previous descriptions. Confirmation by molecular analysis was made through sequencing of the fragment spanning D2/D3 domain of the 28S rDNA gene. The obtained fragment showed high similarity with *M. xenoplax* sequences isolated in China and Japan. Its presence in Portugal was detected for the first time in fig trees in 2008 and since has been also found in different important fruit trees such as almond and walnut. In view of the touristic impact of the regions surrounding Lisbon that rely on extensive areas of gardens, parks and golf courses, it is of great concern and of economic and environmental responsibility to avoid the spread of this parasitic nematode. Therefore, good practices for reducing nematode damages and to prevent loss of amenity value of the affected lawns are of primary importance among which, proper irrigation and fertilisation and avoidance of other stresses on the grass are the most relevant. This is the first report of *M. xenoplax* associated with turfgrass in Portugal and in Europe.

**E67\_First report of a plant-parasitic nematode *Cryphodera* species on *Lagerstroemia indica* in Korea**

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During a survey of nematodes in Korea, specimens of the uncommon genus *Cryphodera* were found from the roots of *Lagerstroemia indica*. Comparative morphological and molecular studies of the nematode revealed that it was similar to *C. coxi*. Integrative molecular data for this species were obtained using rRNA large subunit (LSU) D2-D3 segments and ITS region for the first time. The sequenced LSU D2-D3 segments and ITS region are 792 and 1,028 bp, respectively. A BLASTn search of *Cryphodera* sp. on the LSU D2-D3 segments revealed high-scoring matches 95% (752/792) with *Cryphodera sinensis* (GenBank accession number JX566456), which is the species isolated from the roots of *Boehmeria nivea* in China. A BLASTn search of *Cryphodera* sp. on the ITS region also revealed similarities with *C. sinensis* (JX566457), with only 83% identity (892/1,075).

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**M50\_Nematicidal activity of fluensulfone against *Xiphinema index* and *Longidorus vineacola***

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The nematicidal activity of fluensulfone against *Xiphinema index* and *Longidorus vineacola* was compared with that of organophosphate and carbamate nematicides. Numbers of *X. index* recovered *via* Baermann funnels from infested soil treated with fenamiphos or cadusafos at 2.0 and 4.0 mg l<sup>-1</sup> soil were higher than that recovered from non-treated infested soil. The number of recovered *X. index* after fluensulfone treatment was at the same level of the control. In experiments using fig plants in pots, *X. index* population was much lower after the treatment with fluensulfone than that of fenamiphos-treated soil. The nematode control efficacy of fluensulfone against *X. index* was much higher by pre-planting application than post-planting application. Treatment of *L. vineacola*-infested soil with fluensulfone 2.0 and 4.0 mg l<sup>-1</sup> soil slightly reduced the number of recovered nematodes in one of two trials, whereas fenamiphos and cadusafos did not, or even increased the number of the nematode in the soil. The nematode control efficacy of fluensulfone against *L. vineacola* was much higher than that of fenamiphos or oxamyl on pepper in pots. Again, pre-planting application of fluensulfone was more effective in reducing the *L. vineacola* population than post-planting application. The results suggest that fluensulfone can be effective in control of *Xiphinema* and *Longidorus* species in the field, especially by pre-planting treatments.

### **M51\_Natural, bioactive compounds against *Heterodera schachtii* – the CombiCom project**

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The beet cyst nematode, *Heterodera schachtii*, is a devastating problem in sugar beet production and a serious problem in oilseed rape. Resistant or tolerant sugar beet varieties and resistant trap crops are currently used to manage the problem, but resistance-breaking nematode populations jeopardise this management system. For that reason, eco-friendly and effective alternatives have to be developed. Natural microbial products represent an invaluable source of bioactive compounds, which can be used as such or serve as chemical frameworks for developing new agrochemicals. In our project, we aim to change the paradigm from synthetic chemistry to sustainable microbial production by using synthetic biology approaches. We exploit microbial secondary metabolite pathways to deliver natural compounds by creating synthetic biology-tailored microbial chassis allowing for the sustainable production of novel high-value compounds. Currently, we evaluate these compounds for their activity against *H. schachtii* infective juveniles. Moreover, the compounds impact on nematode parasitism will be investigated using our well established gnotobiotic *Arabidopsis thaliana* - *Heterodera schachtii* system and glasshouse studies. In parallel, the effect of the compounds on plants will be studied by checking growth parameters and by measuring reactive oxygen species burst. Finally, the extent to which a selected compound changes the plant-associated microbial community will be determined.

**M54\_Rice (*Oryza sativa*) genotypes Supa and Komboka are partially resistant to root-knot nematodes at different steps of the infection process**

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The response of the rice (*Oryza sativa*) genotypes 'Komboka' and 'Supa' to rice root-knot nematodes, *Meloidogyne graminicola* and *M. javanica*, was evaluated in plant room conditions. *O. glaberrima* 'TOG5674' and 'CG14' were used as resistant control for *M. graminicola* and *M. javanica*, respectively, while 'UPLRi5' (*O. sativa*) was included as susceptible control for both nematode species. None of the two genotypes under investigation was completely resistant to the root-knot nematodes. However, both genotypes were found to be partially resistant to these nematodes. For both nematode species, a large difference in galling severity was found between the rice genotypes under investigation and their respective susceptible control 'UPLRi5'. 'Komboka' and 'Supa' responded less to galling by these nematodes than the 'UPLRi5'. Further analysis showed that 'Komboka' and 'Supa' were significantly less favourable than 'UPLRi5' for juvenile penetration, development into adult females and reproduction. Nematodes that successfully penetrated and developed in 'Komboka' and 'Supa' showed strange phenotypes illustrating the post infection mechanism of resistance; *M. graminicola* was more aggressive on these rice genotypes than *M. javanica*.

### M55\_Host reaction of tomato rootstocks to *Meloidogyne incognita*

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Root-knot nematodes (RKN), *Meloidogyne* spp., are the most damaging nematodes in horticultural crops, causing significant losses worldwide. Their populations have been traditionally managed through the application of synthetic pesticides, and after the methyl bromide ban, RKN populations have increased in Europe. The COCOoN project (POCI/01/0145/FEDER/016611-PTDC/AGR-PRO/3438/2014) aims to improve sustainable management of RKN in tomato, *Solanum lycopersicum*, through a combination of practices, including plant grafting. Several rootstock cultivars, described by seed companies as having intermediate resistance to RKN, are available for tomato. We assessed the host reaction of 11 different rootstock cultivars from seven major seed companies to *M. incognita*, in pot trials in controlled conditions, at 25°C. Susceptible tomato cultivars 'Coração de Boi' and 'Moneymaker' were used as positive controls. Sixty days after inoculation, the numbers of galls, egg masses, eggs and juveniles in soil were determined and used to estimate gall and egg mass indices, reproduction factor and female fecundity. Of the 11 rootstock cultivars tested, four were resistant ('Arnold', 'Embajador', 'Forzapro' and 'Tronix'), one was hypersensitive ('Silex') and the remaining six were susceptible according to the standard classification (Sasser *et al.*, 1984). However, numbers of galls and egg masses per root system were smaller compared to control plants in 10 out of the rootstock cultivars ( $P < 0.05$ ). Female fecundity was significantly larger in seven of the rootstock cultivars than in control, with each female producing an average of up to 1330 eggs; in these rootstock cultivars, the reproduction factor was greater than 1. In summary, rootstocks generally suffered less damage (root galling) from RKN but more than half of them allowed nematode reproduction. Thus, the success of vegetable grafting in nematode control could be increased by combination with biocontrol agents that target nematode eggs and prevent RKN population increase.

#### Reference

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### **M56\_Pest risk analysis for *Meloidogyne ethiopica* and *M. luci* in Slovenia**

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Root-knot nematodes, *Meloidogyne ethiopica* and *M. luci*, are serious pests of several agricultural crops belonging to monocotyledons and dicotyledons, including herbaceous and woody plants. All previously reported populations of *M. ethiopica* in Europe and Turkey were reclassified as *M. luci*. In Brazil and Chile, *M. ethiopica* is considered to be a damaging species on kiwi and grapevine, as infestations lead to a reduction of plant growth, fruit size and quality. It is widely distributed in vineyards in Chile. In Brazil, *M. ethiopica* has also been reported to cause multiple galls on potato root systems, as well as protuberances on tubers. Similarly, *M. luci* has been reported on several vegetable crops and has also been shown as a potential threat to the potato production in Portugal. *Meloidogyne ethiopica* and *M. luci* can be introduced by several pathways but the highest risk for entry and spread in the pest risk assessment (PRA) area (whole territory of Slovenia) is considered the pathway of host plants for planting with or without soil attached. In addition, plant parts like tubers may present also high risk of spreading in case of pest establishment outdoors. Human assisted spread of the pests is the most important one. The probability of establishment in the protected area is high. *Meloidogyne luci* has already been detected in the open field production in Europe (Mediterranean climate), in corn and kiwi production in Greece, and potato production in Portugal. There is a high risk of *M. luci* establishment outdoors in the PRA area, as this species was proved to overwinter outdoors at the open field in continental and Mediterranean climate. The endangered area in the PRA area for *M. luci* is therefore: protected production area, vegetable production area, potato production area, corn and other cereals production area, and vineyards, kiwi and peach production area.

**M58\_Nitrogen and sulphur inputs affect the performance of biofumigation with Indian mustard (*Brassica juncea*) in the suppression of potato cyst nematode (*Globodera pallida*)**

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Potatoes are an important crop in Great Britain with 121,000 ha planted in 2017 achieving 6.04 Mt. The most important pest affecting this crop are the potato cyst nematodes (PCN) (*Globodera pallida* and *G. rostochiensis*), which occur in 48 and 35% of potato growing land (ware) in England & Wales and Scotland, respectively. Uncertainty over the future availability of nematicides has resulted in research on alternative crop protection measures such as biofumigation. Biofumigation involves the use of brassicaceous inter-crops with a high glucosinolate (GSL) content. When macerated and incorporated into moist soil, an endogenous enzyme, myrosinase (thioglucoside glucohydrolase, E.C.3.2.1.147), hydrolyses foliar aliphatic GSLs into an array of biocidal and volatile compounds including isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidine-2-thiones. As part of a 3-year Agriculture & Horticulture Development Board (AHDB) project investigating biofumigation for PCN management, the effect of variable rates of nitrogen and sulphur on the biomass of Indian mustard 'Caliente 99' (*Brassica juncea*), stem and leaf GSL accumulation and suppression of PCN were investigated under field conditions at a site in Crudgington, Shropshire, UK. Both nitrogen and sulphur application to *B. juncea* resulted in significant reduction in the viability of encysted eggs of PCN, 6 weeks after the brassica residues had been incorporated ( $P < 0.01$ ). Additionally, nitrogen significantly increased the fresh and dry weight of *B. juncea* stems and leaves ( $P < 0.01$ ). Based on the findings of this field experiment, we would recommend applying nitrogen at a rate of 100 kg ha<sup>-1</sup> with sulphur at a rate of 25-50 kg ha<sup>-1</sup> to summer sown Indian mustard for the reduction of PCN. Further analysis of stem/leaf GSLs associated with treatments in this experiment are in progress.



