ASSOCIATION OF APPLIED BIOLOGISTS

President: Stuart Knight

Advances in Nematology

A one day conference
Virtually via Zoom
on 15 December 2020

PROGRAMME

Association of Applied Biologists, Warwick Enterprise Park, Wellesbourne, Warwick CV35 9EF, UK
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Advances in Nematology

on 15 December 2020
Virtually via ZOOM

This conference invites contributions from all areas of nematology (biology, ecology, epidemiology, management of plant parasitic nematodes, entomopathogenic nematodes and diagnostic methods). It actively invites students to participate, both in the platform presentations and the poster session. A prize is given for the best student platform presentation – Bryan Kerry Prize and the best student poster –AAB prize.

PROGRAMME

Tuesday 15 December

10:00 **Welcome and AAB Nematology Group AGM**
STEVE EDGINGTON (CAB International, UK)

10:15 ***Invited speaker***
From trash to cache - the evolution of nematode ecology
ROY NEILSON (James Hutton Institute, Dundee, Scotland)

10:45 **Use of automated image analysis techniques for Globodera species identification and classification**
ROMAIN THEVENOUX, HELOÏSE VILLESSECHE (IGEPP, INRAE, Agrocampus Ouest, Université de Rennes, Le Rheu, France/ANSES – Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, Laboratoire de la santé des végétaux - Unité de nématologie, Le Rheu, France), LAURENT FOLCHER (ANSES – Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, Laboratoire de la santé des végétaux - Unité de nématologie, Le Rheu, France), ERIC GRENIER and NICOLAS PARISEY (IGEPP, INRAE, Agrocampus Ouest, Université de Rennes, Le Rheu, France)

11:00 **Can Entomopathogenic nematodes and fungi be used as a biocontrol option of the Potato leafminer (*Liriomyza huidobrensis*) pupae?**
THABU MUGALA, PIA ADDISON, ANTOINETTE MALAN (Dep Conservation Ecology and Entomology, Stellenbosch University, Western Cape, South Africa) and DIEDRICH VISSER (ARC-VOP, Agricultural Research Council, Pretoria, Gauteng, South Africa)

11:15 **Development and validation of four TaqMan qPCR methods for the identification and quantification of *Pratylenchus crenatus*, *Pratylenchus neglectus*, *Pratylenchus penetrans* and *Pratylenchus thornei***
VALERIA ORLANDO (Harper Adams University, Newport, UK), DAVID ROBERTS (The James Hutton Institute, Dundee, Scotland), SIMON EDWARDS (Harper Adams University, Newport, UK), ROY NEILSON (The James Hutton Institute, Dundee, Scotland), THOMAS PRIOR (Fera Science Ltd, York, UK) and MATTHEW BACK (Harper Adams University, Newport, UK)

11:30 **Towards genetic modification of plant-parasitic nematodes**
OLAF KRANSE, HELEN BEASLEY (Dept of Plant Sciences, University of Cambridge, UK), SALLY ADAMS, ANDRE PIRES DA SILVA (School of Life Sciences, University of Warwick, UK), CHRIS BELL, CATHERINE LILLEY, PETER URWIN (Centre for Plant Sciences, School of Biology, University of Leeds, UK), DAVID BIRD (Entomology and Plant Pathology, NC State University, Raleigh, North Carolina, USA), ERIC MISKA (Wellcome Trust/Cancer Research UK Gurdon Institute and Dept of Genetics, University of Cambridge, UK), GEERT SMANT (Laboratory of Nematology, Dept of Plant Sciences, Wageningen University and Research, Wageningen, the Netherlands), GODELIEVE GHEYSEN (Dept of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Belgium), JOHN JONES (Cell & Molecular Sciences Dept, The James Hutton Institute, Invergowrie, Dundee, UK/School of Biology, Biomedical Sciences Research Complex, University of St Andrews, UK), MARK VINEY (Dept of Evolution, Ecology and Behaviour, University of Liverpool, UK), PIERRE ABAD (INRAE, Université *Côte d'Azur, CNRS, ISA, Sophia Antipolis, France*), THOMAS R MAIER, THOMAS J BAUM (Dept of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa, USA), SHAHID SIDDIQUE (Dept of Entomology and Nematology, University of California, Davis, California, USA), VALERIE WILLIAMSON (Dept of Plant Pathology, University of California, Davis, California, USA), ALPER AKAY (Biomedical Research Centre, School of Biological Sciences, University of East Anglia, Norwich, UK) and SEBASTIAN EVES-VAN DEN AKKER (Dept of Plant Sciences, University of Cambridge, UK)

11:45 **DISCUSSION**

12:00 **Poster flash presentations**

12:20 **LUNCH BREAK**

13.00 **POSTER SESSION – POSTERS SHOWING ON A LOOP**

13:30 **POSTER SESSION – QUESTIONS AND ANSWERS**

14:00 ***Invited speaker***
Are plant-parasitic nematodes taking a sip out of your glass of wine?
INGA ZASADA (Oregon State University, Corvallis, Oregon, USA)

14:30 **The cost-effective mass production of *Steinernema yirgalemense* and *S. jeffreyense*: Replacing the artificial medium diet with cheaper ingredients while maintaining high yields**
MURRAY D DUNN (Dept of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa), PRASANNA D BELUR (Dept of Chemical Engineering, National Institute of Technology, Karnataka Surathkal, Mangalore India) and ANTOINETTE P MALAN (Dept of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa)

14:45 **A screening platform for highly selective chemical biology of parasitic nematodes**
LINDY HOLDEN-DYE, VINCENT O'CONNOR (School of Biological Sciences, University of Southampton, UK), PETER URWIN, CATHERINE LILLEY (Centre for Plant Sciences, University of Leeds, UK), KATARZYNA DUDKIEWICZ and FERNANDO CALAHORRO (School of Biological Sciences, University of Southampton, UK)

15:00 **The occurrence and distribution of potato cyst nematodes in Turkey**
EMRE EVLICE (Plant Protection Central Research Institute, Yenimahalle, Ankara, Turkey), HALIL TOKTAY (Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, Turkey), ATILLA ÖCAL (Atatürk Horticultural Central Research Institute, Turkey), GÖKHAN YATKIN (Plant Protection Central Research Institute, Yenimahalle, Ankara, Turkey) and CIVAN GÜVEL (Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, Turkey)

15:15 ***Bursaphelenchus xylophilus* secretome under *Pinus pinaster* and *P. pinea* stimuli**
HUGO SILVA (CFE-Centre for Functional Ecology, Science for People & the Planet, University of Coimbra, Portugal), SANDRA I ANJO, BRUNO MANADAS (CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Portugal), ISABEL ABRANTES, LUÍS FONSECA and JOANA M S CARDOSO (CFE-Centre for Functional Ecology, Science for People & the Planet, University of Coimbra, Portugal)

15:30 **DISCUSSION**

16:00 **END OF CONFERENCE**

POSTERS

The separated and combined effect of entomopathogenic nematodes on the cotton pests, *Spodoptera littoralis* (Bois) in Israel
NONA MIKAIA (Faculty of Natural Sciences, Mathematics, Technology and Pharmacy, Sokhumi State University, Tbilisi, Georgia)

Impact of ecological and edaphic factors on diversity of soil nematodes
RAWHAT UN NISA (Nematode Biodiversity & Genomics Research Lab, BGSB University, Rajouri, India), AADIL YOUSUF TANTRAY (Institute of Biological and Environmental Sciences, University of Aberdeen, UK) and ALI ASGHAR SHAH (Nematode Biodiversity & Genomics Research Lab, BGSB University, Rajouri, India)

