

# XLIII ONTA

## Annual Meeting

### ABSTRACT BOOK

4-8 SEPTEMBER, 2011  
COIMBRA \* PORTUGAL



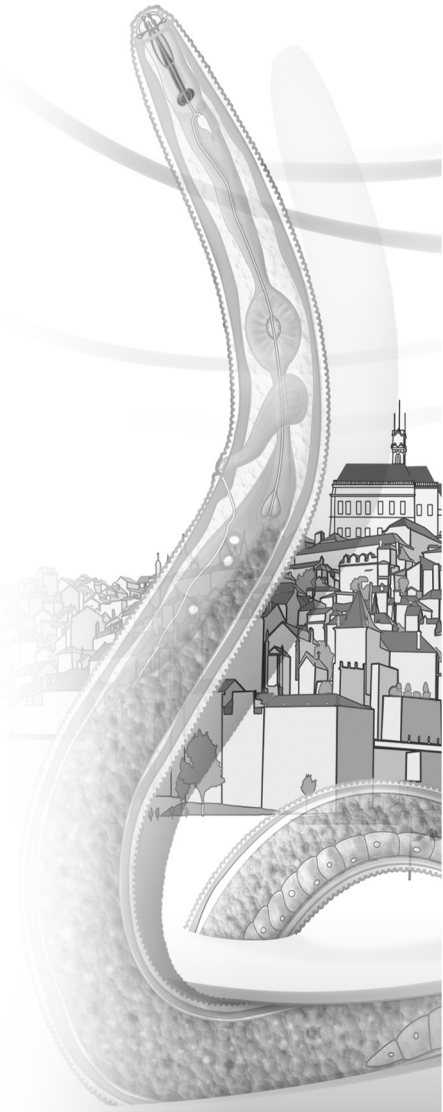
FCTUC FACULDADE DE CIÊNCIAS  
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# XLIII ONTA Annual Meeting

## Welcome

### Message



The Local Organizing Committee would like to welcome all of you and express our sincere appreciation to the ONTA Executive Committee for the choice of Coimbra (“City of Knowledge”), Portugal, as the place for the **XLIII ONTA Annual Meeting**. This meeting will be an international forum to gather researchers from around the world, to share and discuss their findings regarding the broad and interdisciplinary field of Nematology. Therefore, scientific contributions will cover a variety of topics including systematics, biodiversity, ecology and novel control strategies for nematode diseases. Throughout the meeting, we hope to create an atmosphere where everyone, students and professionals, can exchange ideas and establish collaborations.

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

On behalf of the Local Organizing Committee

**Isabel Abrantes**

*President of the Local Organizing Committee*



### **Local Organizing Committee. Portugal 2011**

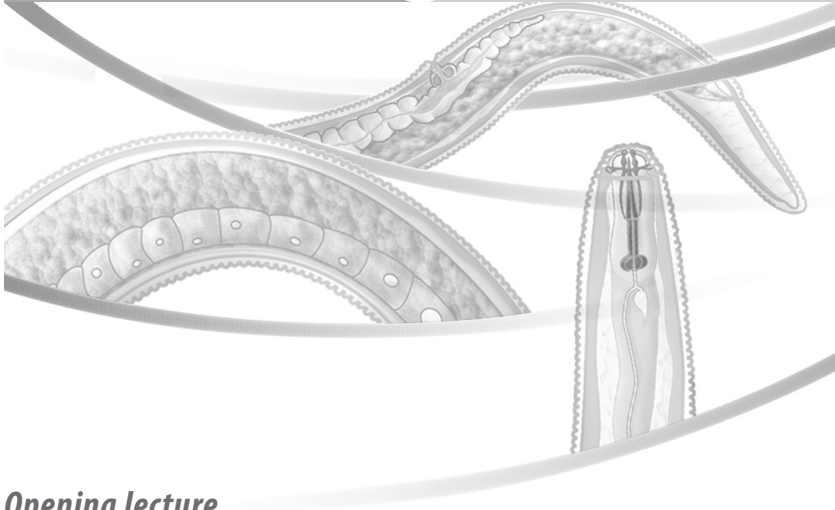
Isabel Abrantes – IMAR-CMA, University of Coimbra, Portugal  
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 Vanessa Carvalho – IMAR-CMA, University of Coimbra, Portugal  
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## *Opening lecture*

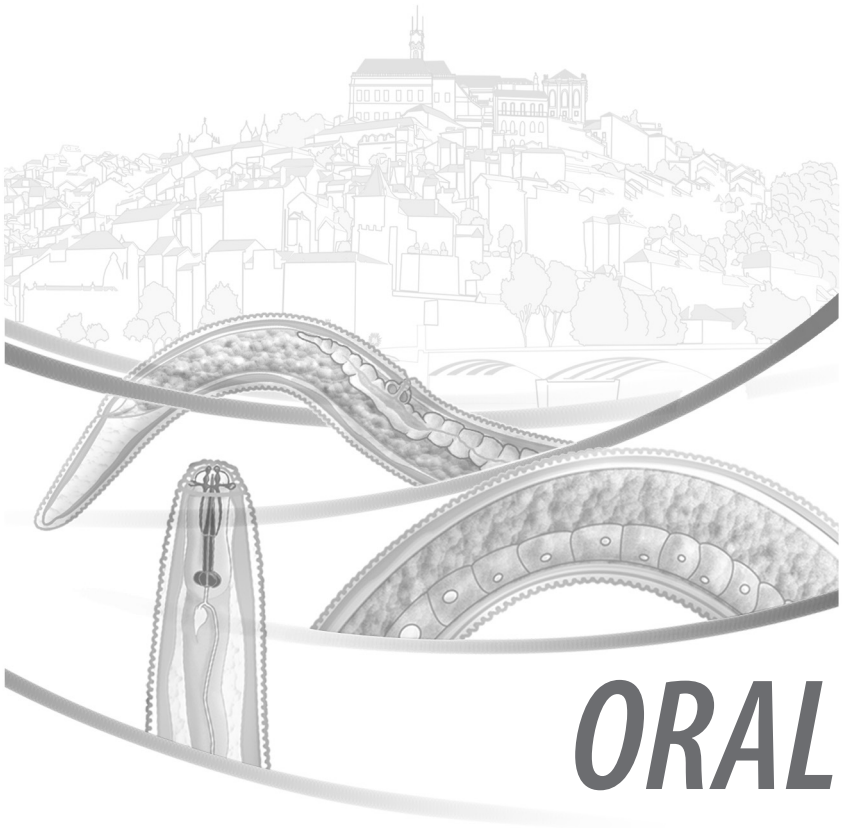
### **Ecology of plant and free-living nematodes in natural and agricultural soil**

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Current concerns of global climate change, pollution and soil loss are prompting a renewed look at the ecology of soil nematodes in arable soils for alternative and effective strategies in disease management and providing plant nutrition. Research questions in population, community and ecosystem ecology are relevant to testing ecological theory of biodiversity and ecosystem stability, ecological succession, and biogeography. The physical habitat constraints of soils add complexity and challenge existing theory and systems models. Essential research is needed to synchronize nutrient release and availability relative to plant needs, test ecological hypotheses, apply optimal foraging and niche partitioning strategies for more effective biological control, blend organic amendments to foster disease suppression, monitor environmental and restoration status, and develop better predictive models for land use decisions.