**M60\_Utilisation of some plant based oils in controlling of root-knot nematode (*Meloidogyne javanica*)**

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Root-knot nematodes are the most important pests in tomato fields because they reduce yield and quality. This study aimed to determine the possibilities of using some essential oils to control *Meloidogyne javanica*, which is harmful to tomato plants. For this reason, five different doses (0.0625%, 0.125%, 0.25%, 0.5% and 1.0%) of four different essential oils (*Laurus nobilis*, *Melissa officinalis*, *Eucalyptus globulus* and *Ricinus communis*) were used to examine hatching inhibition, second-stage juveniles mortality, and the damage level and growth criteria of the tomato plants infested with *M. javanica* in the laboratory and glasshouse. The most hatching inhibition (45.5%) was observed in *E. globulus* with plants doses of 0,5%. The most second stage juveniles mortality (98,5%) was found in *M. officinalis* at treated with doses of 1.0%. When considering the effects of essential oils to the plant growth criteria, the most affected criterion was found in plant height. The most effective application for the tomato plant height (51.3 cm) was *M. officinalis* at 0.25% rate. Growth criteria and damage level of infested tomato plants were affected differently in all applications. *Melissa officinalis* was found the most effective on reduction of root galling and egg mass per root; at the 0.125% dose, root gall index was 0.88 and egg mass number per plant was 0.38 at 0.0625% doses. The *E. globulus* was the next most effective but *R. communis* and *L. nobilis* were found to be less effective in the reduction of galling and egg mass per root. When the results were reviewed together, it was concluded that *M. officinalis* oil and doses may be used as an alternative to synthetic pesticides for the control of *M. javanica*.

**M61\_The host status of some pepper varieties to different initial population densities of *Meloidogyne arenaria* and *M. javanica***

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Root-knot nematodes (*Meloidogyne* spp.) are spread throughout the world and have the main detrimental effect on vegetables. Vegetable varieties respond differently to various initial populations of root-knot nematodes. In this study, the reactions of four different pepper varieties ('Caglayan F1', 'Treasure F1', 'Uslu F1' and 'California Wonder'), widely grown in Turkey, were investigated at three different initial populations of *Meloidogyne arenaria* and *M. javanica* (0, 500, 5000 eggs plant<sup>-1</sup>) in comparison with the 'H-2274' tomato variety known to be sensitive. Pot experiments were conducted in controlled glasshouse conditions with four replicates and two repeats. Eight weeks after nematode inoculation, all plants were uprooted and the number of leaves, plant height, the number of fruits, fresh and dry fruit weight and root weights were determined as plant developmental criteria. In addition, roots were evaluated according to the 0-10 gall index and the reproduction factor ( $R_f = P_f / P_i$ ) was recorded to determine the damage caused by nematodes and the variation in the nematode populations. In general, when the plant development criteria are summarised, the most affected variety from each nematode species was the 'Uslu F1' and the least affected variety was 'California Wonder'. For both nematode species, the plants with the highest gall index were the control plants, while the 'Uslu F1' and 'California Wonder' had the least gall index. Again, for both nematode species, the control had the highest number of eggs per root but 'Uslu F1' and 'California Wonder' had the least number of eggs. The same is true for the  $R_f$  reproductive index. Generally, as the nematode initial population increased for both species, the plant development criteria were not affected equally, while the nematode damage in the plant increased proportionally. Thus, in the vegetable cultivation areas where these nematode species are present, pepper producers can be offered 'Uslu F1'.

**M65\_Managing root knot and cyst nematodes on potato in Kenya with banana paper: ‘Wrap & Plant’, a novel concept.**

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Both potato cyst (PCN) (*Globodera rostochiensis*) and root-knot (RKN) (*Meloidogyne* spp.) nematodes represent a serious threat to potato (*Solanum tuberosum*) production and to food security in the Kenya, where potato is the second most important staple crop and mainly produced by smallholder farmers. The ‘Wrap and Plant’ technology is an innovative concept based on banana fibre paper, as a mechanism to deliver micro-doses of the nematicide abamectin ( $\approx 100 \text{ ng (400 cm}^2\text{)}^{-1}$ ) to the root zone. Seed potatoes, ‘wrapped’ with the impregnated paper at planting, were assessed for nematode management in farmers’ fields and pot studies over two years and compared against a *Trichoderma asperellum* (Real Trichoderma®; Real IPM) impregnated paper and a paper-control (without abamectin), as well as non-paper treatments of abamectin (Tervigo®; Syngenta), *T. asperellum* and an absolute control (farmers’ practice). Results showed that the number of PCN and RKN infective juveniles, and of PCN cysts, were reduced with all the treatments except for the absolute control. The greatest reduction was observed with the ‘Wrap and Plant’ abamectin-treated paper, which was also associated with the largest yields. Potato yields were consistently fourfold higher at  $\bar{\chi} = 26.41 \text{ T ha}^{-1}$  for PCN field trials and  $\bar{\chi} = 20.24 \text{ T ha}^{-1}$  for RKN field trials, than yields observed under farmers’ practices ( $\bar{\chi} = 5.67 \text{ T ha}^{-1}$ ). Surprisingly, potato yields on the ‘Wrap and Plant’ paper-control were also significantly higher than for the absolute control. The ‘Wrap and Plant’ technology also provided the highest profitability for farmers, compared with other treatments. This novel and innovative technique provides a simple and effective option for nematode management in potatoes, in addition to numerous other crops.

**M66\_Host plant status and damage threshold of spinach (*Spinacia oleracea*) for the temperate root-knot nematode *Meloidogyne chitwoodi***

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The deep-frozen vegetable industry in Flanders is ranked first on a global scale. This makes local open field vegetable production an important economic activity. A recent questionnaire showed that 52% of the vegetable farmers were confronted with yield loss due to plant-parasitic nematodes. Major problems are the quarantine root-knot nematode *Meloidogyne chitwoodi* and the root lesion nematode *Pratylenchus penetrans*. Crops with a short growing period limit build-up of populations and allow subsequent management options. Spinach is a vegetable crop that requires 40 to 60 days before harvest. To examine if spinach can be used in rotations on *M. chitwoodi* infested fields host plant status and damage threshold of three commercially important cultivars ('Gnu', 'Meerkat' and 'Whale') were evaluated in a pot experiment. Inoculation densities of freshly hatched second-stage juveniles (J2) were 0, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 per 100 cm<sup>3</sup> soil. After 40 to 50 days shoot weight, root weight and final population density were determined. Root galls were observed on all three cultivars and the numbers of *M. chitwoodi* increased depending on the initial population density ( $P_i$ ). First results show no effect on root weight but shoot weight declined with increasing  $P_i$  indicating a damage threshold between 50 and 150 J2 (100 cm<sup>3</sup> soil)<sup>-1</sup>. Experiments are repeated and the Seinhorst model will be used for calculation of the damage threshold, which will be presented.

## **M67\_The potential effect of soil management techniques on *Meloidogyne* spp. control in Hungary**

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The success of the control of root-knot nematodes (RKN) is greatly influenced by the performance of soil-dwelling organisms, whose activity is vulnerable. Performing soil sterilisation may temporarily reduce it, increasing the supply of organic materials or introducing targeted antagonists into the soil may enhance it. As the first step of our recently launched research project, this presentation is to identify the main soil management techniques applied in small-scale commercial horticulture in Hungary. We aimed to explore what type of innovative solutions have a potential interest from a technical, environmental and financial perspective among the given circumstances and, finally, to determine how to monitor the effect of the innovations. Our presentation gives a review on the possibilities of combining soil sterilisation, organic matter supply, the use of antagonists, and aims also to identify future research areas of the sustainable application of innovative fumigants to widen the use of integrated soil-pest management methods against RKN species. A trial study to support the project is also on-going. Our work and presentation were supported by the EFOP-3.6.3- VEKOP-16- 2017-00008 project.

**M69\_Histological observations in wheat genotypes parasitised by *Heterodera avenae*, *H. latipons* and *Pratylenchus neglectus***

Ece B. Kasapođlu Uludamar and İ. Halil Elekciogđlu

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Cereal cyst nematodes (CCN) threatens wheat productions in many regions of Turkey. Root lesion nematodes (RLN) are also widely distributed throughout country. This study was conducted to wheat varieties which were infected with *Pratylenchus neglectus*, *Heterodera avenae* and *H. latipons* in order to determine cellular variation in wheat cultivars *in vitro* conditions. *Pratylenchus neglectus*, *H. avenae* and *H. latipons* were inoculated on wheat varieties, 'Adana-99', 'Ceyhan-99' and 'Silverstar' together in tube. Plants were grown at 24±1°C for 16:8 light:dark conditions for 12 weeks. After 12 weeks, wheats varieties were harvested and slides were prepared. After root cell preparation, all samples were examined under a light microscope. Cortical cells below the epidermis were damaged in the wheat genotypes. 'Adana 99' had syncytia with destroyed cell wall. This project was supported by TUBITAK 2140419

**M70\_ Multiple resistance of the cereal cyst nematode (*Heterodera latipons*) and root lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*) in wheat**

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Cereal cyst nematodes (CCN) and root lesion nematodes (RLN) are economically important in wheat growing area in the Turkey. Current management strategies are not always practical and economic, so development of alternative control methods would be of significant benefit. The wide distribution and high population numbers had historic adverse impact on wheat production. Most of plant-parasitic nematodes are found together in soil/root ecosystems. Thus, the effect of mixed nematode population on different wheat varieties should be investigated. The objectives of this project were to determine multiple resistance of *Heterodera latipons*, *Pratylenchus thornei* and *P. neglectus* in summer and winter wheat genotypes *in vitro* and semi-field conditions. Twenty-five wheat genotypes were grown in pots and semi-field conditions during 2016-2017 in November-June. Each plant was evaluated for the number of eggs/juveniles and cysts, and adult nematodes. *Heterodera latipons* inhibited *P.thornei* and *P. neglectus* population density. 'Yelken-2000' and 'Yayla-305' genotypes had the lowest population density with numbers of cysts, juveniles and mature root lesions. Root lesion nematodes inhibited cyst nematodes on 'Gerek-79', 'Yayla-305', 'Porsuk 2800', 'P-8-6', 'Lütfibey', 'Tosunbey', 'Uzunyayla', 'Silverstar' and 'Seri-82'. Future work will focus on reactions of different pathotypes of CCN on wheat in mixed populations. This project was supported by TUBITAK 2140419

**M71\_ Multiple resistance of the cereal cyst nematode (*Heterodera avenae*) and root lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*) in wheat varieties**