Distribution pattern and population densities of *Bursaphelenchus xylophilus* in pine tree tissues
DAVID PIRES, JORDANA BRANCO (Mediterranean Institute for Agriculture, Environment and Development (MED), University of Évora, Portugal), ISABEL MIRANDA, CARLA PIMENTEL (Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Portugal) and MANUEL MOTA (Mediterranean Institute for Agriculture, Environment and Development (MED), University of Évora, Portugal)

Nematicidal activity of naphthoquinones on the root knot nematode *Meloidogyne luci*
CARLA MALEITA (CIEPQPF, Department of Chemical Engineering, University of Coimbra, Portugal/CFE, Department of Life Sciences, University of Coimbra, Portugal), IVÂNIA ESTEVES (CFE, Department of Life Sciences, University of Coimbra, Portugal), MARA E M BRAGA, SILVÂNIA MORESCHI (CIEPQPF, Department of Chemical Engineering, University of Coimbra, Portugal), ISABEL ABRANTES (CFE, Department of Life Sciences, University of Coimbra, Portugal) and HERMÍNIO C DE SOUSA (CIEPQPF, Department of Chemical Engineering, University of Coimbra, Portugal)

Ficus microcarpa* 'Tiger bark' bonsai parasitised by *Meloidogyne javanica* and *Helicotylenchus dihystera
DUARTE SANTOS, ISABEL ABRANTES (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal) and CARLA MALEITA(CFE, Dept of Life Sciences, Calçada Martim de Freitas and CIEPQPF, Dept of Chemical Engineering, University of Coimbra, Portugal)

Mycobiota of *Pinus pinaster* associated with Pine Wilt Disease
C VICENTE (MED- Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, University of Évora, Portugal/ INIAV IP – Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal), H BRAGANÇA, L BONIFÁCIO, E SOUSA (INIAV IP – Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal), M MOTA (MED – Mediterranean Institute for Agriculture, Environment and Development, Dept of Biology, University of Évora, Portugal), F NÓBREGA and M L INÁCIO (INIAV IP – Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal)

Integrative taxonomy on *Meloidogye ottersoni* (Thorne, 1969) Franklin, 1971 (Nematoda: Meloidogynidae) parasitizing flooded rice in Brazil

VANESSA S MATTOS (EMBRAPA, Brasília, DF, Brazil), RAYCENNE ROSA LEITE (Depto de Fitopatologia, Universidade de Brasília, Brazil/EMBRAPA, Brasília, DF, Brazil),
ANA CRISTINA M M GOMES (EMBRAPA, Brasília, DF, Brazil), LEANDRO GRIMALDI PY (Rua Frei Humberto Weil, Porto Belo, Brazil), DANIELA A SOUZA (EMBRAPA, Brasília, DF, Brazil),
PHILIPPE CASTAGNONE-SERENO (INRA, Université de Nice, Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France), JUVENIL E CARES (Depto de Fitopatologia, Universidade de Brasília, Brazil) and REGINA M D G CARNEIRO (EMBRAPA, Brasília, DF, Brazil)

Formulation of *Steinernema yirgalemense* by entrapment in alginate beads

ABONGILE NXITYWA and ANTOINETTE PAULA MALAN (Dept of Conservation Ecology and Entomology, Stellenbosch University, South Africa)

Influence of land use on soil nematode trophic structure, diversity and metabolic footprint in Verinag sector of Pir - Panjal mountain range

SHAHID AFZAL, WASIM AHMAD and HUMIRA NESAR (Nematode Biodiversity Research Laboratory, Dept of Zoology, Aligarh Muslim University, India)

Sub-lethal concentrations of isothiocyanates associated with brassica species impedes the host finding activity of the stem nematodes *Ditylenchus gigas* and *D. dipsaci*

NASAMU BAWA MUSA, SIMON EDWARDS and MATTHEW BACK (Agriculture and Environment Dept, Harper Adams University, Newport, UK)

Does the dilution agent affect root lesion nematode mortality evaluation?

PEDRO BARBOSA (NemaLab/MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal), CLÁUDIA VICENTE (NemaLab/MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Portugal/ Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Quinta do Marquês, Oeiras, Portugal), A CRISTINA FIGUEIREDO (Centro de Estudos do Ambiente e do Mar (CESAM Lisboa), Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal (CBV), Lisboa, Portugal) and MANUEL MOTA (NemaLab/MED – Mediterranean Institute for Agriculture, Environment and Development & Depto de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Portugal/Depto Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Lisboa, Portugal)

Distribution patterns of plant-parasitic nematode genera in a football field

SOFIA R COSTA (CBMA – Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal), JOSÉ F AZEVEDO (Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Ponte de Lima, Portugal[2], M TERESA M ALMEIDA, M CLARA VIEIRA DOS SANTOS (CBMA – Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal) and LUÍSA MOURA (Escola Superior Agrária, Instit. Politécnico de Viana do Castelo, Ponte de Lima, Portugal/CISAS-Centro de Investigação e Desenvolvimento em Sistemas Agroalimentares e Sustentabilidade, Instituto Politécnico de Viana do Castelo, Portugal)

Morphometrical characterisation of *Pratylenchus penetrans* isolates from potato

DIOGO GIL, JOANA M S CARDOSO, ISABEL ABRANTES and IVÂNIA ESTEVES (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal)

Host status of cultivated plants to the root knot nematode *Meloidogyne luci*

ANTÓNIO CORREIA (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal), CARLA MALEITA (CFE, Dept of Life Sciences, Calçada Martim de Freitas and CIEPQPF, Dept of Chemical Engineering, Rua Sílvio Lima, University of Coimbra, Portugal), IVÂNIA ESTEVES and ISABEL ABRANTES (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal)

Potato cultivars as new sources of resistance against the root lesion nematode *Pratylenchus penetrans*
JOANA FIGUEIREDO (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal), PAULO VIEIRA (Virginia Polytechnic Institute and State University | VT, School of Plant and Environmental Science, Blacksburg, Virginia, USA), ISABEL ABRANTES and
IVÂNIA ESTEVES (CFE, Dept of Life Sciences, Calçada Martim de Freitas, University of Coimbra, Portugal)

Distribution update of the root-knot nematodes in Portugal
LEIDY RUSINQUE, FILOMENA NÓBREGA and MARIA L INÁCIO (Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P), Quinta do Marquês, Oeiras, Portugal)

Platform

From trash to cache - the evolution of nematode ecology

ROY NEILSON

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

ABSTRACT

Soils are a key natural asset that deliver a broad spectrum of ecosystem services supporting life on earth. However, there are several threats to soil function and biodiversity including a changing climate and intensive agricultural production. Nematodes, the most abundant taxa on the planet, mediate a range of soil processes typically through interactions with other soil taxa. Yet until recent times terrestrial nematology had a twin focus of taxonomy and on plant-parasitic species, in particular their impact on crops and potential management. It was common that once a nematode sample was assessed for plant-parasitic species, the sample was thrown away with the majority of (beneficial) nematodes not considered.

However, during the last three decades the convergence of technological advancement coupled with a deeper understanding of the societal value of soils and a greater appreciation of the role of nematode communities in soil processes has led to significant advances in nematode ecology research. Examples of innovative research across several production systems will be presented to highlight the contribution of nematodes to soil processes and addressing global challenges such as the climate emergency and biodiversity crisis.