# **ORAL**

## *Abstracts*

## Induced resistance as plant defense against plant-parasitic nematodes

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Several nontoxic compounds are known to inhibit pathogens' infection and/or their development in plants. When plant responses are involved, this phenomenon is often called induced resistance. Induced resistance to plant pathogens involves activation of defence signalling pathways, in which salicylic acid, jasmonic acid and ethylene play important roles. Chemically-induced resistance to plant-parasitic nematodes has not been thoroughly studied as compared with other pathogens. Several compounds have been tested as resistance inducers -amino-n-butyric acid, salicylic acid, jasmonic  $\beta$  against nematodes, including DL- acid and their derivatives. In many cases, suppression of nematode infection and/or development has been found in plants treated with the inducers. However, mechanisms of nematode suppression by the inducers still remain unclear. Recent developments of molecular techniques, including use of *Arabidopsis* mutants, enabled to study deeper the mechanisms of induced resistance against pathogens, including nematodes (mainly *Heterodera* and *Meloidogyne* spp.). Another compound, which suppresses nematode development, is phosphite ( $\text{PO}_3^{3-}$ ). This compound, also known as a fungicide, was found to suppress nematode development in plants, especially *Heterodera* and *Meloidogyne* spp. in cereals. Current study using *H. schachtii* and *A. thaliana* suggests that phosphite may inhibit the role of auxin in feeding cell development in plants. Other possible mechanisms will be discussed as well.

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## Defense responses in chilli pepper (*Capsicum annuum*) CM-334 infected by *Nacobbus aberrans*

Damaris Godínez-Vidal<sup>1</sup>, Noé López-Martínez<sup>1</sup>, Ernesto Fenández-Herrera<sup>1</sup>, Edgar-Villar-Luna<sup>1</sup>, Mario Rocha-Sosa<sup>2</sup>, R. I. Rojas-Martínez<sup>1</sup>, Lorenzo Guevara-Olvera<sup>3</sup> and Emma Zavaleta-Mejía<sup>1</sup>

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Plants defend themselves against pathogens through chemical and physical barriers. Resistance (incompatibility) and susceptibility (compatibility) are determined by the speed and magnitude in which plant defenses are triggered, being faster and of higher magnitude in incompatible interactions. In the compatible interaction chilli pepper CM-334–*Nacobbus aberrans*, lower PAL activity was associated with lower amounts of soluble phenolic acids, particularly chlorogenic acid which, *in vitro*, caused mortality of second-stage juveniles (J2) of *N. aberrans*. Likewise, the concentration of the capsidiol phytoalexin was similar to those in control plants and transcripts accumulation of the gene EAS that codes for epi-aristolochene synthase, a key enzyme in the synthesis of capsidiol, was also significantly less in comparison to the incompatible interaction CM-334–*Phytophthora capsici*. Capsidiol had a deleterious effect on *N. aberrans*, *in vitro*. Regarding pathogenesis related proteins, the levels of GLU transcripts and corresponding glucanase activity were similar to those observed in non inoculated plants, whereas such levels were increased significantly in the interaction CM-334–*P. capsici*, and those of peroxidases and PR1 protein increased in nematode infected roots in comparison to the control; however, accumulation was significantly lower as compared to the CM-334–*P. capsici* interaction. A slight accumulation of the peroxidase transcripts was associated with lower enzyme activity in the interaction CM-334–nematode, in comparison to that in the incompatible interaction, and was statistically similar to non inoculated control. The results suggest that the mentioned plant defense responses might play a role in the defense against *N. aberrans*.

**Cyst nematodes (*Heterodera* and *Globodera* spp.)****Vivian Blok<sup>1</sup>, Alex Reid<sup>2</sup>, David Kenyon<sup>2</sup> and Jon Pickup<sup>2</sup>**Corresponding author: [Vivian.Blok@hutton.ac.uk](mailto:Vivian.Blok@hutton.ac.uk)<sup>1</sup>The James Hutton Institute, Invergowrie, Dundee, UK, DD2 5DA.<sup>2</sup>SASA, Roddinglaw Road, Edinburgh, UK, EH12 9FJ.

PCR based molecular diagnostic methods are now available for cyst nematodes and are widely used in conjunction with morphological and host range information for species identification. Molecular data has also been used to provide a phylogenetic framework for understanding inter-specific relationships within the Heteroderinae. However, there are few examples where high-throughput molecular technologies are used routinely of for applied and experimental purposes concerning cyst nematodes. With the coming into force of EU PCN directive 2007/33/EC, the number of soil samples SASA was required to evaluate to meet statutory requirements greatly increased and hence warranted the introduction of an automated cyst extraction carousel, an automated DNA extraction system and a multiplex real-time PCR assay which uses a liquid handling robot. With the implementation of the new processes, SASA staff is now able to handle 3 times as many samples with the same staffing resources. This system also provides a novel resource; the DNA bank that is generated from the statutory samples, for assessing the utility of new molecular assays for surveying intra-specific diversity and the distribution of pathotypes of PCN in Scotland.



## Integrative diagnosis of root-lesion nematodes of the genus *Pratylenchus*

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Root-lesion nematodes of the genus *Pratylenchus* are migratory endoparasites that are distributed worldwide and are regarded as severe constraints of many crops. Proper identification of these nematodes (> 70 species) is critical for their management and phytosanitary purposes. The morphological separation of *Pratylenchus* species is complicated due to their high morphological plasticity, the small number of the differential characters available and high intraspecific variability of some of these characters. During the last decades, new approaches based on biochemical, molecular and phylogenetic analyses have provided powerful tools to nematode systematics and reliable identification of *Pratylenchus* spp. In particular the ITS, 18S and 28S ribosomal regions have been largely used to discriminate among different populations and species of *Pratylenchus*. The need for applying a polyphasic approach to characterize *Pratylenchus* species, and, in addition, to demonstrate the existence of cryptic or complex species, which are morphologically indistinguishable but genetically divergent, has been shown in recent morphological and molecular studies of members of this genus. The findings of these studies, conducted in the last decade, have produced a great number of new sequences and taxa used for testing hypotheses of phylogenetic relationships within *Pratylenchus* and have emphasized the importance of polyphasic taxonomy as a better strategy to delimit species and to infer phylogenetic relationships in this complex group of nematodes.

**Virus vector nematodes (Trichodoridae and Longidoridae)****Wilfrida Decraemer<sup>1</sup>, Thomas J. Prior<sup>2</sup> and Sue H. Hockland<sup>2</sup>**Corresponding author: [Wilfrida.decraemer@naturalsciences.be](mailto:Wilfrida.decraemer@naturalsciences.be)<sup>1</sup>Royal Belgian Institute of Natural Sciences, Vautier street 29, B1000 Brussels, Belgium and Ghent University, Department of Biology, Ledeganckstraat 35, B9000 Gent, Belgium.<sup>2</sup>The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.