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*Heterodera avenae*, the cereal cyst nematodes (CCN), is one of the major economic and ecological threats to wheat all over the world. *Heterodera avenae*, *Pratylenchus thornei* and *P. neglectus* are found together in wheat root ecosystems. Despite the difficulties in controlling cyst nematodes and root lesion nematodes (RLN), control using resistant genotypes can be applied in integrated management approaches to reduce the damages of both CCN and RLN. The aim of study was to test the reproduction of CCN, RLN and the resistance level of wheat genotypes in soil when these nematodes were together. Soil samples were taken from areas where *H. avenae* and *P. thornei*, *P. neglectus* were known to be present. Twenty three (23) wheat genotypes were placed in pots *in-vitro* and semi-field conditions. Summer and winter wheats were sown in November and harvested in June during 2016-2017 in Turkey. Initial results showed that 'Kundurur-1149', 'Uzunyayla', 'Lütfibey', 'P 8-6', 'Porsuk-2800', 'Gerek-79', 'Harmankaya-99', 'Adana-99', 'Ceyhan-99' and 'Fuatbey' were resistant to all species. Root lesion nematodes were inhibited by cyst nematode on 'Porsuk-2008'. Although the number of cysts was low *in vitro* and semi-field conditions, the number of eggs produced was high. Future studies should include different plant-parasitic nematode species, and pathogen fungi and bacteria. This project was supported by TUBITAK 2140419



**M72\_PALADAPT, an EFSA funded project for monitoring and tackling genetic selection in the potato cyst nematode *Globodera pallida***

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Management of plant pathogens is probably the most serious challenge in sustainable food production and the maintenance of food security. Due to the strict regulation of or ban on major categories of pesticide, the potato cyst nematode *Globodera pallida* has been managed by a combination of crop rotation and the potato resistance locus *Grp1*, a relatively narrow range resistance gene that was introgressed into a range of commercial potato cultivars. However, in 2014 *G. pallida* populations were described from Emsland (Germany) that can no longer be controlled by *Grp1*. Since then, several *G. pallida* populations have been identified from multiple locations in the north-eastern part of The Netherlands that showed aberrant multiplication rates on *Grp1* resistant starch potato varieties. Most likely similar highly virulent populations will also emerge in all major potato growing areas in North Western Europe where production practices are very similar. Except for laborious, costly and often moderately accurate pot experiments, there is currently no rapid and reliable method to identify virulent populations. This represents a strong limitation and prevents an accurate and durable management of infestations. The PALADAPT project represents the first step of a European battle plan against the emergence of virulent *G. pallida* populations and aims at improving the methods and tools for a fast identification of virulence outbreaks. The activities planned in this project also include: i) the creation of networks focusing on monitoring emergence of resistance breaking potato cyst nematode populations; ii) the dissemination of knowledge and recommendations to potato producers potato-breeding companies and European and National Plant Protection Organizations; and iii) the organization of a workshop in late 2018 on rapid and reliable methodologies and tools for monitoring virulence in *G. pallida*.

**M73\_Optimising screening studies with wild chickpea genotypes collected from Turkey against the stem and bulb nematode, *Ditylenchus dipsaci***

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Production of cool season food legumes can be severely limited by nematode attack. Symptoms are yellowing, wilting, stunting, decreased biomass and seed yield. The stem and bulb nematode, *Ditylenchus dipsaci* is an economically important pest described as one of the major limiting factors in chickpea production. Nine accession of wild *cicer* species (*Cicer arietinum*, *C. reticulatum* and *C. echinospermum*) were assessed for resistance to *D. dipsaci* according to multiplication rate. A major strategy to develop resistance to *D. dipsaci* in chickpea was to assess and exploit their natural variation. Chickpea is constrained by limited genetic and adaptive diversity. Widening this diversity was the focus of an international collaboration that was collecting, phenotyping and introgressing wild *Cicer* into chickpea involving institutes in Turkey, Australia, the USA, Canada, Ethiopia and India. One of the aim of this study was also to find best initial nematode density, harvesting time and was determined the reaction of difference varieties of chickpea against these nematodes for using in the optimization studies in chickpea. The result of analyzes variance between 3 species with 3 different population density of nematode indicated there was no significant difference at growing times between 16, 20 weeks and inoculum density of 300 and 400 nematodes per plant in *D. dipsaci* ( $p < 0.011$ ) with chickpea species and just observed difference at low population density of 150 nematodes per plant and there was no change indicated as the population rises to 400 nematodes per plant in all species. Therefore inoculum density of 300 of *D. dipcasi* and 16 weeks for harvest time can be used for screening studies for all species. The Grains Research and Development Corporation supplied funding for the research projects that comprised this thesis. I also acknowledge the support the Corporation has given for nematology research in South Australia.

**M75\_Population dynamics of the root lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and *Ditylenchus dipsaci* on chickpea genotypes in Turkey**

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Population dynamics of the root lesion nematodes (RLN), *Pratylenchus thornei* and *P. neglectus*, and *Ditylenchus dipsaci* were conducted at two regions (Ankara and Sanliurfa) in Turkey in 2014 -2016. Twelve accessions of wild *Cicer*, representing three *Cicer* species, were used in the experiments. The population dynamics of the nematodes on different species of chickpea in growing areas were studied. Field trials to assess the effect of *Cicer* varieties on population dynamics of the RLN were important due to their patchy distribution in the soil. The result of analyses of variance indicated that all stages of the nematodes were detected in roots and soil throughout both growing seasons, with the maximum numbers of RLN and *D. dipsaci* extracted from roots and soil during May and July. The population densities of nematodes increased from early spring (March) to mid-summer (July). The number of nematodes tended to decrease from the late summer (August) to the end of winter and starting to increase from the early spring (March) to the mid-summer. In general, results indicated that the population densities of nematodes were lowest in experimental plots during January and February and were highest in plots between June and July. It was estimated that the population density of nematodes increased in the plots in the early spring (March) more than in the first sampling results in mid-summer (July).

### M77\_Trying to keep root-knot nematode under control fostering sustainability

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Considering the major problems caused by traditional nematicides on the environment and human health, it is crucial to find eco-friendly solutions to control nematodes, promoting a more sustainable agriculture. The goal of this work was to find natural alternatives to control root-knot nematodes (RKN), contributing as well to: *i*) value Portuguese native marine macroalgae species; *ii*) promote Circular Economy, valuing agro-industrial waste; and *iii*) control invasive plant species. Nematicidal activity of plant and algae water extracts were evaluated on *Meloidogyne* sp. second-stage juveniles (J2), using excavated glass blocks. For each block, 20 J2 hatched within 24 h were transferred to 0.5 ml of extract. Distilled water and fenamiphos were negative and positive controls, respectively. Immobility and mortality were observed at 24, 48, 96, and 168 h. Four replicates were considered for each treatment and observation time. Immobility/mortality were classified as: strong, higher than 80%; moderate, 80-61%; low, 60-41%; very low, 40-10%; no activity, less than 10%. Data were subjected to a two-way ANOVA, followed by Tukey test, using a significance level of  $P = 0.05$ . The *Ulva rigida* (0.1 g ml<sup>-1</sup>) extract was the most effective, causing 98.8 and 100% mortality, after 48 and 96 h of exposure. The second most effective was the oxidised pod of *Vicia faba* (0.1 g ml<sup>-1</sup>) extract with nearly 100% at 96 h. The fresh pod of *V. faba* (0.1 g ml<sup>-1</sup>) extract was the third most effective, followed by *U. rigida* (0.05 g ml<sup>-1</sup>) and *Acacia dealbata* (0.2 g ml<sup>-1</sup>). *Sargassum muticum* (0.1 g ml<sup>-1</sup>), *Gracilaria gracilis* (0.1 g ml<sup>-1</sup>) and *A. longifolia* (0.2 g ml<sup>-1</sup>) were the least effective. This work can be considered a new insight for the development of promising natural and environmentally-friendly alternative nematicides, promoting sustainable development and the Circular Economy.

## M78\_Increasing sustainability through 'phytonematicides' searching

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Organic Farming (OF) is considered a sustainable production mode for using practices that respect the agroecosystem. Although some species of nematodes are essential for the ecosystem's equilibrium, high populations of phytopathogenic nematodes, particularly root-knot nematodes (RKN), *Meloidogyne* spp., cause severe economic damage in many crops. In order to minimise the imbalances observed in ecosystems, it is crucial to find environmentally-friendly solutions to control those plant enemies. For this purpose, the study of the nematicidal potential of two plants, *Melianthus major* and *Tagetes minuta*, in relation to a RKN species was started. A bioassay was carried out using excavated glass blocks with 0.5 ml of each treatment, *T. minuta* essential oil and hydrolate and *M. major* hydrolate, with 20 second-stage juveniles (J2) for 24 h. Distilled water served as control. There were five replicates for each treatment and control. Observations were made at 24, 48 and 168 h. The numbers of live, immobile and dead J2 were recorded. The cumulative mortality values, at 168 h, were submitted to an ANOVA, and the Tukey test was performed, when significant differences were observed. The obtained results showed an instantaneous nematicidal potential of *T. minuta* essential oil, and a nematostatic effect of the two plants hydrolates. Hence, this work can be considered relevant since, in a general way, the two plants under study, *T. minuta* and *M. major*, have nematicidal potential in relation to this *Meloidogyne* species. It is also important to highlight that, so far, no reference was found for *M. major* effect on RKN.

**M79\_The susceptibility of the stem nematode *Ditylenchus gigas* to isothiocyanates associated with Brassicas**

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Isothiocyanates (ITCs) are produced from the enzymatic degradation of sulfur-containing glucosinolates (GLS) by  $\beta$ -thioglucosidases (myrosinase). Brassica plants contain glucosinolates and myrosinase and are being introduced into cropping systems as biofumigants due to the release of ITCs that are toxic to soil borne pests and pathogens. However, the activity of isothiocyanates against the stem nematode, *Ditylenchus gigas*, is unknown. Hence, the aim of this study was to evaluate the biocidal activity of four synthetic ITCs, sulforaphane, 2-propenyl, 2-phenylethyl and benzyl against *D. gigas* and to determine their efficacy at concentrations of 0.01, 0.005, 0.0025, 0.00125% (w/v) after 24, 48 and 72 h of exposure under *in-vitro* conditions. All the ITCs except sulforaphane caused 100% mortality of *D. gigas* at 0.01 % (w/v) after 24 h ( $P < 0.001$ ). Mortality of *D. gigas* exposed to 2-phenylethyl, benzyl, 2-propenyl isothiocyanates and sulforaphane at 0.005% (w/v) was 100%, 87.7%, 80.4% and 24%, respectively after 72 h exposure. This suggests that Brassica plants containing 2-phenylethyl, benzyl, and 2-propenyl glucosinolates could have suppressive effect on *D. gigas* infested fields.

**M83\_GLOBODERA ALLIANCE (GLOBAL): risk assessment and eradication of *Globodera* spp. in U.S. production of potato**

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A transdisciplinary team of researchers including nematologists, plant breeders, extension specialists and economists are working together to tackle the ongoing threat of *Globodera* spp. to U.S. potato production. This collaborative effort is known as GLOBAL (GLOBodera Alliance). The objective of this research will yield a model management approach to protect the U.S. potato industry from current and future introductions of these nematode pests, and will improve U.S. agriculture, food security, and stakeholders' economic interests, knowledge base, and participation in decision-making. Ongoing research is directed towards: *i*) development and implementation of effective early warning tools for *Globodera*, including improved detection and diagnosis methods; *ii*) use of genomic approaches to characterise pathogen virulence and host resistance for development of resistant cultivars, and for detection and identification of effector genes and broader genetic variability in *Globodera* across its geographic range; *iii*) Identification and deployment of potato germplasm conferring resistance to three species of *Globodera* in economically viable potato varieties; *iv*) coordination with stakeholders and policymakers to co-develop science-based agricultural approaches to deal with the threat of *Globodera* and implement sustainable, environmentally sound agricultural practices for potato production in the context of *Globodera* risk management; and *v*) Increased number of scientists, extension specialists, and educators with the skills and knowledge for effectively addressing the problem of invasive agricultural pests.