Use of automated image analysis techniques for *Globodera* species identification and classification

ROMAIN THEVENOUX[1,2], HELOÏSE VILLESSECHE[1,2], LAURENT FOLCHER[2], ERIC GRENIER[1] and NICOLAS PARISEY[1]

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ABSTRACT

Thanks to novel methods of automatized digitalisation, it is now possible to identify and study more morphologic data, faster and even sometimes more precisely. The automatization and standardisation of morphometric studies are expected to i) make easier the identification of known species and ii) bring evidence about the differentiation inside species complex. Some cyst nematode species belonging to the genus *Globodera* are capable of causing damage on potatoes and are therefore of major economic importance. However, their morphological identification remains complex and requires a high level of expertise. In addition, strong suspicions of cryptic species within a particular species, *G. pallida*, exist based on recent genetic and phylogenetic investigations. The creation of tools capable of exploring the morphological variability within this species complex on one hand and distinguishing species already described on the other hand would be an undeniable asset.

In this study, we developed new metrics, especially in the head of infective juveniles, relevant to automatized digitalisation. To evaluate their interest, we choose 3 populations belonging to the European well-known *G. rostochiensis* and *G. pallida* species, the close species *G. mexicana* and a cryptic species suspected in Chile. For each of these populations 30 individuals were photographed and used to build an automatized method using up-to-date image processing. Using such image analysis we were able to accurately detect anatomical parts, predict simple metrics and distinguish *G. pallida* from *G. rostochiensis*. We were also able to accurately predict metrics for novel infective juveniles and assign them to one of these species with a confidence of 95%. We also showed that thanks to these new metrics and the high number of infective juveniles that were analysed, a morphologic distinction can be observed between *G. pallida* and the Chilean populations which are suspected to belong to a novel species. These data represent a new contribution towards an improved species identification and description inside the *Globodera* genus.

Can Entomopathogenic nematodes and fungi be used as a biocontrol option of the Potato leafminer (*Liriomyza huidobrensis*) pupae?

THABU MUGALA[1], PIA ADDISON[1,] ANTOINETTE MALAN[1] and DIEDRICH VISSER[2]

[1]*Dep Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, Western Cape, South Africa*
[2] *ARC-VOP, Agricultural Research Council, Pretoria, Gauteng, South Africa*

ABSTRACT

Although agriculture is crucial to economic growth in South Africa, it faces several challenges, most include pests and diseases that lead to crop losses. Such losses significantly hinder food security and efforts to reduce poverty and hunger. Therefore, a major concern is to find effective, environmentally friendly control measures for insect pests. Potatoes are among the four most widely consumed vegetable crops worldwide, with more than 315 million ha being grown yearly. The South African potato production industry contributes 60% of the total percentage of vegetables grown in the country, and 4% of total agricultural produce. However, potatoes are infested by various pests and diseases, like the devastating leaf miner, *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae). *L. huidobrensis* has developed resistance against a wide range of synthetic insecticides, hence the need to find an integrated pest management program. In this study, four South African local EPN species, namely, *Steinernema yirgalemense, Heterorhabditis baujardi, Heterorhabditis zealandica* and *Heterorhabditis bacteriophora*, and two EPFs (*Metarhizium robertsii* and *Beauveria bassiana*) against the pupae stage of *L. huidobrensis* in laboratory conditions. The mortality by infection of the entomopathogens was investigated, of which *Heterorhabditis baujardi* was the best performing treatment (> 55% mortality) for pupae of all EPN treatments. In the case of EPF, *Metarhizium robertsii* outperformed *Beauveria bassiana* with >80% mortality.

References

[1] **Ameixa *et al*., 2007**. *Journal of Insect behaviour* **20**(1):137-155.
[2] **Lacey *et al*., 2012**. *Journal of Nematology* **44**(2):218–225.
[3] **Weintraub *et al*., 2017**. *Journal of Insect science* **17**(1):28; 1–27.

Development and validation of four TaqMan qPCR methods for the identification and quantification of *Pratylenchus crenatus*, *Pratylenchus neglectus*, *Pratylenchus penetrans* and *Pratylenchus thornei*

VALERIA ORLANDO[1], DAVID ROBERTS[2], SIMON EDWARDS[1], ROY NEILSON[2], THOMAS PRIOR[3] and MATTHEW BACK[1]

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ABSTRACT

Pratylenchus crenatus, *P. neglectus, P. penetrans* and *P. thornei* are globally the most common species of root-lesion nematodes (*Pratylenchus* spp.). Correct identification and quantification of these nematodes is important for strategic management such as rotation choice and nematicide use. A real-time quantitative PCR can provide a fast and reliable alternative to morphological identification which requires significant taxonomic experience. A TaqMan hydrolysis probe method based on the 28S rDNA D2D3 expansion region was developed and validated for identification and quantification of these four species of root-lesion nematodes. A set of two primers and one TaqMan probe were designed for each species target. After optimization, based on the Ct values obtained, the optimal conditions of the qPCR reaction were: 10 µl SensiFast Probe Hi-Rox Mix (Bioline Reagents), 0.25 µM of each primer, 0.6 µM of probe and 2 µl of DNA template. The amplification conditions were: 95ºC for 3 min followed by 35 cycles at 95ºC for 10 sec with 68–69ºC for 60 sec. Four standard curves were made by plotting known gene copy number, obtained by a log serial dilution of purified plasmids, with the corresponding Ct values. Each standard curve showed a highly significant linearity (R^2 = 0.99) between Ct values and gene copy numbers, demonstrating the specificity of primers and probe. Consistent amplifications for samples with species-targets from different locations were detected, whereas a lack of amplification was found for non-target species such as *P. coffeae, P. pseudocoffeae, P. vulnu*s, *P. fallax*, *Globodera rostochiensis, Meloidogyne hapla, Trichodorus primitivus* and *Bitylenchus hispaniensis*. The high specificity and sensitivity of the method were confirmed also by the consistent detection and amplification among different life stages and increasing numbers of species target. Methods allowed the detection of one and ten individuals from a species target when combined with up to 30 individuals of non-target species. Fifteen samples from potato fields were used to compare estimated abundance from qPCR with traditional counting by microscopy. A significant correlation (R^2 = 0.78, described by the equation y = 0.4x + 36.3) was found between the two approaches, thus confirming the robustness of the methods. In summary, the qPCR TaqMan methods developed in this study appear to provide a highly specific, sensitive, fast and accurate quantification of *P. crenatus, P. neglectus, P. penetrans* and *P. thornei*.

Towards genetic modification of plant-parasitic nematodes

OLAF KRANSE[1], HELEN BEASLEY[1], SALLY ADAMS[2], ANDRE PIRES DA SILVA[2], CHRIS BELL[3], CATHERINE LILLEY[3], PETER URWIN[3], DAVID BIRD[4], ERIC MISKA[5], GEERT SMANT[6], GODELIEVE GHEYSEN[7], JOHN JONES[8,9], MARK VINEY[10], PIERRE ABAD[11], THOMAS R MAIER[12], THOMAS J BAUM[12], SHAHID SIDDIQUE[13], VALERIE WILLIAMSON[14], ALPER AKAY[15] and SEBASTIAN EVES-VAN DEN AKKER[1]

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ABSTRACT

Plant-parasitic nematodes are a continuing threat to food security, causing an estimated 100 billion USD in crop losses each year. The most problematic are the obligate sedentary endoparasites (primarily root knot nematodes and cyst nematodes). Progress in understanding their biology is held back by a lack of tools for functional genetics: forward genetics is largely restricted to studies of natural variation in populations, and reverse genetics is entirely reliant on RNA interference. There is an expectation that the development of functional genetic tools would accelerate the progress of research on plant-parasitic nematodes, and hence the development of novel control solutions. Here, we develop some of the foundational biology required to deliver a functional genetic tool kit in plant-parasitic nematodes. We characterise the gonads of male *Heterodera schachtii* and *Meloidogyne hapla* in the context of

spermatogenesis. We test and optimise various methods for the delivery, expression, and/or detection of exogenous nucleic acids in plant-parasitic nematodes. We demonstrate that delivery of macromolecules to cyst and root knot nematode male germlines is difficult, but possible. Similarly, we demonstrate the delivery of oligonucleotides to root knot nematode gametes. Finally, we develop a transient expression system in plant-parasitic nematodes by demonstrating the delivery and expression of exogenous mRNA encoding various reporter genes throughout the body of *H. schachtii* juveniles using lipofectamine-based transfection. We anticipate these developments to be independently useful, will expedite the development of genetic modification tools for plant-parasitic nematodes, and ultimately catalyse research on a group of nematodes that threaten global food security.