Longidoridae and Trichodoridae are polyphagous root-ectoparasites and are the only nematode families that act as vectors for plant-viruses. Classification and delineation of taxa within both families have undergone a great deal of flux and have not yet reached a general consensus. Longidoridae comprises over 480 species, many of which are of economic importance, with 5% known to be virus vectors. Many species are known only from their type description which may be poor with little or no discussion of species concept used. Moreover, species are often phenotypically very similar, showing morphometric data overlap and there is a lack of comprehensive geographic and ecological data. Trichodoridae has 102 species, 12% of which are virus vectors. Trichodoridae are difficult to fix, showing intraspecific variation in shape and size of diagnostic features, including several cryptic species and showing co-occurrence of at least two species. Increasing trade in plants and plant products may influence the nematofauna worldwide, but many records of passive dispersal are not complemented by subsequent records of established populations. Consultants in crop protection need to be able to identify species and be aware of their ecology, including interactions with plants and their ability to transmit viruses. Therefore, recent integrated approaches including comparative morphological studies and molecular analyses of e.g. the D2-D3 expansion segments of 28S facilitate identification, including an example of the quarantine listed *Xiphinema americanum sensu lato* (Annex IAI of Council Directive 2000/29/EC) (Longidoridae) and the trichodorid fauna of the Iberian Peninsula, an apparent centre of speciation as case studies will be discussed.

## Morphology and molecular phylogeny of Aphelenchoidea: their divergence and convergence

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The superfamily Aphelenchoidea is a group of nematodes with various life history traits, e.g., there are mycophagous, plant-parasitic, predator and entomoparasitic species and inhabit dead wood and soil. The superfamily is morphologically well-defined, i.e., they have a stylet with small, indistinctive basal knobs or swellings and a well-developed pharyngeal median bulb (= metacarpus). The superfamily is separated into two families, Aphelenchidae and Aphelenchoididae, and each is distinctive based on their nerve ring position; i.e., the nerve ring only surrounds the pharyngeal gland in the Aphelenchidae while surrounding pharyngeal gland and intestine in the Aphelenchoididae. In other words, the pharyngo-intestinal junction is located anterior and posterior to the nerve ring in Aphelenchidae and Aphelenchoididae, respectively. However, according to recent molecular phylogenetic analyses, these two families do not form a well-supported monophyletic clade, and Aphelenchidae belongs to the Tylenchoidea clade, whereas Aphelenchoididae forms a well-supported clade within an infraorder of Tylenchomorpha with no clearly resolved sister taxa. Although there is a significant morphological similarity or convergence in gross stylet and metacarpus morphology in Aphelenchidae and Aphelenchoididae, these two families need to be examined for their ultrastructure and apomorphic patterns integrating recent molecular and morphological studies. In the present work, the morphological variation among six subfamilies of Aphelenchoididae is compared with their molecular phylogeny, and their convergence and divergence are discussed.

## Challenges facing the management of Potato Cyst Nematodes require collaboration between continents!

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Potato Cyst Nematodes (PCN) (*Globodera pallida* and *G. rostochiensis*) are native to South America and a few populations are likely to have been introduced to Europe in soil adhering to seed potatoes in the 19<sup>th</sup> century. A new EU Council Directive 2007/33/EC, as well as the EU Plant Health Directive 2000/29/EC, provide legislation for the unilateral suppression of PCN in Europe. Each species needs to be identified, both for legislative purposes and for management programmes. With increasing pressure to increase world trade in potatoes knowledge of the risks involved in the associated spread of these pests and subsequent problems in management is essential. Plant breeding programmes in Europe have produced potato cultivars with a range of resistance to a relatively limited level of genetic variability in PCN here compared to that in its original home range of the Andes; they comprise a principal component of the statutory PCN management programmes in the European Union. The difficulty in identifying populations with greater virulence has highlighted the need for statutory measures to prevent the risk of their introduction into Europe, as well as monitoring populations to detect unusual virulence. Additional measures to reduce population levels and prevent spread include sampling seed potato fields prior to planting, rotation, surveys to monitor presence and distribution of each species, disinfestations of certain plants for planting and disposal of infested waste in approved facilities.

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## When nematodes met geochemistry: modification of geochemical multivariate tools for nematodes species discrimination

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The advantages of multivariate methods of analysis are indisputable. However, the interpretation of these results is not always simple and straightforward and a certain level of expertise is usually required. The graphical representation of the resulting data from the Fourier Transform Infrared (FTIR) spectral analysis (cluster) only shows how many differences there are between samples. There is not information about which variables are responsible for the majority of the variation. Star diagrams have been used traditionally to discriminate gas-chromatography samples in order to assess oil reservoirs continuity. This method was modified for being used for discrimination of FTIR/ATR (Attenuated Total Reflexion) nematode samples. The results demonstrate that the proposed method it is potentially useful for separating nematodes species, exhibits a strong repeatability and could be an interesting tool for classification purposes. The best characteristics of this methodology are related with the easiness of data processing, the view of the graphical results and the identification of the variables involved in sample differentiation.

## Vegetable production in the absence of methyl bromide

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Vegetable producers in Florida have been seeking viable alternatives to methyl bromide (mbr) since the early 1990's. During the past few years there have been numerous rounds of critical use exemptions (CUE) granted allowing continue use of mbr but they are becoming difficult to obtain. It is possible that no CUE will be granted for 2013. Thus, growers have reached a crossroad whereby they must turn to alternative methods of managing soilborne pests and pathogens. *Meloidogyne* spp. and *Belonolaimus longicaudatus* are the plant-parasitic nematodes that are of a main concern in vegetable production. New nonfumigant and fumigant nematicides are under development for vegetable nematode management, but unfortunately none offer the characteristics whereby they would serve as a drop in replacement for mbr. In addition, the *Mi-1* gene in tomato is under investigation as an alternative to using chemical control. Four tomato cultivars with the *Mi-1* gene have been evaluated in spring and autumn trials. Evaluations included fumigated and nonfumigated treatments and all trials included a root-knot nematode susceptible tomato cultivar. There is a lack of consistence in fruit yield of the resistant cultivars when planted in nonfumigated soil even though galling indices were very low. The low percentage galling of resistant cultivars vs. susceptible cultivars indicates that the root-knot nematode resistant *Mi-1* gene was not broken by soil temperature.

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## Impact of plant parasitic nematodes on fruit growth and productivity

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The goal of any management strategy of controlling plant parasitic nematodes should be centered on the roots of the cultivated plants rather than the direct control of the nematode. The amount of fruit produced by a plant and the quality of the fruit depends on the root system. In seeking the "perfect equation" that enables the coexistence between plants and nematodes, there is little known about the root system. The roots and their role in the production process in agriculture are the most essential factors to consider. Chemical treatments have been widely criticized recently for their negative impact on the environment. When deciding on a nematicidal treatment it is important to know the benefit it will have on the productivity of the plant, rather than just its action on the nematode. There is a clear and proven correlation between the amount and quality of plant roots and their potential in the agricultural yield. Modern irrigation techniques have promoted the best conditions for the nematodes in certain soils receiving water more frequently, or with a more permanent water percentage. A poorly watered root system with water and nutritional imbalances will eventually end up in a poor production.

## «The principal plant nematodes of crops in the French West Indies» What 's new since this first review of Scotto La Massèse in 1968?