### M84\_Damage potential of *Meloidogyne incognita* populations on selected tomato genotypes in Ethiopia

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Reliable data are required to relate the effect of a range of  $P_i$  on plant growth, biomass and yield for specific crop-nematode associations under local conditions. In Ethiopia, no information is available on the effect and relationship between initial population densities ( $P_i$ ) of *M. incognita* and damage to tomato cultivars. Hence, the effect of a series of  $P_i$  of Babile and Jittu *Meloidogyne incognita* populations on four tomato cultivars ('Assila', 'Chochoro', 'Moneymaker' and 'Tisey') and one breeding line ('CLN-2366B') for growth, yield and the relationship with final population densities ( $P_f$ ) were studied. Each tomato cultivar was inoculated with a geometric series of  $P_i$  (0, 0.125, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 J2 (100 g dry soil)<sup>-1</sup>) and was allowed to grow until the crop reached senescence. The relationship between  $P_i$  and  $P_f$  was fitted to the Seinhorst population dynamics model, while the effect of  $P_i$  on different plant parameters considered was fitted to the Seinhorst yield model. Based on the damage model, all the tested plant parameters were found negatively affected by both populations of *M. incognita*. The Jittu population was found to have more effect on the majority of parameters compared to the Babile population. As the reproduction factors (RF) obtained for the tested tomato cultivars were high the tested cultivars were considered as good hosts for both populations of *M. incognita*. The highest RF for *M. incognita* populations was obtained at lower  $P_i$  (0.125 J2 (100 g dry soil)<sup>-1</sup>) and reduced with increasing  $P_i$  on all the tested cultivars. Severity of root galling and number of egg masses per root system increased with increasing inoculum levels of both nematode populations. The tomato genotypes, *M. incognita* population and initial population density ( $P_i$ ) had a highly significant ( $P < 0.001$ ) effect on all the plant and nematode data parameters considered. The seedlings of all the tested tomato genotypes were dead at the higher  $P_i$  value (256 J2 (100 g dry soil)<sup>-1</sup>) except for 'Assila' whose seedlings survived even with the highest  $P_i$  values for both *M. incognita* populations. Among all the cultivars tested 'Tisey' was highly susceptible to both nematode populations and all the seedlings were dead at ( $P_i \geq 16$  J2 (100 g dry soil)<sup>-1</sup>), worse than the susceptible control 'Moneymaker' where seedlings died at  $P_i \geq 64$  J2 (100 g dry soil)<sup>-1</sup>. For all the plant parameters studied 'Tisey' was found to have a lower damage threshold,  $T$ , while 'Assila' (except for root weight) had a higher  $T$ . A difference was observed for the tested tomato genotypes on their minimum yield ( $m$ ) for the different plant parameters studied against the two *M. incognita* populations. Determination of  $T$  and  $m$  of a given crop variety for the prevailing *Meloidogyne* species in fields to be planted (local setting) is vital.



**M85\_The effect of the essential oil and hydrosol of *Cuminum cyminum* against *Meloidogyne incognita* and *M. javanica***

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Root-knot nematodes (RKN; *Meloidogyne spp.*) cause serious problems to a large number of economically important glasshouse crops in Greece. Since the withdrawal of methyl bromide, only few synthetic nematicides are left in use. Therefore, there is an urgent need for development of alternative nematode control methods. Among these alternatives is the use of natural plant products, such as essential oils or other plant extracts. In this study, we evaluated the efficacy of the essential oil and the hydrosol of *Cuminum cyminum* against the root-knot nematodes *M. incognita* and *M. javanica*. Also, the plant extracts isolated from *C. cyminum* were analysed for their chemical composition in a Gas Chromatography-Mass Spectrometry (GC-MS) system. The nematicidal activity of the essential oil has been tested in paralysis trials, inhibition of egg differentiation and hatching bioassays, as well as in pot experiments where the essential oil was incorporated in nematode infested soil at different concentrations. The nematicidal activity of the hydrosol has been tested only in paralysis trials. The results of these experiments showed high toxicity of the plant extracts against second-stage juveniles (J2) of these two RKN species even in low concentrations ( $EC_{50} < 62.5 \mu\text{l l}^{-1}$ ) and there was no phytotoxicity. The essential oil inhibited the differentiation of the eggs and also inhibited the hatch of J2 from the egg masses. Chemical composition analysis might help towards finding the most effective compounds of these plant extracts for further studies.

**M86\_Benefits of oil radish (*Raphanus sativus conv. oleiformis*) and white mustard (*Sinapis alba*) as a catch crop for beet cyst nematode *Heterodera schachtii***

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The beet cyst nematode (*Heterodera schachtii*) is one of the major pathogens in sugar beet, causing significant sugar yield losses worldwide. This nematode forms a feeding site (syncytium) through which it obtains all plant nutrients required for completing its life cycle. After copulation and completion of the life cycle, white females become brown dead cysts containing more than 200 eggs. Cysts can survive in the soil up to 10 years until the proper host is in the field again. Therefore, crop rotation only slowly results in the decrease of nematode densities and since resistant sugar beet is limited and many nematicides are banned, control of this nematode is very difficult. With selecting, breeding and registration of oil radish and white mustard for nematode-resistance, P.H. Petersen redefined a whole field of application for cover/catch crops. Ever since then, the company has been the market leader in Europe, breeding and providing new varieties with resistance, not only for *H. schachtii*, but also for the wide range of other plant-parasitic nematodes, such as *Meloidogyne* ssp. and *Pratylenchus* ssp. The key to the company's success is to understand plant-nematode interaction in field, glasshouse and *in vitro* laboratory situations, and to combine breeding with agricultural practices. Only by taking into consideration all systems, can new breeding methods and strategies for fighting plant-parasitic nematodes be developed. Some of the results with cover crops oil radish, mustard and bristle oat will be presented, and future prospects will be discussed. In addition to nematode control, cover crops also contribute for improving soil fertility by additional organic matter, efficient nitrogen fixation, erosion control, enrichment of the biodiversity and beneficial soil microorganisms and thus help for sustainable farming and environmentally friendly crop production.

## Posters Biological Control 2

### **B31\_ Examination of background factors to decrease the damage by *Meloidogyne incognita* in an open-field tomato experiment**

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In 2016, the effect of different agricultural practices (mulching, irrigation, mycorrhiza inoculation) was examined on an artificial *Meloidogyne*-infection on open-field tomato. Among the practices, only mulching decreased the number of root galls. At the same time, however, the examined background factors (soil organic matter, soil pH, predatory nematodes), which could be correlated with mulching and decreasing damage, were found to have no influence on that effect. The experiment was repeated in 2017 with the same treatment combinations. This time our goals were to examine more background factors: soil temperature, soil compaction and soil moisture content. In addition, yield, *Meloidogyne* damage, the density of earthworms and free-living nematodes, and mycorrhiza colonisation were evaluated. Similarly to 2016, mulching treatment reduced the number of *Meloidogyne*-induced galls on plants. Yield was not influenced by the artificial *Meloidogyne*-infection. Although mulching increased the overall density of predatory nematodes (this effect was not found in 2016) and the number of earthworms, these factors did not correlate with the reduced number of root galls. As very high mycorrhiza colonisation was measured for all plants in the field, the applied mycorrhizal product was not able to encourage colonisation in the soil. Mulched plots warmed up and cooled down more gradually than non-mulched plots. Moreover, mulching increased soil moisture and decreased soil compaction. Soil temperature and moisture were more strongly correlated than soil compaction with *Meloidogyne* damage. Our results showed that while the examined biotic factors had no influence on *Meloidogyne* damage, abiotic factors did. We assume that the initially lower temperature in the mulched plots possibly hindered the reproduction of *M. incognita* that requires warmth. Our work and presentation were supported by New National Excellence Program of the Ministry of Human Capacities (ÚNKP-16-2) and the EFOP-3.6.3- VEKOP-16- 2017-00008 project.

**B33\_Pathogenicity and reproductive potential of *Steinernema abbasi* CS40 (Rhabditida: Steinernematidae) against *Helicoverpa armigera***

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Entomopathogenic nematodes (EPN) are effective against a diverse number of agricultural pests, especially against soil-dwelling pests. Many of the species of EPN have tremendous potential for biological control of insect pests. The ability of EPN to seek out and kill insects in agricultural habitats, where chemical insecticides fail, makes these biocontrol agents especially attractive. Isolate CS40 (*Steinernema* sp.) was isolated from the soil of village Rohana around the rhizosphere of crop wheat, District Muzaffarnagar, Uttar Pradesh, India located at 29.4727° N and 77.7085° E and elevation at 276 m a.s.l. For the evaluation of the efficacy of these isolates four different concentrations viz. 25, 50, 100, 200 infective juveniles (IJ) larva<sup>-1</sup> were used against the insect larvae *Helicoverpa armigera* reared in the laboratory. Mortality occurred after 12 h post infection period (PIP) at 25, 50 and 100 IJ larva<sup>-1</sup> but no mortality was observed at 12 h PIP at 200 IJ larva<sup>-1</sup>. The percentage mortality at 12 h PIP was 20, 20, 10 and 0% respectively from lower to higher doses; however, at 36 h 90, 90, 100 and 100% mortality was recorded. Total mortality was observed at all concentrations only after 36 h PIP. No mortality was observed in the control group. The mean IJ production was found highest at 200 IJ larva<sup>-1</sup>, which was  $74 \times 10^3$  IJ larva<sup>-1</sup> followed by 50 IJ larva<sup>-1</sup> dose ( $=64 \times 10^3$  IJ). The least IJ production was found at 100 IJ larva<sup>-1</sup> doses ( $61 \times 10^3$ ). At 25 IJ larva<sup>-1</sup> the mean IJ production was  $62 \times 10^3$  IJ larva<sup>-1</sup>. These findings demonstrated that isolate CS40 showed good efficacy against the tested insect, *H. armigera*, and therefore could be used for the management of this pest. For using this isolate as a biological control agent, tests on other insect pests as well as in glasshouse and field conditions need to be evaluated.