Are plant-parasitic nematodes taking a sip out of your glass of wine?

INGA ZASADA

USDA-ARS Horticultural Crops Research Unit
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ABSTRACT

Wine grape production in the Pacific Northwest of the United States (Oregon and Washington) is valued at approximately US$450 million. Similar to other wine grape producing regions in the world, vine productivity is impacted by plant-parasitic nematodes. Nematodes of concern include *Meloidoygne hapla*, *Mesocriconema xenoplax*, and *Xiphinema* spp. Vines parasitized by *M. hapla* and *M. xenoplax* can have dormant pruning weights reduced by up to 50%, which in subsequent years will impact grape yield. The goal of research in the region is to generate biological data on plant-parasitic nematodes in wine grape vineyards and to use this information to inform management decisions. To this end, it was determined that *M. hapla* undergoes a single generation in Pacific Northwest vineyards with juvenile population densities in soil highest from fall to spring. When the distribution of nematodes in soil was considered, it was discovered that *M. hapla* is aggregated in irrigation zones to a depth of 30 cm, while *Xiphinema* sp. is distributed across the vineyard to depths of 120 cm. This biological data combined with information on host status of rootstocks for nematodes, fumigant efficacy, and cover crops provides a basis for the management of plant-parasitic nematodes in wine grapes vineyards in the Pacific Northwest.

Cost-effective mass production of *Steinernema yirgalemense* and *S. jeffreyense*: Replacing the artificial medium diet with cheaper ingredients while maintaining high yields

MURRAY D DUNN[1], PRASANNA D BELUR[2] and ANTOINETTE P MALAN[1]

[1]*Department of Conservation Ecology and Entomology, Faculty of AgriScience, Stellenbosch University, Stellenbosch, South Africa*
[2]*Department of Chemical Engineering, National Institute of Technology Karnataka Surathkal, Mangalore 575025, India*

ABSTRACT

The *in vitro* liquid mass production of entomopathogenic nematodes for biological control of soil-dwelling pests is a costly endeavour. The initial phase of liquid flask production is small and unsuitable for commercial application. Only with the initiation of the scale-up phase, extending large bioreactors of 10 000 L to 100 000 L, does the cost of production start to decrease. However, prior to the scale-up phase, the cost of production can also be substantially decreased by means of sourcing relatively cheap ingredients of the liquid medium diet. The present study shows that comparatively cheap ingredients can be used, while maintaining high yields, as well as substantially increasing the yield. Three experiments were conducted on *Steinernema yirgalemense*, with one experiment being conducted on *S. jeffreyense.*

For both species, two alternative protein sources (egg yolk and milled black soldier fly protein) were used, instead of the usual soy powder protein source. Though the insect protein produced greatly reduced yields for both species, for *S. jeffreyense*, the yields for the egg yolk and soy powder protein were not significantly different (approximately 110 000 IJs ml), leading to the far cheaper egg yolk being chosen as the protein source. For *S. yirgalemense*, the egg yolk showed a significant increase in yields compared to the soy powder and insect protein, with mean yields of 308 000, 62 194 and 25 755 IJs ml, respectively. A trial was also conducted to establish the lipid source of the medium diet, using the much cheaper canola oil versus the usual expensive olive oil. Results showed that using either lipid source obtained high yields, with olive oil achieving a mean yield of 378 833 IJs ml and canola oil achieving 410 833 IJs ml. Canola oil was, thus, seen as the optimal lipid source, which, again, significantly reduced production costs. A trial on the source of nitrogen was conducted, comparing expensive laboratory-grade yeast extract with low-grade brewer's yeast and Marmite. Results showed yeast extract to be the optimal choice.

A full cost analysis, which was conducted for each alternative ingredient, showed how high yields can be obtained with far cheaper dietary ingredients than usual and highlighted the need for optimisation of the *in vitro* liquid mass production of EPNs. Moreover, this study indicates how EPNs can be mass-produced cost-effectively and, with further optimisation, could come to compete with the cost of production for synthetic pesticides.

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A screening platform for highly selective chemical biology of parasitic nematodes

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ABSTRACT

Chemical treatments are successfully used to mitigate the deleterious effects of parasitic nematodes that impact human, animal and plant health. Many of these chemicals act as neurotoxins via evolutionary conserved transmitter pathways providing potential for off-target effects. The 5-HT-gated chloride channel MOD-1 has a constrained phylogenetic distribution and is apparently found only in the Nematoda. It has potential to harbour distinct pharmacophores that may be targeted by highly selective chemicals. Here, we rationalize MOD-1 as a *bona fide* candidate harbouring pharmacophores that will potentially promote chemical treatment strategies. We establish a whole organism bioassay of *mod-1* function in *C. elegans*, by implanting a heterologous sensitivity of MOD-1 receptor function into the essential neuron M4 which is a key regulator of feeding. Activation of MOD-1 heterologously expressed in the M4 neuron imposes a pharmacologically tractable 5-HT dependent development arrest. We couple this to a simple green fluorescence robust read out of organism growth that provides a flexible assay of *mod-1* function in intact worms. We take advantage of this bioassay and develop a liquid based bioassay suited to higher throughput micro-titre plate approaches. This PharmacoGenetic Targeting of M4 neuron (PhaGenTM4) provides a binary assay of MOD-1 receptor activation and inhibition.

We use this experimental approach to benchmark the pharmacology of MOD-1 and identify quipazine and 5-carboxyamidotryptamine (5CT) as a lead route to define activators and methiothepin as a corresponding antagonist. We show that the platform has the ability to identify agonists and antagonists, as well as probe this in a system with an embedded tone that will facilitate identification of chemicals that also act as allosteric modulators of the target function. This assay has the intrinsic ability to filter out off target effects and can be extended to the investigation of other classes of membrane receptors and modulators of neuronal excitation, as well as to accelerate the discovery of new effective and selective anthelmintics with a reduced environmental impact and side effect bias.

Given the value of phylogenetic selective agonists/antagonists and allosteric modulators in parasitic pest control, the PhaGenTM4 assay will help approaches aimed at extending utility of candidate targets and classes of chemicals capable of selectively targeting the modulation of membrane receptors and determinants of the neuronal excitation.

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The occurrence and distribution of potato cyst nematodes in Turkey

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ABSTRACT

Potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* are amongst the most damaging potato pests and can cause severe yield losses and both species have worldwide regulatory concern. In Turkey, the first reported occurance of *G. rostochiensis* and *G. pallida* was in 1996 in Bolu (Dortdivan district) province (Enneli & Oztürk, 1996). After that time, *G. rostochiensis* has been found in seed potato fields of Afyon, Konya, Kayseri, and Sivas provinces. All infestations are not widespread, being localised to individual fields, but strict phytosanitary measures have been implemented to eradicate PCN from all these areas following detection. *G. rostochiensis* has also been detected in ware potato fields in the Ödemiş district, İzmir province (Ulutaş *et al*., 2012).