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The first surveys of plant-parasitic nematodes in the French Caribbean islands began in the sixties. These surveys in Guadeloupe and Martinique islands demonstrated that nematode damages occur mainly on banana and pineapple commercial plantations and on some food crops such yams and vegetables. The numbers of identified nematodes species were 14 and 15, respectively, in Martinique and Guadeloupe. Nematode control on commercial crops was mainly based on pesticide applications belonging to eight authorized compounds, fumigant and non-fumigants (brominated products and organophosphates). Forty-three years after this first review, the numbers of identified nematode species has increased to 87 and 83, respectively, in Martinique and Guadeloupe. The total number of nematode species reported in the Caribbean islands is presently 126 with 7.1 % of endemic species, confirming the high level of biodiversity of these Caribbean Islands. This communication reviews i) the present status of some invasive nematodes species (*Radopholus similis*, *Scutellonema bradys*, *Helicotylenchus multincinctus*) and ii) the importance and impact on crops of some nematode genus in the Caribbean such as *Meloidogyne*, *Pratylenchus* and *Rotylenchulus*. Then, the future of nematode control in these Caribbean islands is discussed in a context of the decline of homologated nematicides, parallel to the decline of the number of nematologists in the Caribbean since 1968, while some emerging pests are still arising.

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## Perception of the impact of nematode-caused diseases on the horticultural protected crops of southeastern Spain: a quantitative survey analysis

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The coastal areas of southeastern Spain, in particular the province of Almería, are the major horticultural growing areas in Europe with more than 37 000 dedicated hectares, and a total yield about three millions tons. Although there are a few studies on perception of the impact of plant pathogens on agricultural systems, there are no data for the case of the nematode diseases on horticultural crops in this area. In this context, a survey on perception of nematode-caused diseases was undertaken in January 2011, with face-to-face interviews to 120 field advisors in the horticultural growing area of Almería, Spain. The poll was performed to evaluate, among others, the opinion of field advisors about incidence and severity of nematode diseases and efficacy of the most usual approaches for nematode management. Results indicated that 22% of the fields were infested by root-knot nematodes and that patches occupied on average 19% of the cultivable area within a field, which account for 2% of the total area dedicated to growing vegetables. Average yield losses were estimated around 31%. Forty percent of the interviewed advisors responded that nematode problems had significantly increased in the last 5 years. The most frequently used control methods were soil fumigants (28%), non-fumigant nematicides (21%), soil solarization + nematicides (17%), soil solarization (13%), root growth promoters and biological products (13%), grafting and resistant cultivars (5%), and soil solarization + biofumigation (3%). The most efficient treatments against nematode diseases, according to the field advisors' opinion were the application of soil fumigants plus soil solarization and grafting in resistant rootstocks.

## Soil ecosystems in the presence of extensive root piercing may result in replant problems

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Two orchards 600 m apart on sandy loam soil were replanted following three soil treatments. Recorded history of Site 1 was 60 years of Thompson Seedless vineyard followed by 22 years of Plum on Nemaguard. Only a few *Pratylenchus hexincisus* per sample were present at this replant site. Site 2 had been 40 years of Thompson Seedless vineyard followed by 25 years of black walnut rootstock, then 23 years of peach on Nemaguard. First noted when in walnuts, *Pratylenchus vulnus* appeared uniformly and abundantly across Site 2. Soil treatments at both sites were: 1) Telone II fumigation, 2) Roundup herbicide to old trees plus one year of fallow and 3) untreated check. Site 1 was replanted to almond on Nemaguard, Viking and HBOK-50 rootstocks. Site 2 was replanted to plum on Nemaguard, Krymsk1 and HBOK-50 rootstocks. Two years after replanting there were still no damaging nematodes and only a minor replant problem in Site 1. Roundup treatments produced trees at least as good as those grown in the fumigated and both these treatments resulted in significantly better trees than the untreated. Two years after replanting Site 2 the biomass from Nemaguard stocks planted to fumigated or Roundup treated soil was 4X and 2.5X that of the untreated. Roundup plus waiting one year provided notable and significant enhancement of tree growth. Biomass attributable to switching of rootstocks (Viking or HBOK-50 instead of Nemaguard) was most apparent due to their similar growth in untreated soil compared to those planted to Roundup treated soil. Presence of plant parasitic nematodes has been reported to increase root leakage. At these neighbouring sites the intensity of first and second year root rejection appeared related to abundance of nematode feeding.

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## Effect of temperature on the development of *Heterodera schachtii* on oilseed rape (*Brassica napus* L.)

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Oilseed rape (OSR; *Brassica napus* L.) is a crop of increasing world importance. In the UK the area grown has expanded since the 1970s and is currently 642,000 ha. The yield average is 3.4 t ha<sup>-1</sup> which is below the potential of 6.5 t ha<sup>-1</sup>. This shortfall is attributed to agronomic, physiological, environmental and genetic factors. Yield loss can also occur when OSR is invaded by *Heterodera schachtii* and/or *H. cruciferae* and can be high under short rotations. *Heterodera schachtii* is particularly a problem in the UK as sugar beet is often grown in rotation with OSR. Rising soil temperatures in the UK could favour completion of multiple nematode generations on OSR in a growing season producing high population densities which could cause severe crop damage and yield loss. The effect of different temperatures on hatch, root invasion and development of a population of *H. schachtii* on two winter OSR cultivars (Flash and Castille) was investigated in six thermostatically-controlled water baths in a glasshouse. The total degree days, the base, optimum and maximum temperatures for development and the duration of the life cycle were investigated. In this study temperature had a profound influence on the development of *H. schachtii* on OSR. Leachate from both OSR cultivars stimulated more J2 hatch than distilled water and no significant cultivar differences were observed at the different temperatures.

## Identification of putative plant parasitism functions in nematodes

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The Southern root-knot nematode (RKN) *Meloidogyne incognita* is a mitotic parthenogenetic parasite capable of infecting the roots of almost all cultivated plants, which possibly renders this species the most damaging crop pathogen in the world. We have deciphered the genome of this nematode species which represented the first entire genome sequence assembly and annotation for a plant-parasitic nematode. This not only represented the first genome for a plant-parasitic animal but also the first genome for a metazoan species that reproduces exclusively via parthenogenesis. The genome of *M. incognita* showed a spectacular presence of an extensive set of plant cell wall-degrading enzymes (CWDE), which has no equivalent in any animal studied to date both in terms of diversity and abundance. We performed phylogenetic analyses for all these CWDE and showed that all have close relatives in soil bacteria, some of these bacteria being notorious plant-pathogens. Our analysis also showed that these genes underwent massive duplication and gained introns early after a most likely acquisition by multiple independent (horizontal gene transfer) HGT events. Genes, initially defined as RKN-specific based on a comparative analysis with seven other eukaryotic genomes, have been further compared to a set of 25 eukaryotic genomes (including *M. hapla*). We showed that more than half of the predicted proteins in *M. incognita* could not be assigned an ortholog to any of the eukaryotic genomes considered, based on reciprocal best BLAST hits criteria. Such a protein set could therefore represent a putative wealth for identifying specific targets to develop sharp strategies against these pests.