### **B36\_Peptidomics of *Steinernema* spp., entomopathogenic nematodes**

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As in many nematodes, behavioural changes are regulated by neuropeptidergic signalling (e.g., *Caenorhabditis elegans*). Emerging evidence suggests that behavioural changes associated with host finding - like observed in entomopathogenic nematodes (EPN) - may be regulated by neuropeptides. Hence, peptidomic discovery in EPN, like *Steinernema* species, provide a fundament upon which differential and functional work can build. The peptidomic similarities between the well-known *C. elegans* peptidome and predicted *S. carpocapsae* peptidome are profound, indicating similar behavioural regulations. This, combined with the extensive peptidomic knowledge of our laboratory, gives us a broad fundament for peptidomic research in other nematodes. An in-house method based on acidified methanol was used to extract endogenous neuropeptides of *Steinernema carpocapsae*. Neuropeptide identification was done by state-of-the-art sub-Dalton high accuracy tandem mass spectrometry coupled online to an ultra-high performance liquid chromatograph (UHPLC-MS/MS). The current state of the *S. carpocapsae* peptidome will be presented at the conference. *Steinernema* spp. are used as a potent and environment-friendly alternative for chemical pesticides to combat pest insects. For now, EPN only have a limited applicability and there is a need for host-range and ecological niche expansion, while controlling host specificity. Knowledge on peptidomic regulation of host-finding strategies will help us understand how EPN regulate their behaviour, hence contributing to improving EPN applicability and host specificity.

**B37\_ *Heterorhabditis indica*- based management of tea semiloopers, cutworms and termites infesting tea plantations in India.**

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The Northeastern Indian states of *Assam and West Bengal* are the two major *tea-producing* states with their *combined production* accounting to 83% of the total output. In recent years the seasonal occurrence of green tea-semiloopers (*Argyrogramma signata*) and cutworms (*Spodoptera litura*) coupled with the perennial presence of belowground subterranean termites are decimating the plantations. Application of chemical pesticides is largely prohibited due to prolonged residues in the leaves and therefore alternative management avenues are being explored. The entomopathogenic nematode, *Heterorhabditis indica*, showed high pathogenecity against the three pests. In preliminary laboratory assays *H. indica* caused 100% mortality within 24 to 36 h in last instar larvae of green tea-semilooper or cutworms, whereas *Steinernema abbassi* caused similar mortality in 42-48 h. *Heterorhabditis indica* was further selected for field trials either as foliar and soil surface spray using a sponge-based product, implantation of *H. indica*-infected *Galleria mellonella* cadavers near the root zone or simultaneous application of both. A single foliar application coupled with simultaneous spraying of soil surface resulted in up to 82% reduction of semilooper population. A recommendation of gentle passing of a rope over the leaves during the day time resulted in the dislodging of the semi-loopers which further took *H. indica* infection from the treated soil. Higher mortality percentage (89%) was observed in case of cutworms, which spent more time in the soil. There was a visible reduction in the formation of termite mud tunnels on the trunks of treated shrubs as compared to untreated control.

**B38\_Chitosan soil drench treatment to control root-knot nematode, *Meloidogyne incognita***

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Plant-parasitic nematodes infect various plant organs, causing a great impact on crop productivity. The root-knot nematode *Meloidogyne incognita* is one of the major constraints infecting tomatoes and causing high levels of damage. Control strategies include cultural practices, the use of chemical and biological nematicides and also growing resistant cultivars. However, all those practices have their limitations. Crop rotation is difficult due to the broad host spectrum of *M. incognita*, resistance is often lost due to the selection of resistance-breaking pathotypes and synthetic nematicides are expensive and harmful to the environment. An alternative control measure is seen in the use of chitin and chitosan, which both have shown nematostatic and nematicidal potential in agricultural and horticultural applications. Chitosans are partially deacetylated polysaccharides derived from chitin, which can be obtained from the outer shell of crustaceans and cell walls of fungi. Chitosans are known to induce plant defence mechanisms. In this study two different chitosans with different degrees of acetylation were tested as soil drench on tomato plants ('Moneymaker') with two different times of application, 24 and 48 h before the inoculation with *M. incognita*. Both chitosans reduced the penetration of *M. incognita* into the roots by about 50%. Fresh root, shoot and total weight was also recorded.

**B39\_Chitosan biopolymers as nematicidal agents of the root-knot nematode *Meloidogyne incognita***

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Chitosans are well-studied linear polysaccharides, containing the monomers 2-acetoamido-2-deoxy-B-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN). Chitosans with a high portion of GlcN are soluble in slightly acidic media. Chitosan is considered one of the most interesting natural polymers. For agricultural purposes, it is produced from crustaceous shell waste. Chitosans can control pathogenic microorganisms by preventing growth, sporulation, spore viability, germination and disrupting cells as well as inducing different defence responses in host plants and/or inhibiting different biochemical activities during the plant-pathogen interaction. Nematicidal activity of chitosans has not been well studied. The biological activities of chitosans typically depend on the concentration, molecular weight, degree of acetylation and some other properties. There are almost no studies related to the direct effect of chitosan on nematodes. In the current *in vitro* study, four chitosans with different degree of acetylation and polymerisation were tested towards *Meloidogyne incognita* second-stage juveniles (J2). The percentage of active nematodes was recorded every 24 h for a period of 96 h. The results show a high nematicidal effect of two chitosans with almost complete inhibition of J2 activity.



**B40\_ Characterization of the heat shock protein 90 gene of *Heterorhabditis bacteriophora* and its expression in response to different temperature stress**

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Heat shock proteins (HSP) belong to a protein family that are rapidly synthesised in response to a series of environmental stressors. Among all HSPs, HSP90 is the most conserved and abundant in cells and is involved in response to temperature stress. Temperature affects life-stage development and behaviour of entomopathogenic nematodes, with different species and strains having optimum temperatures for penetration, feeding, survival and reproduction. Thus, temperature is an important factor in controlling generation time of EPN. In the present study, the full-length cDNA and the corresponding gene of HSP90 of *Heterorhabditis bacteriophora* were cloned and sequenced. The unexpected finding of intron presence-absence in the *Hb-hsp-90* genes of *H. bacteriophora* opens some questions about their function and their evolutionary significance. The *Hb-hsp90* gene was constitutively expressed in all developmental stages of *H. bacteriophora* but at higher level in adult males and females. The *Hb-hsp-90* expression in response to three different temperatures was also investigated. Larvae of *Galleria mellonella* were infected with infective juveniles (IJ) of *H. bacteriophora* and exposed at 12, 23 and 30°C for 10 days in order to explore the impact of an adverse temperature on reproduction and infectivity of *H. bacteriophora* and on *Hb-hsp-90* expression in IJ and adult stages. Results obtained in this study are reported and discussed.

**B41\_Biolog FF MicroPlate characterisation of substrate utilisation by *Pochonia chlamydosporia* isolates**

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*Pochonia chlamydosporia*, a parasite of nematode eggs, is a promising biological control agent for root-knot nematodes, *Meloidogyne* spp. However, genetic and phenotypic variation among isolates of the fungus can affect performance in nematode control, and bioassays are required to select isolates with appropriate combinations of rhizosphere colonisation, chlamydospore production and egg parasitism ability. A broad-range assessment of nutrient sources exploitable by *P. chlamydosporia* could contribute to a better understanding of the fungal metabolism and enzymatic activity to complement its characterisation. The BIOLOG system offers high-throughput, concurrent assessment of utilisation of 95 different carbon substrates by fungi using FF MicroPlates, with easy comparability of results among laboratories. We have characterised carbon substrate utilisation by 10 Portuguese isolates and one reference (Vc10) isolate of *P. chlamydosporia*. Altogether, the 11 isolates used all of the 95 substrates, but only 70 were used by all fungal isolates: 4 out of 6 amines/amides; 9 out of 13 amino acids, 37 out of 44 carbohydrates, 12 out of 17 carboxylic acids, 3 out of 5 polymers and 5 out of 10 miscellaneous compounds. Six of the substrates were used by fewer than half of the isolates, namely n-acetyl-d-mannosamine, sedoheptulosan, d-galacturonic acid, d-lactic acid methyl ester, alpha-cyclodextrin and beta-cyclodextrin. Average well colour development, indicative of substrate utilisation, also varied between isolates. Unweighted Pair Group Method with Arithmetic Mean hierarchical cluster analysis of reads allowed the separation of reference isolate Vc10 from all Portuguese isolates, and further divided the latter into two main clades depending on carbon substrate utilisation. As part of the COCOON project (POCI/01/0145/FEDER/016611-PTDC/AGR-PRO/3438/2014), we are working tentatively to relate carbon substrate utilisation profiles with biological characteristics of the isolates. BIOLOG characterisation of *P. chlamydosporia* for rapid isolate selection will need to be validated using isolates that differ in respect to geographical origins, hosts, biology and physiology.

**B42\_Biocontrol potentials of *Trichoderma* spp. isolates on *Meloidogyne luci***

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*Meloidogyne luci* has become the most common root-knot nematode species especially in glasshouse vegetable production areas, particularly in the Black Sea Region, Turkey. While evaluating the chemical alternatives to the control this species, *Trichoderma* spp., which had obtained from the Region for the previous studies, were used to search the efficiency of isolates in controlled glasshouses as a pot experiment. In the experiments, sensitive tomato seedlings were suspended in the suspensions prepared from *Trichoderma atroviride* (T4-5), *T. asperellum* (T11-25), *T. hamatum* (F4) and *T. harzianum* (11TTR2) isolates  $1 \times 10^6$  conidia ml<sup>-1</sup> for 10 min. In order to see the effects of *Trichoderma* isolates on plant growth, negative and positive control groups were prepared with four replicates and repeated twice. Ten days after the inoculations of isolates, 2000 egg per plant were applied to pots. Sixty days after nematode inoculations, all plants were rooted and number of leaves, fresh and dry root weights, shoot height, fresh root weight was determined. Roots were evaluated using 0-10 gall index and eggs were counted under the microscope to use obtain the reproduction factor ( $R_f = P_f / P_i$ ). As a result, it has been found that in general, all isolates promoted tomato plant development and *T. atroviride* was the most effective. *Trichoderma harzianum* (25%) was followed by *T. asperellum* (21.8%) and *T. hamatum* (16.6%) in the isolates. The most significant reduction in *M. luci* was again found in the plants to which *T. harzianum* was applied. The *T. atroviride* isolate had no significant effect on the reproduction index and the nematode eggs counted per root.

**B45\_Effect of different inoculum densities of *Trichoderma viride* against common plant-parasitic nematodes in northwestern marmara region of Turkey**

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Plant-parasitic nematodes are important pests of crop plants. During our nematode survey in Northwestern Marmara Region of Turkey species belonging to 27 genera were identified in several crop plants. The present study was carried out to determine the comparative efficacy of *Trichoderma viride* on common plant-parasitic nematodes that are highly distributed in agricultural areas in Northwestern Marmara Region of Turkey. The efficacy studies were conducted *in vitro* and under glasshouse conditions. Fungal *Trichoderma viride* was isolated from plane tree (*Platanus* spp.) and purified by several dilutions in PDA medium after incubation at 24°C for 14 days. Petri studies were performed with approximately 100 nematode individuals and fungus was applied at different doses (10<sup>2</sup>, 10<sup>4</sup>, 10<sup>6</sup>, 10<sup>8</sup> spore (g soil)<sup>-1</sup>). The pot experiment with several crop plants was conducted at the same dose and nematode inoculum level to investigate fungus, nematode and host plant relationship. Chickpea, tomato, sunflower, eggplant, sorghum, grapevine and sugarbeet plants were used in the experiments. *In vivo* and *in vitro* studies were carried out with three replicates and two controls. In all experiments *T. viride* was highly effective on nematodes except 10<sup>2</sup> spore (g soil)<sup>-1</sup>. The fungus was found suppressive on *Mesocriconema xenoplax*, *Longidorus elongatus*, *Xiphinema index*, *X. pachtaicum*, *Helicotylenchus digonicus*, *H. multicinctus*, *H. dihystra*, *Pratylenchus thornei*, *P. neglectus*, *Ditylenchus dipsaci*, *Merlinius brevidens* and *Meloidogyne incognita* collected from planting areas in Tekirdağ province. The efficacy of fungi increased as inoculum densities increased. The suppressive effect of fungus was observed 1 week after nematode inoculation. Furthermore plant growth was also promoted by fungus application.