In this study, in order to determine the distribution of PCN in Turkey, 1054 soil samples were taken in nine provinces covering 66.1% of the Turkish potato production areas in 2017–2019. Identification of *Globodera* spp. populations was then conducted based on: (i) morphological and morphometrics characteristics of cyst vulval area and J2; (ii) ITS region analysis, amplified using ITS5, PITSr3, and PITSp4 primers and detected in multiplex PCR (Bulman & Marshall, 1997). The provinces of Konya, Kayseri, Sivas, and Aksaray, which are important seed potato production areas, were found free of *G. rostochiensis* infestation and only 2 ware potato fields were found infested with *G. rostochiensis* in the province of Afyonkarahisar. However, 26.9%, 41.4%, 12.4% and 1.1% of the soil samples taken from Niğde, İzmir, Nevşehir and Bolu provinces respectively -where seed potato production is prohibited- were infested with *G. rostochiensis*. While PCN cysts were not detected in Konya, Sivas and Kayseri provinces, non-viable PCN cysts were detected in some areas without live PCN cysts in Afyonkarahisar and Bolu provinces.

The results of present study demonstrate that *G. rostochiensis* was found in 12.1% of soil samples. However, no evidence for the existence of *G. pallida* was recorded in Turkey. As a result, it is necessary to take urgent control measures in infested areas, in particular encourage use *G. rostochiensis* resistant varieties, to prevent further PCN spread.

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Bursaphelenchus xylophilus secretome under *Pinus pinaster* and *P. pinea* stimuli

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ABSTRACT

The pinewood nematode, *Bursaphelenchus xylophilus* (*Bx*), the causal agent of pine wilt disease, is a migratory endoparasitic nematode skilled to feed on pine tissues and on fungi that colonize coniferous trees. In order to study the *Bx* secretome under different pine aqueous extract stimuli, nematodes from a Portuguese isolate were exposed to the aqueous pine extract from *Pinus pinaster* (susceptible host) and *P. pinea* (less susceptible host) during 16 hours. Secretomes were analysed by SWATH-MS and 776 secreted proteins were quantified in both secretomes. From these, 501 proteins were found increased in the *Bx* secretome under *P. pinea* stimulus and 22 proteins increased under *P. pinaster* stimulus. Functional analyses of the 501 proteins revealed an enrichment of proteins with binding activity. On the other hand, from the 22 proteins found increased in *P. pinaster* stimulus, proteins with peptidase, hydrolase and antioxidant activity were the most represented. The differences found in the secretomes highlight diverse responses from the nematode to overcome host defences with different susceptibilities and provide a better understanding of the mechanism behind the pathogenicity of this nematode, which may help identify possible new targets for nematode control.

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Posters

The separated and combined effect of entomopathogenic nematodes on the cotton pests, *Spodoptera littoralis* (Bois) in Israel

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ABSTRACT

The Egyptian cotton leaf worm *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae), is an important and widespread agricultural pest in the subtropical and tropical ranges. It infests cotton, tomato, lettuce, strawberry and other vegetables throughout Africa, Middle East and Mediterranean basin (Pineda *et al*., 2007; Shairra & Nouh, 2014). The pest causes serious and considerable economic losses to many crops in both greenhouses and open fields (Abd El-Razik & Mostafa, 2013) and it is the most important, damage causing pest in Israel today. The effects of entomopathogenic nematodes *Steinernema carpocapsae* isolate "Zi" (Germany) and strain *Steinernema carpocapsae* (Israel) were studied against cotton leaf worm *S. littoralis* (Bois) under laboratory conditions. Two hundred juveniles IJ/ml of *S. carpocapsae* isolate "Zi" (Germany) and strain *S. carpocapsae* (Israel) were used separately and in combination against cotton leaf worm *S. littoralis* with water used for the control treatments. The investigation showed a high effect of all pest treatments on survival of 4th and 5th instar larvae of *S. littoralis* recorded on the 7th day after treatment with *S. carpocapsae* isolate Zi (Germany) and *S*. *carpocapsae* (Israel) in combination. Separately, 4th instar larval mortality was 58 % after treatment with *S*. *carpocapsae* isolate Zi, and 72 % for *S*. *carpocapsae* (Israel). Mortality imposed by a combination of both strains was 84%.

Separately, the 5th instar larval mortality caused by *S*. *carpocapsae* isolate Zi (Germany) was 64 % and by *S*. *carpocapsae* (Israel) was 78%, whereas a combination of both strains imposed mortality of 89%. There was no larval mortality in control treatments. These findings might be taken into consideration in evaluating the success of entomopathogenic nematodes as pathogenic insecticides for *S. littoralis,* an economic pest of cotton plants. The work opens new approaches to develop highly efficient combined biological products on the basis of entomopathogenic nematodes as bio-agents.

In conclusion, it was determined that *S. littoralis* (cotton leaf worm) can be more effectively controlled by *S. carpocapsae* isolate Zi (Germany) and *S*. *carpocapsae* (Israel) in combination and further studies should be conducted in field and greenhouse conditions.

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Impact of ecological and edaphic factors on diversity of soil nematodes

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ABSTRACT

In soil nutrient cycling nematodes play an important role by decomposing organic matter. Other than the parasitic damage caused by some nematodes, they are also considered as bioindicators of the soil health by ecologists. Previous microbiome project have enlightened most of the soil microbes, among them one class is nematode which is poorly identified and enlisted (Thompson *et al*., 2017). The diversity of nematodes varies under different habitats due to changes in ecological and edaphic factors. The aim of our study was to determine the nematode diversity under a range of ecological factors in different soil types. Our results depict that forest soil has higher nematode diversity than rice field soil, apple orchard soil, pasture and alpine soil. Nematode trophic levels showed that bacterivores dominate in all the soil types. Ecological factors, such as temperature and moisture have significant impact on nematode diversity; the highest nematode trophic levels were observed above 21°C, and above 30% moisture. Soil nutrients such as, nitrogen (N) and phosphorus (P) have depicted a detrimental role in nematode richness at each site, where nematode diversity and richness of genera were higher at abundant soil N and P but decreased at low soil nutrients. Calculated ecological indices like diversity index (DI), maturity index (MI), Shannon-Wiener Index (H') and enrichment index (EI) will predict the deviation on nematode community structure and soil health quality (Ferris *et al*., 2001). Our present study suggests these indices may be helpful as soil monitoring tools for sustainable ecosystems.