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## A candidate nematode effector from root-knot nematodes with a role in *Mi*-mediated resistance in tomatoes

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The tomato resistance gene *Mi* provides highly effective resistance against three species of root-knot nematode, *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*. *Mi*-mediated resistance is characterized by a hypersensitive response. Because *Mi* is cytoplasmically localized, we hypothesized that the nematode is secreting molecule(s) into the plant cytoplasm that are involved in triggering the *Mi*-mediated hypersensitive response. To address this hypothesis, we used an avirulent strain of *M. javanica* for several rounds of selection on *Mi*-resistant plants. From this selection, we isolated a virulent strain of *M. javanica*. A comparison of their DNA fingerprints and DNA blots indicated that the newly-acquired virulent strain was closely related to its avirulent progenitor. To further understand the genetic basis of this virulence, we compared transcript profiles between the virulent and avirulent strains using cDNA Amplified Fragment Length Polymorphism analysis. We found that the two strains were nearly identical at the transcript level, but differed in a cDNA fragment called Cg1 that was present in the avirulent but absent from the virulent strain. We cloned the gene corresponding to this cDNA fragment and discovered that it encodes a small, 32 aa peptide with no similarity to known proteins. When avirulent nematode juveniles were soaked in dsRNA for Cg1, they and their progeny were virulent on *Mi*-tomatoes, suggesting that Cg1 plays a role in *Mi*-mediated resistance.

## Root-knot nematode feeding site development is impaired by cyclin-dependent kinase inhibitors

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Plant-parasitic nematodes of the genera *Meloidogyne* trigger the formation of giant cells that undergo recurring acytokinetic mitosis and endocycles. Expression analyses of key cell cycle genes showed their early induction in the nematode feeding site (NFS). Additionally, disturbance in NFS development and juvenile maturation were observed during treatment of infected roots with cell cycle inhibitors. Intense DNA synthesis and enlarged nuclei demonstrated that giant cells undergo additional endocycles. How precisely nematodes manipulate the cell cycle in their favour remains to be understood. A systematic comparison of the temporal and spatial expression pattern of core cell cycle genes between uninfected roots and in galls of *Arabidopsis thaliana* resulted in the identification of a collection of genes up-or downregulated in NFC. Among them, negative regulators are candidates to control the cell cycle in NFC. Previous work has shown that KRP2, a member of the cyclin-dependent kinase/kip-related proteins (ICK/KRP), regulate mitosis-to-endocycle transition in plant cells, and is expressed in endoreduplicating cells. The KRP2 gene showed to be expressed during gall development. Therefore to study the relevance of the KRP cell cycle inhibitor genes (7 in *Arabidopsis*) for NFS ontogeny, mutant lines over-expressing and knocked-out are being tested to determine their effect on feeding site development. *In vivo* subcellular localization studies have been carried out to better understand the dynamics of these proteins during giant cell development. Based on these data, three KRP genes are perceived to control giant cell size and consequently nematode reproduction.

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## Global transcript profiling and comparative expression analysis of *Meloidogyne javanica* infecting soybean (*Glycine max*) using 454 pyrosequencing

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To perform a large-scale analysis focusing on the nematode infection on soybean we analyzed root transcriptome in response to infection with *Meloidogyne javanica*. Based on *in silico* approach we are interested in transcripts expressed under several time courses comparing a resistant plant genotype PI595099 to a mock-inoculated reference sample. Total RNA from five biological replicates were pooled and pyrosequencing produced a substantial EST dataset. A total of 1,123,977 reads was successfully aligned to *Glycine max* reference genome to predict 40,441 gene models. 38,810 (96%) were coincident with previously annotated genes. Statistical significance testing accurately identified 27,775 genes with sufficient reads to enable differential expression analysis. Comparative transcript profiling analysis based on the distribution of the relative gene count abundance between the treatments allowed the identification of 478 differentially expressed genes with adjusted p-value cutoff of 0.01. 290 genes were up-regulated in the nematode infected sample, whereas 188 were down-regulated. Domains annotated according to Pfam classification showed that for up-regulated genes the most distinctive situation occurs for domains involved in oxidation-reduction reactions, especially peroxidase domains. It was also detected an increase in expression of several genes containing multicopper oxidase domain families, lipid transfer protein (LTP) family and a pectinesterase family protein. We observed that several biological processes were assigned to cope with the infection, such as cellular growth, polysaccharide biosynthesis, phenylpropanoid biosynthesis and response to oxidative stress. Supported by EMBRAPA, CAPES and CNPq.

## ***Mi*-gene virulence status of field populations of *Meloidogyne* collected from grafted tomatoes**

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The *Mi* a/virulence status of 17 field populations of *Meloidogyne* collected from grafted tomatoes cultivated in plastic houses in south-eastern Spain was determined by comparing nematode reproductive factors in *Mi*-resistant and susceptible tomato cultivars. Eggs from nematode infected roots of the respective field populations collected at the end of the growing season were extracted and used to inoculated plants of the resistant Caramba and susceptible Roma. Individual plants were inoculated with aliquots of the egg suspension as to give 2 eggs per cm<sup>3</sup> of soil, and maintained in an air conditioned growth chamber set at 25 °C. Plants were harvested after completion of one nematode generation as indicated by temperature probes placed in the potted soil. The populations were identified as *M. javanica* (15 populations), *M. arenaria* and *M. incognita* (one population each). Based on the reproduction index (RI) of the nematode (eggs/g root on resistant cultivar divided by eggs/ g root on susceptible cultivar ×100), eight populations were considered as avirulent for the *Mi* gene (RI<6%), four as partially virulent (RI=31% to 43%) and five as *Mi*-virulent populations (RI>55%). In the resistant cultivar, *Mi*-avirulent populations showed lower ( $P<0.05$ ) infection frequency (egg masses/egg inoculum), number of egg masses, egg production, and multiplication rate (eggs per plant/egg inoculum) than the partially virulent followed by the virulent populations. In the susceptible cultivar, none of the reproductive factors differ among populations irrespective of their virulence status.

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## Assessment of entomopathogenic nematode efficacy in combination with chemical and biological insecticides for control of the corn earworm (*Lepidoptera: Pyralidae*) in southwestern USA

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In Arizona, chemical pesticides still remain a widely used tactic for insect control. Therefore, alternative options are needed for sustainable management of insect pests in desert crops. One of these options is the consideration of entomopathogenic nematodes (EPN) which have shown to curatively control many insect pests when used alone or combined with other pathogens and/or chemical pesticides. In this study, we evaluated the efficacy of two Arizona-native EPN strains: *H. sonorensis* (Caborca) and *S. riobrave* (SR-5) in combination with three chemicals (Imidacloprid [Merit® 75 WP], Dinotefuran [Scorpion™], and Indoxacarb [Avaunt]) and one biological pesticide (*Bacillus thuringiensis* subsp. *kurstaki*) for the control of *Helicoverpa zea*. Third instar larvae of *H. zea* were considered for laboratory assays. For each pesticide considered, lethal dose (LD50) was calculated and used for all experiments. Combined effect of EPN and the different pesticides were evaluated considering different application times. Insect mortality, IJ penetration and progeny production were considered to measure EPN virulence in combination with different pesticides. Treatments were organized in blocks and each block was repeated three times. Larval mortality was corrected using Abbot's formula and analyzed using logistic regression. Means were compared using Tukey's test. Preliminary results indicate that virulence of both EPN strains increases when they were combined with imidacloprid. For dinotefuran 35, increased larva mortality was observed in combination with *H. sonorensis*, but not for *S. riobrave* (SR-5). This and additional pesticide data will be presented and discussed.