**B46\_A potential biocontrol strain *Aspergillus japonicus* ZW1 against root-knot nematodes**

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are important pathogens on vegetable and other food crops worldwide. The year-round planting in the ground in plastic film glasshouses has led to the situation that 'every glashouse has RKN'. These nematodes have become a major obstacle to vegetable production in China. Biological control is frequently considered as an environmentally friendly approach to mitigate nematode-caused diseases. In this investigation, a fungal strain isolated from soil was evaluated for its potential to control the root-knot nematode *in vitro* based on *in vitro* toxicity of its fermentation broth against second-stage juveniles (J2) of RKN. After 2 weeks growing in Czapek Dox broth, the fungal filtrate and up to a 40-fold dilution exhibited a significantly higher mortality rate against J2 of *M. incognita* than the broth (no fungus) filtrate or the water control ( $P < 0.01$ ). The mortality rate was 100% after 6 h at 2-fold dilution, 12 h at 5- and 10-fold dilution, and 93.8% after 48 h exposure to 20-fold dilution. The nematode body content was degraded, and the J2 became transparent with increasing time and filtrate concentration. In a germination assay, seeds of various crops were exposed to the fermentation broth in a moist chamber and incubated for several days in the dark at room temperature. The filtrate of the fungal strain did not affect the germination of corn, wheat, rice, cowpea, cucumber and tomato. The results suggested that the strain ZW1 produced and excreted metabolites toxic to root-knot nematodes but without a negative effect on seed germination. This fungal strain was identified as *Aspergillus japonicus* based on morphological and molecular methods. Further studies will attempt to identify the nematicidal metabolite and to evaluate the *in vivo* efficacy of the strain against RKN.

**B48\_Preliminary studies on soil suppressivity against root-knot nematodes (*Meloidogyne* spp.) in Hungary**

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Tomato landraces and their pests, including root-knot nematode (RKN) damage, were studied and compared to control varieties in a field trial and under polytunnel (2015-2017) on two organic farms. The level of infestation was low on the roots of every landraces and control varieties, despite factors like previous crops and irrigation may have accounted for the accumulation of, and damage by, RKN. We assumed that a certain suppressive effect of the soil was one of the reasons, if not the main reason, to explain our findings. For further investigations of this phenomenon, a pot experiment was set up. We used the two types of medium loam soils collected from these two farms and a control soil, and grew artificially infected and non-infected plants in the different soil treatments. During the experiment, we ensured that the invasive RKN juveniles could spread among the pots with irrigation. Quite interestingly, in the control soil, *Meloidogyne* colonisation was higher in the non-infected than in the infected treatment, suggesting that RKN juveniles were able to spread successfully with irrigation water in this soil. The soils from the two organic farms showed certain suppressivity to RKN, but their colonisation was not inhibited, just their spread. In 2017, we tested two less susceptible tomato landraces in a new extensive tomato cultivation system on sandy soil preferred by *Meloidogyne* in a field trial. In the artificially infected treatments, *Meloidogyne* colonisation reached 5-7 value on Zeck scale, but this did not affect the yield. Based on our results, soil texture has the largest impact on *Meloidogyne* colonisation. Although soil suppressivity is a complex of several biotic and abiotic factors, according to our experience, serious RKN damage can be prevented with maintaining soil biodiversity and soil structure. The work is supported by the EFOP-3.6.3- VEKOP-16- 2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

## Posters Plant-Nematode Interaction 2

### **P48\_Proteomic changes during the development of nematode-induced syncytium on tomato roots**

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*Globodera rostochiensis* is a plant pathogenic nematode, infesting potato, tomato and other members of Solanaceae family. It is one of the most deleterious pests of these hosts and causes significant crop losses all over the world. *Globodera rostochiensis* belongs to the cyst nematode subfamily and during pathogenesis it induces the formation of specialised feeding structures called syncytia on infected roots. The process of *G. rostochiensis* pathogenesis still remains obscure, and especially little is known about the regulation of this process at the protein level. We aimed to define the dynamics of tomato proteome during the course of formation of syncytium on *G. rostochiensis* infected roots. In this study we used Mass Spectrometry methods to determine alterations at the level of protein quantity and protein phosphorylation. The changes were monitored at three stages of syncytium development. In order to obtain a greater insight into this process, we included analysis of resistant tomato lines carrying the resistance gene *Hero*. Several candidates were evaluated at the transcriptional level and were selected for further studies. Our findings give a new insight in the field of plant-pathogenic nematode interactions. They may suggest new levels of regulation of syncytium development, which include alterations in protein abundance and their post-translational modifications.

**P52\_A comparative study of the development and reproduction of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species**

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The root-knot nematode species, *Meloidogyne enterolobii*, is present in local fruit, grain and vegetable production areas. It was first discovered in South Africa during 1997 in guava roots. No studies on the life cycle duration and reproduction potential of South African populations of the species, compared to that of *M. incognita* and *M. javanica*, has been done and was attempted during this study. Second-stage juveniles (J2), 48-h old, of single-species populations of *M. enterolobii*, *M. incognita* and *M. javanica* were inoculated on roots of susceptible tomato seedlings. Two days after J2 penetration, seedlings were obtained for the first sampling interval, while the others were lifted and their roots rinsed with tap water to get rid of all J2 that may have been present in their rhizospheres. Each seedling was transplanted into fumigated soil in pots in a glasshouse (ambient temperature range of 14 – 24 °C; 14L:10D photoperiod). The same procedure was followed for a second trial where infected tomato seedlings were transplanted to a fumigated field site (Mbombela; ambient temperature range of 25 – 28°C). The development of different life stages of each of each species was determined at sampling intervals of 2, 5, 10, 15, 20, 25 and 30 days after inoculation. Differences in the life-stage development of the three species were substantial for the different sampling intervals and will be discussed. Experiments will be done next at lower temperature ranges known to occur in another area where *M. enteroblobii* was also found to infect potato and maize. This information represents the first on the life-cycle of a local *M. enterolobii* population, in relation to its predominant thermophilic counterparts species that infect crops in South Africa, and can contribute towards employing optimal management strategies to combat this species.



**P55\_Genetic diversity and phylogeny of South African *Meloidogyne* populations using genotyping by sequencing: first report**

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Root-knot nematodes (*Meloidogyne*) as the first ranked plant-parasitic nematode genus is an economic important pest worldwide. Genetic host plant resistance is one of the most effective management strategies successfully applied to reduce damage caused by these pests. However, some root-knot nematode resistant genes are overcome by more virulent species such as *Meloidogyne enterolobii*. Therefore, comprehensive and detailed knowledge about the genetic composition of species is essential. In this study, it was endeavoured to obtain detailed information regarding to genetic relation among South African populations of *M. enterolobii*, *M. incognita* and *M. javanica* by using genotyping-by-sequencing and Pool-Seq techniques. As a result, 653 common SNPs were identified from populations investigated of the three species, and used. The allelic frequencies of 33 of these SNPs sequences were consistently different between *M. enterolobii* and the other two species (*M. incognita* and *M. javanica*). Principal component analyses and phylogeny revealed that the *M. enterolobii* populations grouped together into one clade that was distantly related to *M. javanica* populations. Both species shared genetic links with the *M. incognita* populations that were placed in the middle in the phylogenetic analysis. Hence, genotyping-by-sequencing was confirmed as a good tool to identify SNPs that successfully discriminate these three species. Results ultimately indicated the presence of some alleles that were present only in the genome of *M. enterolobii* and, therefore, such variants should be further explored to evaluate whether they are involved in virulence.

**P60\_Programmed cell death in *Arabidopsis thaliana* roots during *Heterodera schachtii* parasitism**

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Plant-parasitic nematodes are widespread pests of many crops causing substantial losses in agriculture. Their parasitism relies on injecting gland secretion that induce the developmental and metabolic reprogramming of plant cells. As a result the syncytia are established, which are the feeding structures being a sole food source for growing nematodes. Programmed cell death (PCD) is a major cellular process of host plants; in the case of avirulence (hypersensitive response) it still plays a role during compatible plant-nematode interaction but is limited to some cells. Several regulators of PCD were analysed to shed light on its role in compatible plant-nematode interaction. First, we monitored the cyst nematode performance in the mutant of *LSD1* gene (negative and conditional regulator of PCD), which showed a significant reduction of syncytium size and overall susceptibility. The syncytia induced on *lsd1* roots compared to Col0 showed significantly retarded growth, modified cell wall structure, increased vesiculation and multilamellar structures present at 7 and 12 days post-infection. To combine morphological and ultrastructural analyses of *lsd1* mutant with underlying molecular mechanisms, the RNA-sequencing analysis of infected and uninfected roots was performed. During nematode infection, the number of transcripts with changed expression in *lsd1* was approximately three times smaller than in wild-type plants (1440 versus 4206 differentially expressed genes, respectively). The ubiquitin-like protein ATG8 is a central player in the autophagy network that is required for autophagosome formation. Genes, that belong to ATG8 family were down-regulated in syncytia in *LSD1* dependent manner and their mutants showed increased susceptibility to *H. schachtii*. Our results show a complex and precise fine-tuning of PCD regulatory machinery in compatible plant-nematode interaction. This work was supported by National Science Centre (grants no. 2014/13/B/NZ9/02070).

**P63\_Sucrose metabolism in *Serendipita indica*-nematode-plant interaction**

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The beneficial root endophyte *Serendipita indica* (= *Piriformospora indica*) is an orchid mycorrhiza that forms mutualistic relationships with many different plants including the model plant *Arabidopsis thaliana*. During this interaction, the endophyte promotes host plant growth, biomass and seed production. Further, the resistance to abiotic (e.g., drought, salt, toxins and heavy metals) and biotic stresses (e.g., pathogens) is significantly increased. It can be speculated that, similar to arbuscular mycorrhizal fungi, *S. indica* receives carbohydrates (preferably hexoses) from the host in exchange for this service. In this study qRT-PCR of *AtSUS* and *AtINV* genes as well as multiple *sus* and *inv* mutant lines of *A. thaliana* were carried out to assess their importance during fungus-plant interaction. The results showed an overall upregulation of *AtSUS* and *AtINV* genes in roots, whereas shoots showed at first a phase of downregulation followed by an upregulation. Growth promoting effects were only observed in colonised wild type plants of *A. thaliana*, whereas multiple *sus* and *inv* mutant lines showed no growth promoting effects indicating the importance of these genes for successful interaction. To determine the importance of *AtSUS* and *AtINV* genes for host finding of *Heterodera schachtii*, attraction assays with exudates sampled from different multiple *AtSUS* and *AtINV* mutants were carried out. The obtained data show no alteration in attractiveness between multiple *sus* and *inv* mutant lines in comparison to the wild type. Additionally, a nematode-development assay was carried out in three-chamber dishes. One half of the wild type root was inoculated with *S. indica*, the other one with the nematodes. Interestingly, higher female number on plants colonised with *S. indica* was observed. This work significantly increases our knowledge on *S. indica*-nematode-plant interaction with special emphasis on sugar metabolism.