Key words: Ecological factors, edaphic factors, nematode diversity, soil types, nematode indices

Distribution pattern and population densities of *Bursaphelenchus xylophilus* in pine tree tissues

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ABSTRACT

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934; Nickle 1970), is a quarantine organism in the European Union and the causal agent of pine wilt disease (PWD), a serious threat to pine forests worldwide, leading to rapid decline and death. In Europe, this invasive pest was first reported in Portugal in 1999, on *Pinus pinaster*. Due to its economic importance and worldwide distribution, an enormous amount of effort is devoted to research on *B. xylophilus* and PWD. However, studies of bottom-up factors on the epidemiology of PWD have been somewhat neglected. Here, we hypothesize that the variation of chemical and structural composition of hosts drives the seasonal patterns of PWN abundance. To test this, we determined PWN population densities from different sections of healthy and declining *P. pinaster* – considered very susceptible to PWN – and *P. pinea* – considered resistant. Wood samples from healthy *P. pinaster* and trees displaying symptoms of PWD were collected at Serra da Lousã (Leiria, Portugal) and Herdade da Apostiça (Sesimbra, Portugal), at the lower (DAP), middle (M) and upper (T) sections of the trees and kept in individual plastic bags to avoid cross contamination. Twigs in the canopy were also sampled. *Pinus pinea* was not sampled at Serra da Lousã, just at Herdade da Apostiça. PWN were extracted from wood material using a modified Baermann tray method and if present were counted under a stereoscopic microscope. DAP and M sections of affected trees presented higher densities than T sections in Serra da Lousã. Samples from Herdade da Apostiça had lower population densities than those from Serra da Lousã, overall. Twigs generally had higher densities than other tree sections in both sites. *Bursaphelenchus. xylophilus* were extracted from seemingly healthy *P. pinaster* trees, while *P. pinea* samples were free of the PWN. Chemical and structural composition of wood samples obtained from the studied trees are presently being analyzed. More sampling will be carried out in the upcoming months to understand the seasonal variation of the constitutive composition of host tissues and its potential interaction with seasonal patterns and epidemiology of PWD in pine trees.

Nematicidal activity of naphthoquinones on the root knot nematode *Meloidogyne luci*

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ABSTRACT

Meloidogyne species (root knot nematodes, RKN) are among the top 10 plant-parasitic nematodes (PPN) in plant pathology with major ecological and economic impacts worldwide. In Portugal, the RKN *Meloidogyne luci* has been detected in potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*) roots. *Meloidogyne luci* was added to the European Plant Protection Organization Alert list in 2017 as it represents a threat to the production of several crops. The scarce availability of efficient nematicides to manage this PPN, together with environmental and health-related issues, has recently encouraged research towards the development of novel, safer and effective natural-origin nematicides. Naphthoquinones, derived from husks of walnut *Juglans nigra*, possess nematicidal activity and are being explored as potential alternatives to synthetic nematicides. This study aimed to assess the effects of two naphthoquinones (juglone and 1,4-naphthoquinone (1,4-NTQ)) on the hatching, mortality and root penetration of *M. luci*. Eggs/second-stage juveniles (J2) were exposed to compound concentrations 20, 50, 100, 150, 200 and 250 ppm and nematode mortality/hatching was monitored at 72 h and15 days. J2 root penetration was assessed at sub lethal concentrations (20 and 50 ppm). J2 were exposed to the compounds for 3 days and penetration was evaluated 3 days after inoculation of 200 J2/tomato plant. Each treatment consisted of four replicates and included tap water and Tween 80 (2500 ppm) as controls. Both compounds had negative impacts on hatching, with a reduction of 40–95% in the number of hatched J2, when compared to the controls. 1,4-NTQ was the most active compound, causing 76 and 99% J2 mortality at 150 ppm and 250 ppm, respectively, within 72 h. During the first 24–48 h of exposure to low concentrations of either compounds, J2 showed visible symptoms of toxicological effects and reduced mobility as concentration increased. Results also revealed a negative effect on J2 infectivity, with a significant reduction in number of nematodes inside the roots at 50 ppm. Juglone and 1,4-NTQ have the potential to be employed in the development of nematicidal products for the sustainable management of *M. luci*. In addition, walnut residues can be valorized by the extraction of their bioactive/nematicide compounds.

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Ficus microcarpa 'Tiger bark' bonsai parasitised by *Meloidogyne javanica* and *Helicotylenchus dihystera*

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ABSTRACT

A *Ficus microcarpa* 'Tiger bark' bonsai tree, acquired in a Shopping Center of Coimbra region (Portugal), showed the typical symptoms of infection (galls) caused by root knot nematodes (RKN), *Meloidogyne* spp. Therefore, a few roots and a soil sample were collected from the pot. Roots were then observed and, egg masses handpicked from roots were propagated on tomato cv. Coração-de-Boi. After two months a pure nematode culture was established and the RKN species identified using biochemical and molecular characters. The esterase isoenzyme phenotype resulted in three bands of esterase (J3), which is the characteristic phenotype exhibited by *Meloidogyne javanica*. Biochemical identification was further confirmed by PCR-RFLP of the mtDNA region between COII and 16S rRNA genes with C2F3 and MRH106 primers, and by SCAR-PCR with the species-specific primers Fjav and Rjav. Roots were stained with acid fuchsin and, besides RKN, *Helicotylenchus* specimens were also found. Afterwards, nematodes of this genus, extracted from roots and soil, were propagated on the same tomato cultivar and used for species characterization/identification, which was based on the morphological characters of females and ribosomal DNA sequencing. Females had the body spirally curved, with up to two turns after relaxing with gentle heat, a key feature of *H. dihystera*. Identification was confirmed using the D2D3 expansion region of the 28S rDNA, which revealed a similarity of 99.99% with available sequences of the common spiral nematode *H. dihystera*. To our knowledge, *M. javanica* and *H. dihystera* are reported for the first time parasitising *F. microcarpa*. Our findings highlight the importance of regular inspections to detect PPN, mainly with quarantine status, in order to prevent their dissemination. Acknowledgements: Projects UIDB/04004/2020, UIDB/00102/2020 and PTDC/ASP-PLA/31946/2017.

Mycobiota of *Pinus pinaster* associated with Pine Wilt Disease

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ABSTRACT

Pine wilt disease (PWD), one of the most threatening diseases to coniferous forests worldwide, results from the complex interaction between the causal agent, the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, the insect-vector belonging to the genus *Monochamus* and the *Pinus* spp. tree host. In the later stages of PWD, PWN undertakes an obligatory mycetophagous phase, feeding on the fungi that colonize the declining trees. Limited studies available indicate that the fungi dominating dead pines, in particular blue-stain fungi assigned to the order Ophiostomatales, may mediate the development and the population of PWN carried by the insect vector. Our aim is to identify and characterise the culturable fungal community of *Pinus pinaster* putatively associated with the PWN lifecycle. A total of 109 fungal isolates were obtained from symptomatic and asymptomatic *P. pinaster* collected in a PWD-affected area in Seia (northwest of Portugal) from which representative isolates of each putative species were characterized on the basis of morphology and selected molecular markers (e.g. internal transcribed spacer, ITS; partial b-tubulin gene, tub; and translation elongation factor 1-alpha gene, tef) for phylogenetic inference. Our preliminary data show that fungal communities of symptomatic *P. pinaster* are less biodiverse and dominated by Ophiostomatales in contrast with the communities of asymptomatic *P. pinaster* trees. Exploring the diversity of multispecies interactions in PWD provides valuable insights into the successful invasion and adaptation of PWN. This work was conducted as part of the national project PineEnemy: Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease (LISBOA-01-0145-FEDER-028724) which focus on the characterisation of the structure and dynamics of the nematode-fungi interactions and to which extent can be targeted to disrupt the disease cycle.