## Infrared spectroscopy as a tool for nematological studies

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Infra-red spectroscopy is a technique which allows the study of the vibration and rotation of the molecules in the infrared region of the electromagnetic spectrum. When a sample is irradiated in those wavelengths, every functional group is excited to vibrational levels and a spectrum is built where the characteristic bands are represented; therefore, the resulting spectrum is unique and can be seen as a fingerprint. The infrared spectroscopy has been used in many fields with different purposes, from detection of diseases to determination of metabolic pathways; its uses in nematology have been neglected and only some spectral portions of *Caenorhabditis elegans* and *Dithylenchus dipsaci* are known. The results obtained in recent studies of infective juveniles of entomopathogenic nematodes (EPN) have shown differences that can be used for identification at species level. On the other hand, different stages of EPN have been analyzed and the results may explain how some of the nutrients can be deployed in their development (like reserve lipids). The advance in the studies of nematodes through infrared spectroscopy would be used for developing new techniques to help in the identification of species and their populations, to explore some metabolic pathways which can explain host-parasite relationships or even for quarantine controls.

## Entomopathogenic nematodes: progress in research-innovation, use and challenges in Cuba

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Entomopathogenic nematodes (EPN) are efficient biological control agents of agricultural pests. In Cuba, EPN are one of the most commonly used organisms for pest management. However, production issues yet remain limited due to *in vivo* methods for their formulation. Cost for EPN production in the Mass Rearing Laboratories for Biological Control Agents (MRLBC) are low therefore, EPN are easily used by farmers to control a number of important pests of several crops including coffee, sweet potato, cabbage and others crops. Specifically, for the management of coffee berry borer (*Hypothenemus hampei* Ferr.), sweet potato weevil (*Cylas formicarius* Fab.) and diamondback moth (*Plutella xylostella* L.), respectively. Use of EPN in management programs, has reduced the economic threshold of damage of these three pests  $\leq 2\%$  for potato and coffee and less than 0.4 larvae/plant of diamondback moth, as stipulated by the Cuban National Plant Protection System. Currently, EPN are also considered in national pest management strategies for several food and ornamental crops. Cuba is now involved in transformations in their agricultural system and the many challenges for research are now related with the optimization of EPN production including *in vitro* liquid mass rearing methods and improvement of the formulation of EPN.

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## Advances in research of entomopathogenic nematodes in Venezuela

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The National Institute for Agricultural Research (INIA-CENIAP), in the frame of Integrated Agreement of Collaboration between Cuba and Venezuela developed two years ago initiated a project on the "Production of entomopathogenic nematodes (EPN) for agricultural pest management in Venezuela". The program involves the survey, identification and assaying of new species and strains of EPN in vivo production in *Galleria mellonella* as well as the evaluation of their virulence against local pests. Another goal of this program involves farmer assessment on the use of EPN and the education of growers, stakeholders and general public on the consideration of biological control agents including EPN for control of agricultural pests in Venezuela. Until now, 25 *Heterorhabditis* and one *Steinernema* isolates have been collected. Several agricultural pests, including fruit fly (*Anastrepha* sp.), *Cylas formicarius* F., *Hypotenemus hampei* Ferr., *Spodoptera frugiperda* Smith and *Aeneolamia*, among others, have been tested to assess their susceptibility to the native EPN. Molecular diagnostics of the recovered isolates is currently being conducted considering RAPD markers and other methods. Results of these studies will be presented and discussed.

## Use of liquid swine manure for the disinfection of soils – management of the potato cyst nematode

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The disinfection effect of liquid swine manure [LSM] for management *Globodera rostochiensis* populations was evaluated under laboratory conditions. LSM was applied at: 0.4, 2.0, 4.0, and 6.0% (v/w) to 300 g nematode-infested soil under microcosms thermally sealed and opened, with four replicates per treatment. After 30 days at 30 °C bacterial-feeders and fungal-feeders predominated in the nematode community, while the least opportunistic groups had very low occurrence. The proportion of bacterial-feeders increased after manure application. LSM reduced significantly *G. rostochiensis* populations and affected hatching activity while increasing soil fertility. Results suggest that soil disinfection with LSM is an efficient non-chemical alternative for the management *G. rostochiensis* populations, especially when it is applied in closed microcosm. The treatment offers the additional advantages of avoiding organic residue accumulation and decreased contamination problems with no extra costs to the farmer. The effectiveness of biodisinfection fundamentally depends on the method of application and not exclusively on individual factors such as doses, composition or distribution of the organic matter in the soil. Successful biodisinfection requires retention of the gases produced during the biodecomposition process and accurate determination of the costs for the method employed to reduce doses and transport costs. The biodisinfectant material, also, stimulates biological activity of the soil acting as a bioimprover.

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## Agroecological basis for biodisinfection of soils

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Biodisinfection of soil is an alternative for nematode management, not only plant parasitic but also saprophagous, predators and other nematodes with high value as environmental indicators, such as dorilaimids. It is a wide concept that encompasses not only the disinfectant action of the gases produced during organic matter decomposition (biofumigation) but also the complementary effect of soil solarization (biosolarization), which together with anaerobic processes, are related to the biological activity in the soil (biodisinfestation). Biodisinfection can regulate plant pathogens, not only nematodes but also fungi, bacteria, virus and weeds, with a selective action that increases saprophagous and antagonistic organisms for biological control of plant pathogenic populations. The work has been focused on plant parasitic root-knot nematodes of the genus *Meloidogyne* that cause important economic losses in agrarian production in warm and temperate climates. Several agrarian, garden and food industry wastes have been evaluated under laboratory conditions and in commercial greenhouses. In general *Meloidogyne* populations, were reduced significantly as were root galling indices, saprophagous nematodes increased and soil fertility improved. The alternative is complemented by the agronomic management of diversity through its biocide or biostatic selective action, which affects crop production quality, since it can be applied in integrated and ecological programs, assessed by technicians and scientists.

## Chemical control: the search for economically sound practices

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Traditional successful management of phytopathogenic nematodes [PPN] is based *inter alia*, on a combination of cultural practices, resistant cultivars, and application of commercial nematicides. Recent elimination of most classical nematicides has resulted in limiting the use of the remaining commercial nematicides to very few crops and situations. Principal among cultural practices, there are cropping systems that incorporate organic matter [OM] for maintenance of soil fertility and suppression of nematodes and other soilborne pests [SBP]. Some types of OM contain pesticidal compounds that are released in the soil and result in significant suppression of PPN and other SBP's. There are numerous by-products of agricultural and other industrial activities, that have been used to disinfest soils either by direct incorporation into soil or as basic materials for fermentation and production of nematicidal compounds. It is possible to develop economical small scale production of some of these compounds by effecting on-farm fermentation of select materials available in situ or within economical distance. This novel approach to nematode management opens the possibility for eliminating or significantly reducing, the need for external inputs to manage PPN in agricultural and horticultural enterprises. The greatest challenge today is to identify practical means for production of nematicidal compounds from local resources in a manner consistent with acceptable environmental and social considerations.