**P64\_Characterisation of cell wall-degrading enzymes of the root lesion nematode *Pratylenchus penetrans***

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Root lesion nematodes (RLN; *Pratylenchus* spp.) are economically important pathogens that inflict damage and yield loss to a wide range of crops (e.g., alfalfa and potato). Like other plant-parasitic nematodes (PPN), RLN require close association with their host to gain access to nutrients. The successful infection of PPN relies on the secretion of a repertoire of proteins (often called effectors) with diverse parasitism-related functions. One of the aims of this study was a detailed characterisation of a set of cell wall-degrading enzymes (CWDEs) of *P. penetrans*. We conducted a data mining of different sets of nematode transcripts generated by Illumina mRNA-seq analyses collected directly from the nematode and potato infected plants. A core set of different families of CWDEs was identified, including a suite of genes encoding for endo-1,4- $\beta$ -glucanases (GH5), pectate lyases (PL3), arabinogalactan endo-1,4- $\beta$ -galactosidases (GH53), xylanases (GH30) and expansin-like genes. Surprisingly, we identified and report here for the first time a pectinesterase-coding gene from a PPN, which is actively transcribed in different tested plants (e.g., alfalfa, corn, potato). The spatial expression of transcripts coding for different CWDEs within the pharyngeal glands of *P. penetrans* was confirmed by in situ hybridisation. RT-qPCR analyses highlight the dynamic expression of *P. penetrans* CWDE genes during plant infection. The importance of individual *P. penetrans* CWDE coding genes was studied by in planta RNA interference (RNAi) assays using soybean hairy root lines.

**P65\_Silencing of *Pratylenchus penetrans* candidate effector genes by RNAi nematode-soaking assays**

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*Pratylenchus penetrans* is a migratory endoparasitic species that feeds and migrates within the root cortical tissue, causing a reduction in root growth after infection, accompanied by the formation of lesions, necrotic areas, browning and cell death. Recently, we provide novel insights into the catalogue of candidate effector genes of *P. penetrans*, covering different functional categories of known plant-parasitic nematode (PPN) effector genes, but also a significant number of genes encoding proteins with unknown functions. The use of RNA interference (RNAi) as an approach for the identification of critical parasitism-related genes of PPN has shown promising results regarding their importance during nematode-plant interaction. This study aims to evaluate the silencing effect of five pioneer effector genes previously localised in the pharyngeal glands of *P. penetrans* and actively transcribed during plant-nematode interaction. Mixed stages of *P. penetrans* were incubated in soaking solution with 1 mg ml<sup>-1</sup> dsRNA of each nematode candidate gene, 50 mM octapamine, 3 mM spermidine for 24 h, with the silencing gene effect validated by RT-qPCR. Aliquots of 50 nematodes exposed to the dsRNA soaking solution were inoculated into sterilized carrot discs and maintained at 25°C for a period of 6 weeks. Silencing of two of the five targeted nematode genes led to a significant reduction (75-98%) on the total number of nematodes recovered from the carrot discs in comparison to the control treatments. The expression of these genes during interaction with the plant and the impaired development of the nematodes inside the host suggest an important role of these genes during infection.

**P67\_ The role of nicotinamide adenine nucleotide (NAD) in tomato resistance against root-knot nematode**

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are among the most damaging pests to tomato production in the United States and worldwide, with damage ranging from 25-100% yield loss. Host resistance conferred by the *Mi* gene in tomato is effective against some species of RKN (e.g., *M. incognita*, *M. javanica* and *M. arenaria*); however, there are virulent species and lines, including *M. hapla* and *M. eterlobii*, that break *Mi*-mediated resistance. Triggering innate plant immunity using chemical elicitors is a proven strategy to combat plant pathogens and we believe this method may augment or supplement *Mi*-resistance in tomato against virulent RKN infection. Nicotinamide adenine dinucleotide (NAD) is one such chemical elicitor that regulates plant defence responses to different biotic stresses. In this study, we investigated the role of NAD in the context of induced tomato innate immunity and RKN pathogenicity in two tomato cultivars, 'VFN' and 'Rutgers', with and without *Mi*, respectively. Single soil drench application of NAD 24 h before nematode inoculation significantly induced defence response pathways, reduced infective-juveniles penetration, and increased plant mass in both cultivars. However, there was no significant difference in gall numbers compared to control. Importantly, we observed no direct toxic effects of NAD on nematode viability and infectivity. The results presented here suggest that NAD induces resistance against RKN pathogenicity in presence or absence of tomato *Mi* gene, likely through accumulation of tomato basal defense responses rather than direct effect on the infective-juveniles behaviour.

### **P68\_ Exploring the genomic structure of the root-knot *Meloidogyne enterolobii***

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Root-knot nematodes (genus *Meloidogyne*) are obligatory plant endoparasites that cause huge economic loss in the agricultural industry and impact the global food supply. The most virulent and widely distributed *Meloidogyne* species worldwide reproduce asexually. *Meloidogyne enterolobii* is an obligatory asexual species. In order to understand further the high level of parasitic success despite the absence of sexual reproduction, we will explore and analyse its genomic structure. Using PacBio and Illumina sequencing technologies, we assembled the most contiguous *Meloidogyne* genome to date. Using MCScanX, we found that 59.49% of the genes are part of duplicated collinear blocks, possibly as a result of hybridisation or multiple segmental duplications. We will look at how collinear genes evolve in these regions and their possible effects in the species parasitic success. In some instances, the duplicated blocks form palindromic structures within the same scaffold. The genome structure is consistent with the allopolyploid structure recently described by our team in other *Meloidogyne* species with obligatory asexual reproduction (e.g., *M. arenaria*, *M. javanica* and *M. incognita*). The palindromes, also observed in *M. incognita* and *M. arenaria* are consistent with the absence of segregation between homologous chromosomes during meiosis. These results could shed light into the surprising parasitic success and adaptation of *Meloidogyne* asexual species in multiple environments and host.

**P69\_Functional characterisation of the candidate effectors identified in the rice parasitic nematode *Meloidogyne graminicola***

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The root-knot nematode, *Meloidogyne graminicola*, is a major pest of rice (*Oryza sativa*) in Asia and in Latin America, causing important crop losses. Successful infection is probably achieved by effector proteins produced in the nematode pharyngeal gland cells and released in the host plant cells. The goal of this study was to identify and characterise *M. graminicola* pathogenicity genes expressed during the plant-nematode interaction. Using the dual RNA-seq strategy, we generated transcriptomic data of *M. graminicola* samples covering several points of the nematode lifecycle. In the absence of a reference genome, a *de novo* *M. graminicola* transcriptome of 76.228 contigs was obtained from those reads that were not mapped on the rice genome. A 'secreted protein prediction' pipeline combined to a comparative transcriptomic approach enabled identification of 15 putative effectors, including two homologues of well-characterised effectors from cyst nematodes (CLE-like and VAP1). By quantitative RT-PCR, we confirmed that these candidate effectors are up-regulated in the early parasitic stages and showed by *in situ* hybridisation that some are expressed in the pharyngeal gland cells. We assessed their sub-cellular localisation in plant cells by transient expression of GFP-effector constructs in *Nicotiana benthamiana* leaf cells, as well as their ability to suppress cell death. To gain insight into their putative function in plant cells, we conducted a search for host interacting proteins. A high-throughput protein-protein interaction screen showed that the candidate effector Mg24 interacts with an *Arabidopsis thaliana* MAP-kinase belonging to a family previously described to be crucial for immunity and development and that Mg10 interacts with a DNA-binding protein. Further experiments are ongoing to identify the rice orthologues of the *A. thaliana* interactants and to assess the role of the candidate effectors in rice infection.



**P73\_Ascorbate oxidation determines the jasmonate level in rice during its interaction with parasitic root-knot nematodes**

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Ascorbate (L-ascorbic acid (AsA)) is a major metabolite in plants that is involved in cell expansion, cell division, growth, defence and, best known, antioxidant metabolism. AsA (reduced form of ascorbate) in the apoplast gets oxidised to dehydroascorbate (oxidised form of ascorbate) during the oxidative burst phenomenon, caused by environmental challenges or pathogen attack. The reduction/oxidation state of the apoplastic ascorbate pool is known to be regulated by ascorbate oxidase (AO). Ascorbate also plays a role as a co-factor for many enzymes, including many dioxygenases, ascorbate peroxidase and violaxanthin de-epoxidase. mRNA-seq studies on root knot nematode (*Meloidogyne graminicola*)-induced galls and giant cells in rice roots, revealed that genes involved in ascorbate biosynthesis, oxidation/reduction and transport are differentially expressed, in comparison with non-infected rice root cells. Based on these observations, this research was set-up to investigate the role of AsA in the interaction between plants and sedentary root-knot nematode *M. graminicola*. Measurement of AsA using HPLC-UV technique showed accumulation of AsA in galls at 3 and 7 days after infection, while the remainder of the infected root system contained similar AsA levels as non-infected roots. Gene expression confirmed AA pathway activation in nematode-induced galls. Infection experiments on rice AA-biosynthesis and AA-peroxidase mutants showed that AA deficiency leads to increased susceptibility to nematodes. Hormone measurements on these mutants revealed disturbances in their hormonal profile, mainly in their root jasmonate level, which could explain their enhanced susceptibility. Furthermore, external application with 20 mM AsA and 20 U ml<sup>-1</sup> AO, showed that oxidised AsA, but not reduced AsA, triggers a strong defence response against root-knot nematodes in rice. In conclusion, our research shows an important role for oxidised ascorbate in hormone biosynthesis and plant defence in the course of the rice-root-knot nematode interaction.

**P74\_The effector MiCTL1 of *Meloidogyne incognita* suppresses pepper resistance and enhances pathogenicity in virulent populations**

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Host resistance is considered the most efficient method for control of *Meloidogyne incognita*. Me-virulent nematodes can overcome host *Me3* resistance and reproduce in pepper. In order to find the effector responsible for overcoming *Me3* resistance in *M. incognita* Me-virulence populations, we compared the transcriptomes of avirulent and Me-virulent near-isogenic lines and found that the secretory protein gene *MiCTL1* of Me-virulent nematodes had significantly increased expression over the avirulent line. Real-time qPCR also indicated that expression levels of *MiCTL1* were significantly higher in pre-parasitic and parasitic second-stage juveniles. *MiCTL1* belonged to C-type lectin gene family, localised in the cytomembrane of plant cells, and was expressed in the subventral glands of nematodes. *MiCTL1* suppressed the programmed cell death triggered by the pro-apoptotic protein *BAX*. Similarly, Virus-induced Gene Silencing of *MiCTL1* in infected host plants reduced the pathogenicity of Me-virulent nematodes. Overexpression of *MiCTL1* increased the susceptibility of *Arabidopsis thaliana* to virulent nematodes. This study revealed that *MiCTL1* is an important effector gene for Me-virulent nematodes, having an immunosuppressive function, and thus shedding light on the mechanism of *M. incognita* virulence necessary to overcome the cognate *Me3* resistance in pepper.