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Formulation of *Steinernema yirgalemense* by entrapment in alginate beads

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ABSTRACT

Large scale use and commercialisation of entomopathogenic nematodes (EPNs) as biological control agents is impaired by their short shelf life. It is therefore of importance to develop a preservation method that can improve storage, without negatively affecting their infectivity against the target pest insects. *Steinernema yirgalemense* infective juveniles (IJs) were formulated in sodium alginate as a method to improve their entrapment and storage capability. To improve entrapment inside the beads, 2% and 4 % sodium alginate, 0.5% and 2 % calcium chloride with a hardening time of 20 and 60 min were investigated. The beads were stored in a 25ºC incubator and monitored weekly for a period of 6 weeks. The infectivity of the nematodes and soil experiments of these formulated beads with regard to escapement of the nematode and disintegration of the beads were evaluated for pathogenicity after 6 weeks using the *Tenebrio molitor* as the trapping host. The disintegration with escapement of IJ and nematode infection was also investigated. The highest number of IJs that escaped from then beads was with the treatment of 2% sodium alginate and 0.5% $CaCl_2$, with a 60 min hardening time, with a mean of 127± 11 escaped nematodes. The treatment recorded with the least nematodes escaped after 6 weeks was 4% sodium alginate, 2% $CaCl_2$, 20 min with mean of 33 ± 16 escaped nematodes. All the treatments did not differ on the infectivity of *T. molitor*. Beads applied to orchard soil, successfully infect the mealworms and disintegration took place after 2 weeks. The results indicated that alginate was the most important factor in the prevention of IJs from escaping from the beads.

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Influence of land use on soil nematode trophic structure, diversity and metabolic footprint in Verinag sector of the Pir - Panjal mountain range

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ABSTRACT

The phenomenon of environmental degradation by inappropriate land use is a worldwide problem, with modifications of above ground land use leading to many changes in below ground soil ecosystems. This study was undertaken with the objective of determining the occurrence of relative abundance of nematode trophic groups, diversity and metabolic footprint in soil under four different land uses. Soil samples were collected from natural forest (NF), scrubland (SL), grassland (GL) and cropland (CL) which are the main land uses in the Verinag sector of the Pir - Panjal mountain range in India. Relative abundance of all the nematode trophic groups changed significantly in the four land use types. Relative abundance of bacterivore nematodes was higher in all four land uses. However, bacterivore abundance was significantly high in GL (59%), fungivores in SL (29.61%), herbivores in CL (29%) and omnivores-predators least abundant in CL (7.3%) compared between the four land uses. Simpson's index (1-D) Shannon's index (H') and Evenness index (J') indicated nematode communities in SL and GL were significantly more diverse as compared to other land uses. Sigma maturity index (ΣMI) indicated nematode communities in NF and SL are stable compared to GL and CL, which is due to reduction in relative abundance of omnivores-predators. Composite metabolic footprint did not show any effect of land use, but functional and trophic shifts in metabolic footprint are found. This study has established that the change in nematode trophic structure, diversity and metabolic footprint is a reflection of change in soil characteristics which is due to different land use.

Key words: Land uses, trophic groups, soil nematodes, metabolic footprint

Sub-lethal concentrations of isothiocyanates associated with brassica species impedes the host finding activity of the stem nematodes *Ditylenchus gigas* and *D. dipsaci*

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ABSTRACT

Stem nematodes, *Ditylenchus gigas* and *D. dipsaci* are the most economically relevant pests of *Vicia faba,* causing up to 60% yield loss. Currently, there are no recommended management measures for their control in *V. faba* production. A potential sustainable solution that has been used successfully for other plant parasitic nematodes is biofumigation using brassica cover crops. The active ingredient in biofumigant brassicas are isothiocyanates (ITC), which are produced from glucosinolates after disruption of brassica tissues. Isothiocyanates have an affinity for proteins, and can therefore cause mass disruption of the functional systems within target pests including nematodes. It is believed that concentrations of 50 mg L^{-1} ITC are achievable in biofumigated soils. *In vitro* studies with allyl ITC, 2-phenethyl ITC, benzyl ITC and sulphoraphane identified 50–100 mg L^{-1} caused up to 100% mortality against the two stem nematodes after 24 h exposure for all ITC except sulphoraphane. Experiments to understand the effect of sub-lethal ITC concentrations were developed. Stem nematodes were exposed to 3–100 mg L^{-1} of the four ITC for 24 h, then ITC were washed off and the nematodes were allowed to recover for 24 h in distilled water. In a 23% pluronic gel, these stem nematodes were examined for their ability to move to the roots of their host, *V. faba*. At 25 mg L^{-1}, allyl ITC reduced migration towards the host by 90%, while benzyl and 2-phenethyl ITC reduced migration by 50–80%. Sulphoraphane had no effect on the stem nematodes' ability to find their host even at 100 mg L^{-1}. Stem nematodes treated with sub-lethal concentrations of ITC showed symptoms of paralysis in addition to being disorientated. The failure of nematodes to migrate towards their host at sub-lethal isothiocyanates concentration further adds to the potential of biofumigation in stem nematode control in *V. faba* crops.

Does the dilution agent affect root lesion nematode mortality evaluation?

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ABSTRACT

Phytochemicals are known for their profuse and well documented biological and pharmaceutical capabilities. They have been extensively tested in nematode, insect or bacteria mortality bioassays. Since some of these compounds have a very low solubility in water, choosing the right dilution agent or technique is of the utmost importance. The selected agent should allow a dilution that does not significantly alter the properties of the tested compound, have no effect on the mortality of the organism to be tested, and finally, have low impact on the environment or on human health. The dilution effect should be, from the macroscopic point of view, complete. Additionally, if the goal is to apply a selected compound as a biological control agent, the dilution agent should itself be as much as possible neutral to the environment and easily applicable to soil, plants or trees.

In this research, standards of naturally occurring phytochemicals were individually tested in five dilution agents (96% alcohol, pure acetone, 70% acetone, 5 mg ml^{-1} Triton-X and 10% DMSO). Thymol solutions prepared in Triton-X or DMSO required heating at 50ºC for 15 min prior to testing for total solubilization. The effect of each dilution agent was evaluated on *Pratylenchus penetrans*, the root-lesion nematode (RLN) prior to dilution. Since 96% alcohol attained 49.5% mortality it was not further used in the assay. The RLN mortality attained with benzaldehyde, carvacrol, octanol and thymol, at 2 mg ml^{-1}, was assessed according to the existing methodology. Overall, 100% mortality was achieved in dilutions with pure acetone, followed by dilutions with 10% DMSO. In face of the environmental impact that pure acetone may have, DMSO may

provide the best relation between full dilution and mortality effectiveness, although unable to achieve 100% nematode mortality at 2 mg ml^{-1}.

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Distribution patterns of plant-parasitic nematode genera in a football field

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ABSTRACT

Plant-parasitic nematodes (PPN) have been reported in sports turfs from several locations around the world, and affect turfgrass rooting and vigour, making it more susceptible to other pests and diseases. Patches of PPN-induced turfgrass decline in football fields change turf performance and management options are limited. We sampled a professional football field in Portugal whose *ca*. 8-yr old turf had weed encroachment and plant health issues, including PPN attack, and was scheduled for replacement. Our objective was to assess the identity and distribution of PPN in the turfgrass to inform turfgrass management decisions.

The field was divided into four quadrants and composite samples were randomly collected in each quadrant, at two different depths: an upper layer mainly consisting of a dense root mat (ca. 0–10 cm), and a deeper layer with sparse roots in sandy substrate (ca. 10–20 cm). Plant-parasitic nematodes extracted from samples were identified to genus level, and their abundance analysed by Generalized Linear Models, considering the effects of field quadrant (horizontal distribution) and sample depth (vertical distribution).