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## **New environmentally friendly strategies for the management of plant parasitic nematodes in the production of wine and table grapes**

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The management of plant parasitic nematodes of the vine [*Vitis vinifera*] requires analyses of the many variables that influence root-nematode relationship. Among these some such as soil type and degree of compaction, may determine the type of nematodes present by promoting or inhibiting the activities of roots. Soil type, temperature requirements of the roots, cover crops, or other specific soil properties or cultural practices, e.g., chemical or biological control agents, can define environments that result in nematode suppression. The use of chemical agents requires a thorough study of details of application methods, such as delivery with modern irrigation systems. Efficient and efficacious application of a product, chemical or biological, requires determination of the minimal dose of product that once applied to a specific volume of soil achieves a concentration of active ingredient, or colony forming units, that can protect new roots at times when plant roots are generating new growth. When the chemicals are applied at doses too dilute they not only do not protect the roots, but can result in pollution of waterways. The times in which products should be applied must concordant with the periods of root growth activity - fruit set, and postharvest. Often producers think they are doing great things and designing new strategies for product applications, but these do not conform to the science base recommendations and knowledge.



## Methionine for nematode suppression on annual ornamentals

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*Meloidogyne* spp. are important pathogens of many annual ornamentals used in commercial and residential landscapes. In the United States, there are currently no effective conventional nematicides labeled for use on landscape ornamentals. Methionine has been shown to suppress plant-parasitic nematodes and might offer an option on certain crops where conventional nematicides are lacking. Microplot and field plot trials evaluated the efficacy of DL-methionine against *M. incognita* on impatiens (*Impatiens wallerana*), a common annual ornamental used in the southern United States. Treatments evaluated were: untreated control, fenamiphos at 11.2 kg a.i./ha, DL-methionine with two applications of 112 kg/ha each, and DL-methionine with a single application of 1120/kg/ha. Population density of *M. incognita* J2 in soil, root gall index (0-5), *M. incognita* eggs/g of root, and plant shoot biomass were used to evaluate the effectiveness of the treatments. In microplots DL methionine at 1120 kg/ha reduced J2 in soil, galling, and eggs/g of root compared to the untreated controls ( $P \leq 0.05$ ). Though fenamiphos reduced galling in field plots, none of the treatments reduced J2 in soil or eggs/g of root compared to the untreated controls ( $P \leq 0.05$ ). Both methionine treatments improved shoot biomass compared to the untreated controls in microplots and in field plots ( $P \leq 0.05$ ). Even though the results of these studies were inconclusive, they do indicate that further research on this topic is warranted.

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## Soil nematodes as indicators of the sustainability of minimum tillage in tropical vegetable systems

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Vegetables grown in tropical areas are located in environmentally sensitive areas of Australia. There is increasing pressure for farmers to improve soil management practices. Conventional management includes multiple tillage operations, polythene mulch, drip irrigation and no cover crops or organic matter inputs. This has led to a decrease in organic C and increasing pest and disease problems. An alternative vegetable production system is being developed that includes minimum tillage, with semi-permanent beds, controlled traffic, sub-surface drip irrigation and a forage sorghum (*Sorghum bicolor* x *S. sudanense*) cover crop which is slashed to form a mulch for the following vegetable crop. Soil nematode communities under the two systems were compared in field trials. The results suggest that soil nematode community diversity and structure was maintained in the alternative system, but declined in the conventional system when vegetables were cultivated. The nematode community in the conventional system was dominated by plant-parasitic nematodes (*Meloidogyne* spp. and *Rotylenchulus reniformis*). The results suggested that plant roots were the main channel for C inputs into the soil in the conventional systems via organisms, such as plant-parasitic nematodes. In the alternative system, the decomposition of organic material, via fungal or bacterial pathways, altered the soil nematode community and helped to suppress plant-parasitic nematodes. Further work is required to demonstrate that the alternative vegetable production system not only reduces the impact of farming on the environment but suppresses pests and diseases.

## Nematicidal properties of supernatants containing products from anaerobic fermentation of bioglycerin-based compositions

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Purified, neutralized bioglycerin from the biodiesel production was used as the carbon source for fermentation of an aqueous solution containing organic N and other essential nutrients. The solution was mixed with field soil in 120L barrels which were then sealed and allowed to ferment. The supernatant [SP] was removed after 3 wks, filtered, and used in greenhouse, microplot, and field plot experiments to drench soils infested with a variety of phytopathogenic nematodes [PPN], weeds, and other soilborne plant pests. SP applied pre-plant controlled reniform nematode [*Rotylenchulus reniformis*], root-knot nematode [*Meloidogyne incognita*] and other PPN in experiments with 'Tiny Tim' tomato [*Solanum lycopersicum*], 'Marketmore '76' cucumber, and 'Conqueror III' squash [*Cucurbita pepo*]. Yields and growth of all crops were markedly improved. These and other results with related experiments, indicate that it is possible to produce pesticidal products from materials readily available through relatively simple fermentation on farm or with small scale operations. All ingredients used in the formulations are natural and should not require stringent registration.

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## Field performance of glycerin-based compositions for control of weeds, phytopathogenic nematodes, and other soilborne plant pests

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Biodiesel production results in accumulation of crude glycerin [CG], an alkaline water soluble liquid composed principally of glycerin and volatile compounds. CG applied directly to soil [ $>2\text{-}8\text{ MT/Ha}$ ] can suppress a variety of plant pathogenic nematodes [PPN], and other soilborne plant pests [SBPS]. Results from greenhouse and microplot experiments at Auburn University indicate that a number of formulations based on partially purified and neutralized CG can be economically prepared and used for control of PPN, SBPS and weeds and deliver significant plant nutrients. A preliminary field test indicated that drenching VERDISOL<sup>®</sup>, one of several possible formulations, on established bermudagrass [*Cynodon dactylon*] turf controlled a variety of PPN and stimulated density, lushness, appearance and quality of the turf. Other field and microplot tests with the same formulation applied to 'Marketmore '76' cucumbers [*Cucumis sativus*] and 'Conqueror III' squash [*Cucurbita pepo*] resulted in PPN control and increased growth and yield. The data strongly suggest that VERDISOL and similar formulations, can be developed as a soil improvement products that provide plant nutrients with broad spectrum pest control properties. All ingredients used in the formulations are natural and should not require stringent USDA/US EPA registration.

## **Economic optimization of glycerine based compositions for control of weeds, phytopathogenic nematodes and other soilborne plant pests**

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Greenhouse and microplot experiments at Auburn University indicate that a number of formulations based on partially purified and neutralized crude glycerin can be prepared economically and used to suppress a variety of plant pathogenic nematodes [PPN], other soilborne pests, and weeds. These formulations also promote beneficial nematodes and deliver significant plant nutrients. A wide range of formulations appears cost effective compared to soil fumigants typically used in turf and high value fruit and vegetable production (chloropicrin, dichloropropene & metam sodium), but various formulations appear to differentially impact different pest species or plant nutrients. Likely pest infestations, the economic importance of pests and beneficials, and plant nutrient needs vary substantially by crop, location, and time. Formulations can be economically optimized based on crop, soil, time of application, and history of each pest species.