**P75\_Functional characterisation of a highly expanded superfamily of dorsal gland effector proteins in cyst nematodes**

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Cyst nematodes are one of the most damaging groups of phytonematodes to agricultural production worldwide. These obligate parasites deliver a suite of effector proteins *via* a stylet to host cells to establish and maintain a metabolically hyperactive feeding site or syncytium. Stylet-secreted effectors hijack host cellular machinery to promote syncytium formation by modulating various aspects of plant development, physiology and defence. However, the functions of a large percentage of identified cyst nematode effectors remain unknown. Two original members of the soybean cyst nematode *Heterodera glycines* parasitome, 16B09 and 2D01, have yet to be characterised. This novel class of dorsal gland expressed effectors represent members of the same superfamily and share conserved protein domains. A genome analysis found this class of effectors to be highly expanded in cyst nematode genomes, suggesting a dynamic role in plant-cyst nematode interactions. Host-induced gene silencing of 16B09 demonstrated a requirement of this effector protein for successful parasitism of the beet cyst nematode *Heterodera schachtii* on *Arabidopsis*. Toxicity of 16B09, but not 2D01, to the growth of yeast suggests that this effector may be modulating an important eukaryotic cellular process. While 16B09 toxicity to yeast precluded the use of a yeast two-hybrid approach to identify potential host targets, an identified 2D01 host target is currently under investigation.

**P77\_ Identification and functional analysis of a novel effector gene Me-3C06 from the root-knot nematode *Meloidogyne enterolobii***

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*Meloidogyne enterolobii* is considered as one of the most damaging root-knot nematode species due to its wide host range, high reproduction rate, and ability to parasitise several crops carrying resistance genes. This nematode is becoming the dominant species in Hainan Island and causing serious yield losses on fruit and vegetable crops in the tropic regions of South China. However, *M. enterolobii* is still poorly characterised at the molecular level. Effector proteins, which are secreted by root-knot nematodes during plant parasitism, have been demonstrated to play a key role in suppressing the plant's defence responses, altering plant signalling pathways and inducing the formation of giant cells. Therefore, identifying and studying the function of these effectors are extremely important for understanding the molecular mechanisms involved in nematode parasitism and pathogenicity. In this study, a new effector gene *Me-3C06* (KY386298) was isolated from *M. enterolobii*. Southern blot analysis revealed that the *Me-3C06* is a member of a multigene family. Furthermore, we used a series of techniques and methods including *in situ* hybridisation, real-time PCR, overexpression, subcellular localisation immunohistochemistry and RNAi to investigate the gene's expression pattern and function. The results confirmed *Me-3C06* transcripts specifically accumulated in the pharyngeal gland cells of the second-stage juveniles, and the transcript abundances were high in the motile juvenile stages and low in the sedentary stage of the nematodes. Knocking down *Me-3C06* using RNA interference reduced nematode infectivity by 46%, indicating that *Me-3C06* plays an important role in nematode parasitism. Based on these results, it is concluded that *Me-3C06* is related to the feeding cell formation and host defence inhibition during early infection.

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**P78\_Changes in root architecture of *Arabidopsis thaliana* induced by the cyst nematode *Heterodera schachtii***

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Low levels of infection by cyst nematodes on some resistant crop varieties result in significant loss in yield, while other heavily infected varieties show hardly any symptoms at all. This difference in responses suggests that some plants tolerate biotic stress by plant-parasitic nematodes better than others. The objective of our current research is to investigate whether *Arabidopsis thaliana* can be used to unravel the genetic architecture and molecular mechanisms underlying differences in tolerance to plant-parasitic nematodes in plants. To this end we first looked at changes in root architecture upon inoculation with increasing numbers of the beet cyst nematode *Heterodera schachtii*. This revealed how plants could compensate for damage induced by cyst nematodes in roots at low inoculation densities. Altogether, our data may demonstrate if *Arabidopsis* can be used as a model to study tolerance to cyst nematodes in plants.

**P79\_Characterisation of PLETHORA during gall formation of the root-knot nematode *Meloidogyne incognita***

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The parasitic root-knot nematode *Meloidogyne incognita* manipulates developmental processes in plants through formation of large tumour-like structures, designated as galls, in the roots of their hosts. The transcriptional profile of these galls show significant overlap with that of the formation of lateral roots (Cabrera *et al.*, 2014). Key regulators in the formation of lateral roots are the PLETHORA (PLT) transcription factors PLT3, PLT5 and PLT7. They are induced by the sustained presence of the phytohormone auxin. These genes work together to regulate positioning and outgrowth of lateral root primordia (Hofhuis *et al.*, 2013). The auxin efflux proteins PINFORMED (PIN) are thought to play an important role in this process and PLTs are known to regulate them in plants (Prasad *et al.*, 2011). During the initial development of nematode-induced galls an accumulation of auxin is observed (Karczmarek *et al.*, 2004). Additionally PIN proteins are known to be involved in gall formation (Kyndt *et al.*, 2016). However, how the PIN proteins are regulated upon root-knot nematode infection is unknown. Hence, we studied the possibility that the PLT-PIN module is used during the formation of feeding sites of *M. incognita*. Mutant lines of the PLT genes showed a decrease in the number of infections and in number of reproducing females as compared to the wild type lines. Additionally, in several mutant lines galls developed that were significantly increased in size. Moreover, the auxin exporter PIN3 in the mutant line of the three PLT genes was no longer localised at the plasma membranes of cells within the feeding site in contrast to the wild type lines. We conclude that the three PLT genes are involved in feeding site initiation and development and could potentially achieve this through regulation of PIN.

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[Poster other Subjects 2](#)

**O11\_Direct DNA isolation of *Pratylenchus* spp. from potato tubers and roots**

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The sanitary health of seed tubers is an important issue in potato production and the accurate identification of plant-parasitic nematodes (PPN) is crucial to define effective and sustainable integrated pest management programmes. Procedures for the simultaneous detection and/or identification of PPN that require less time and costs are desirable in routine diagnostics and quarantine inspections. In this study, potato tubers and roots infected with an isolate of the root lesion nematode *Pratylenchus penetrans*, an important PPN with a wide host range including potato, were used to isolate nematode DNA, without the need of prior nematode extraction. Potato tubers and roots were inoculated with different levels of *P. penetrans* (1, 5, 10, 50, 100, 1000 nematodes tuber<sup>-1</sup> and 10, 25, 50, 200, 500 and 1000 nematodes (20 mg root)<sup>-1</sup>) and total DNA isolated with Flinders Technology Associates, Whatman, GE Healthcare (FTA<sup>®</sup>) cards. Potato peels, from infected tubers, and infected roots were either directly crushed onto FTA<sup>®</sup> or homogenised in phosphate saline buffer and applied to the cards. After purification, the DNA was successfully amplified, using *P. penetrans* specific primers, from infected tubers and roots. The detection threshold levels using FTA<sup>®</sup> were 10 nematodes tuber<sup>-1</sup> (direct prints) and 1.25 nematodes (mg root)<sup>-1</sup> (homogenised roots). The results demonstrated that FTA<sup>®</sup> cards can be useful tools to detect *Pratylenchus* species directly from potato tubers and roots.

## **O12\_ Nematology lab of INIAV, a 5-year overview of services and technical support**

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NEM-INIAV, the nematology lab of INIAV (National Institute for Agrarian and Veterinary Research) located in Oeiras, near Lisbon, integrates the National Reference Laboratory (NRL) for Plant Health in Portugal. The role of this laboratory is to detect plant-parasitic nematodes aiming to prevent the spread and introduction of nematodes of plant material and plant products and also to determine whether these are complying with local and EU legislation. As a NRL, NEM-INIAV is responsible for the testing of samples coming from the national surveys. Several thousands of wood and insect samples for detection of the pinewood nematode (*Bursaphelenchus xylophilus*) have been processed. Similarly, hundreds of soil cores were tested for the presence of potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) and the burrowing nematode (*Radopholus similis*). The diagnostics are based on the nematodes morphology and confirmed by molecular studies whenever needed. Additionally, the lab performs the detection of quality nematodes such as the rice leaf nematode (*Aphelenchoides besseyi*) from seeds, and for the certification of orchards, vineyards and ornamentals. Technical support has been given to private farmers, farmers' associations and forest owners through phytosanitary diagnostics and consultancy. Besides this activity, NEM-INIAV provides support to several ongoing research projects both at national and international levels. The teaching and training of students is also a paramount engagement of the staff. Here we present the distribution of the different activities and the evolution in what concerns number of tested samples over the last five years in NEM-INIAV.



### **O13\_COPAS VISION flow cytometer captures images and sorts**

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We have developed instrumentation for large-particle flow cytometry that can capture images in flow. Adding imaging capability to flow cytometry greatly enhances the phenotyping of samples by providing morphological and spatial information of the sample constituents not collected by conventional flow cytometers. Traditional measurements of size, optical density and fluorescence, as well as Profiler data, are also collected, and these measurements are used for making sorting/dispensing decisions. The collected images and flow cytometry measurements are synchronised so that objects dispensed to wells of multiwell plates can be traced back to their corresponding image. Our COPAS technology platform is designed for large particles, making it ideally suitable for large single cells, cell clusters, and small model organisms. The COPAS *VISION* instrument is based on this platform and is ideally suited for samples made up of particles of varying sizes and shapes. Our data from the COPAS *VISION* shows proof-of-principle support for increased level of phenotyping of these types of samples, including the small model organism *Caenorhabditis elegans*, marine meiofauna samples, seeds, etc.

**O14\_A practical species-specific real-time PCR primer for *Heterodera schachtii***

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The sugar beet cyst nematode, *Heterodera schachtii*, is a nematode parasite of plants that poses a serious threat to areas producing sugar beet, spinach, and cruciferous vegetables around the world. This species was discovered for the first time in Japan in September 2017, and there is increasing concern about it spreading to other areas. Therefore, a method for the rapid identification of *Heterodera* spp. isolated from soil is necessary to investigate their distribution and determine the effectiveness of control measures. However, *H. trifolii* (clover cyst nematode) and *H. glycines* (soybean cyst nematode) are also distributed in Japan, and it is not easy to distinguish these and *H. schachtii* morphologically. Therefore, in this study, we developed a practical, species-specific PCR primer for *H. schachtii*, which can also be used for real-time PCR. Several species-specific primers for *H. schachtii* have previously been reported, but their PCR amplification was poor in our trials. In addition, PCR product size was sometimes too large for use in real-time PCR. The primer we developed amplifies a part of the mitochondrial COI region and has a length of 159 bp. By using this primer, we expect that the detection of *H. schachtii* and estimation of its density in soil will become easier.

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