Six genera of PPN were detected in total, of which *Hemicycliophora, Heterodera, Meloidogyne*, *Nanidorus* and *Pratylenchus* were more abundant, and *Helicotylenchus* and *Tylenchorhynchus* were rarely detected. The density of sedentary endoparasites was higher in the deeper layer, whereas that of ectoparasites was higher in the upper layer ($P<0.05$), indicating that not only the turfgrass mat but also the sandy substrate should be replaced. *Hemicycliophora*, *Heterodera* and *Pratylenchus* nematodes were more abundant in the most damaged southeast quadrant, *Meloidogyne* in the northwest and *Nanidorus* in the southeast quadrants ($P<0.001$). The differential and highly significant horizontal and vertical distribution patterns of the ecto- and endoparasitic nematodes suggests overall niche differentiation in the football field, which would allow for damaging effects of the different genera. Most *Meloidogyne* juveniles had *Pasteuria* endospores attached to their cuticles, and were frequently aggregated by their tails in worm-star formation (Vieira dos Santos *et al*., 2020). Such top-down control mechanisms could potentially be exploited for PPN control in turfgrass.

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Morphometrical characterisation of *Pratylenchus penetrans* isolates from potato

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ABSTRACT

Pratylenchus penetrans is an economic important root lesion nematode, since it can cause significant damage in a wide range of cultivated plants. In Portugal, this species is found frequently in potato but despite the previous detection in this crop, little information is available on the morphometric characteristics of isolates. The objective of this study was to evaluate the morphometric variability of *P. penetrans* from Portugal. Fifteen morphometric characters from females and thirteen characters from males were compared in five *P. penetrans* isolates obtained from potato root samples and maintained in carrot discs. The stylet length in both females and males and the position of the vulva in the females had the lowest coefficient of intra and inter-isolate variability. This low variation confirms that these characters are important in the diagnostics of this species. A high degree of inter-isolate variability was detected in other characters of females (body length, distance from anterior end to excretory pore, body width at anus, vulva-anus distance, tail length and ratios b', c and c') and males (body length, distance from anterior end to median bulb, anterior end to excretory pore, spicule length, tail length and a, b', c and c' ratios).The data gathered in this study contribute to increase the understanding about the variability of *P. penetrans* in Portuguese potato crops.

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Host status of cultivated plants to the root knot nematode *Meloidogyne luci*

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ABSTRACT

The root-knot nematode (RKN) *Meloidogyne luci* is currently included in the *Alert List of the European Plant Protection Organization* due to the potential negative impact of this nematode on economically important crops, in Europe. The identification of cultivars, resistant to *M. luci*, is thus important for RKN management. The host suitability of 22 commercial plant cultivars of eight botanical families (Amaranthaceae, Apiaceae, Asteraceae, Cruciferae, Cucurbitaceae, Fabaceae, Passifloraceae and Solanaceae) to *M. luci* was evaluated in a pot assay. The tomato cv. Coração-de-Boi was used as positive control. Five plants from each cultivar were inoculated with 5,000 *M. luci* eggs (Pi, initial population density). The pots were maintained in a growth chamber with environmental controlled conditions (23±2°C, 12 h photoperiod, ±60% relative humidity). Sixty days after inoculation, the number of galls/plant, final population density (Pf) and the reproduction factor (Rf=Pf/Pi) were determined. Host suitability was assessed on the basis of root gall index and Rf. The presence/absence of the RKN resistance *Mi-1.2* gene was evaluated by PCR in the tomato cultivars. Cabbage cv. Kalé, chard, faba bean, lettuce cv. Folha-de-Carvalho, pepper cvs Rainbow and Yoacali, sweet melon cv. Galia F1, tomato cv. Coração-de-Boi, watermelon cv. Sugar baby and zucchini cvs. Black beauty and Nova Zelândia were recorded as susceptible to *M. luci*. Cabbage cvs Bacalan, Coração and Lombarda, carrot, lettuce cv. Cocktail, passion fruit and spinach cv. Tayto were hypersensitive while tomato cvs Actimino, Briomino, Veinal and Vimeiro, containing the *Mi-1.2* gene, were considered as resistant. The resistant cultivars can be included in crop rotation systems to prevent *M. luci* population increase and possible crop losses.

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Potato cultivars as new sources of resistance against the root lesion nematode *Pratylenchus penetrans*

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ABSTRACT

Potato (*Solanum tuberosum L.*) is an important food staple all over the world due to its high nutrient content, ease of cultivation, high productivity, suitability for storage over long periods of time and low cost. However, the potato plant is highly prone to abiotic and biotic stresses like pathogens, being often critically damaged by plant-parasitic nematodes that can severely reduce yield and quality. The root lesion nematode (RLN), *Pratylenchus penetrans*, is a polyphagous root endoparasite that can affect potato development. Apart from direct damage to roots/tubers, the concomitant presence of RLN with other pathogens can develop into synergistic disease complexes, impacting on marketable quality of potatoes. Current measures to manage this nematode are limited and sustainable strategies to control this nematode are needed. The aim of this work is to identify natural potato plant resistance responses to *P. penetrans,* which are of critical importance to generate effective and stable RLN resistance. Ongoing work is being carried out to evaluate the susceptibility of potato cultivars to *P. penetrans*, through the assessment of nematode reproduction rate in roots and soil and analysis of root cellular changes following infection. Afterwards, for the most promising cultivars molecular analysis will be performed in order to elucidate and identify the biological processes during potato-nematode interaction.

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Distribution update of the root-knot nematodes in Portugal

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ABSTRACT

Plant-parasitic nematodes (PPN) represent an important constraint to agricultural production, as they contribute to severe losses worldwide. Among the PPN, root-knot nematodes (RKN), *Meloidogyne* spp., are one of the oldest known parasitic nematodes of plants and considered serious pests of economically important crops (Dong *et al.,* 2001; Trudgill & Blok, 2001). The *genus* comprises more than 90 species (Hunt & Handoo, 2009) and many have been reported in Portugal: *M. arenaria* (Neal 1889, Chitwoodi 1949); *M. chitwoodi (*Golden *et al*., 1980*)*; *M. hapla (*Chitwoodi, 1949*); M. hispanica (*Hirschman, 1986*)*; *M. incognita (*Kofoid and White, 1919; Chitwood, 1949); *M. javanica (*Trub, 1885; Chitwood, 1949*)*; *M. lusitanica* (Abrantes and Santos 1991), *M. luci (*Carneiro *et al*., 2014*)* and *M. enterolobii* (Yang *et al.,* 1983) [Abrantes *et al*., 2008; Conceição *et al,* 2009; Maleita *et al*., 2018; Santos *et al.,* 2019). From 2017, a field survey has been carried out in areas of vegetable and potato production. Soil samples were collected and the nematodes were extracted according to the protocol PM 7/119 (1) (EPPO, 2013). The suspension was observed under a stereomicroscope and suspected specimens of *Meloidogyne* observed using a bright-field light microscope for confirmation. For positive detections of *Meloidogyne* it was necessary to perform bioassays in order to obtain material for identification. Bioassays were carried out using tomato plants cv. Oxheart, in the remaining soil from the analysed sample and maintained in a quarantine greenhouse for two months. Females and egg masses were handpicked from the infected tomato roots and use for biochemical identification. From 537 soil samples, 110 tested positive and bioassays were performed. The results show that *Meloidogyne* is present in the following councils: Centre region - Coimbra, Castelo Branco, Santarém, Leiria; South region - Setúbal, Évora, Beja, Faro. Seven species remain in the territory, and new detections were made. For instance, *M. luci* included in the EPPO alert list was found for the first time in the island of Azores and *M. hapla* was found parasitising grapevine. Due to the threat some species of *Meloidogyne* pose to Portuguese agro-systems, different projects (RiceAlert, MaisSolo, Know-luci EUPHRESCO-Melotrop) are ongoing, to detect, identify and manage their presence in national territory.

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