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## Management zone development in cotton against concomitant infestation with *Meloidogyne incognita* and *Rotylenchulus reniformis* for the site-specific application of nematicides

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Both reniform nematode (*Rotylenchulus reniformis*) and Southern root-knot nematode (*Meloidogyne incognita*) are major pest problems of cotton production in Louisiana. These two nematodes did not usually occur together in cotton fields in the past (<5% of the time) and one species usually dominated when present together. Recently, more fields are now showing up with serious problems from both species in the same field. Crop rotation with corn during the past decade is suspected as the likely contributor to this problem. A test field was identified in 2007 that had serious problems with both nematodes. The greatest damage from Southern root-knot and reniform nematodes together was found in the lightest soil texture of the field (Bruin silt loam considered coarse-silty). Reniform nematode became the dominant species as soil texture changed to a heavier soil texture (Commerce silt loam considered fine-silty). Apparent electrical conductivity was used to precisely define the various soil zones within the field. Verification strips (treated and untreated rows with 1,3-dichloropropene at 28 L/ha) were applied in 2009 and 2010. Although the greatest damage occurs in the coarser soils where both species are present, reniform nematode caused severe damage in zones having apparent electrical conductivity (ECa) deep reading of up to 75 mS/m. The presence of reniform nematode greatly changes the dynamics of management zones requiring treatment of soils with much ECa readings than have been utilized for root-knot nematode.

## Assessment of molecular tests for identification purpose of tropical *Meloidogyne* species

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In the context of intensive worldwide trade with possible exchange of nematode populations and restricted management options of pests, reliable identification of introduced pest is challenging. With climate change and global warming, the establishment of tropical nematodes, and especially *Meloidogyne* genus, would be possible in unexpected areas, thus the necessity arose to evaluate the performance of molecular identification tools for tropical *Meloidogyne* species. Seven different PCR or PCR RFLP tests were evaluated according to consensus methodology (i.e EPPO PM 7/98). The target species considered were *M. arenaria*, *M. incognita*, *M. javanica* and *M. enterolobii*. The tests were evaluated against more than 40 genus and species of plant parasitic nematodes. Most of the tests were highly specific for their target species, able to detect their different populations available. Few tests showed cross reactions with non target genus or species. The sensitivity of tests varied between one individual to 10 individuals. Continuous evaluation of these tests against new populations or tropical *Meloidogyne* species should be undertaken as to guarantee their reliability. Such validated tests are necessary especially in the context of survey of possible new pests' emergence.

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## The importance of *Meloidogyne ethiopica* Whitehead in Europe

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Root-knot nematode *Meloidogyne ethiopica* Whitehead was discovered for the first time in Europe infecting tomato plants in Slovenia in 2003. Species was isolated from heavily infested plants showing clear above ground symptoms of stunting, wilting, early flower and fruit drop. *Meloidogyne ethiopica* is a polyphagous pathogen, which can parasitize at least 80 different woody and herbaceous host plants belonging to dicotyledonous as well as monocotyledons, including many economically important agricultural crops. It was reported to be the major root-knot nematode pest of grapevine, kiwi and other crops in Chile, already causing crop damage and economic losses. In a three-year outdoor experiment carried out in Slovenia proved that *M. ethiopica* is able to survive open field winter conditions in continental and sub-Mediterranean climates despite several instances of temperatures falling below freezing level. This study indicates that *M. ethiopica* may establish and spread in the southern and central parts of the EPPO region, therefore, it was recently listed on the EPPO Alert list of harmful organisms and a pest risk analysis (PRA) is planned. This nematode species would be difficult to control because of its polyphagous nature and lack of resistance in several cultivated plant species. However, studies have showed that *Mi-1* gene has significant effect on *M. ethiopica* reproduction, suggesting that the use of resistant tomato cultivars could be an appropriate control measure when used properly.



## The importance and distribution of *Meloidogyne ethiopica* in Brazil and Chile: diversity of isolates from these countries

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*Meloidogyne ethiopica* is a root-knot nematode which causes serious economic damage to grape-vines in Chile. Although this species occurs infrequently only on kiwi and other crops in Brazil, it can spread throughout the country and has the potential to become a damaging parasite of important agricultural commercial crops. The objective of this study was to evaluate the genetic variability of *M. ethiopica* using AFLP and RAPD analyses. Fifteen isolates were obtained from different geographic regions and/or hosts in Brazil and Chile. For all isolates, the isoenzyme analysis revealed the typical pattern of esterase E3 (Rm: 0.9, 1.05, 1.20) for *M. ethiopica*. Three isolates of *Meloidogyne* sp. showing an atypical esterase phenotype L3 (Rm: 1.05, 1.1, 1.25) were included in the DNA analyses. The phylogenetic tree obtained for both types of marker showed a high level of homogeneity among the *M. ethiopica* isolates analyzed, which clustered together in the tree (82% of bootstrap support). The three *Meloidogyne* sp. isolates also showed a high homogeneity and clustered separately from *M. ethiopica* (100% of bootstrap support). These molecular data indicate close genetic relationships between *M. ethiopica* isolates, regardless of their different geographical and/or host plants origins.

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**Distribution, hosts and identification of *Meloidogyne partityla* in the USA**

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Pecan, *Carya illinoensis*, is an economically important nut crop and member of the Juglandaceae native to the southern USA. Discovered in South Africa in 1986, *Meloidogyne partityla* was first found infecting pecan in USA in 1996 and currently occurs in Texas, New Mexico, Georgia, Arizona, Oklahoma and Florida. Above-ground symptoms of *M. partityla* infection in commercial pecan orchards include dieback of young branches, stunted growth, mouse-eared leaves due to nickel deficiency, and gradual tree decline have been reported in mature orchards in Texas, New Mexico, Georgia, Arizona and/or Oklahoma. Below-ground symptoms include root galling associated with egg masses. This nematode has a narrow host range which includes pecan, hickory (*C. ovata*) and walnut (*Juglans hindsii* and *J. regia*). More recently, this nematode was also found infecting laurel oak (*Quercus laurifolia*) in Florida. However, in the field conditions in the USA *M. partityla* has only been found infecting pecan. Phylogenetic studies using nuclear and mitochondrial DNA from seven geographically distinct *M. partityla* populations from all infested states have shown the species to be most similar to *M. hapla* when compared to 19 other *Meloidogyne* species. Isozyme analysis of individual female nematodes, especially esterase phenotype (Mp3), provides a fast and reliable diagnosis of the pecan root-knot nematode. A species-specific PCR primer set has also been developed for rapid DNA-based diagnosis of all life stages. Additionally, morphological features of high diagnostic values include stylet knobs of the males and females deeply grooved longitudinally and the presence of a swollen rectum and deeply grooved in the second-stage juveniles.

## A new species of root-knot nematode parasitizing different crops in Brazil, Chile and Iran

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A new root-knot nematode parasitising vegetables, flowering ornamentals and fruit trees in Brazil, Chile and Iran, is described. The mature female has an oval to squarish perineal pattern with a low to moderately high dorsal arc, and no shoulders. The female stylet is robust and 15.0-16.0  $\mu\text{m}$  long, the distance from the dorsal oesophageal gland orifice to the stylet base (DGO) is 3.0-4.0  $\mu\text{m}$ . Males have a high, round head cap continuous with the body contour. The labial disc is fused with the medial lips to form an elongated lip structure. The head region is not marked by incomplete annulations. The male stylet is robust, 20.8-23.0  $\mu\text{m}$  long and has round knobs; the DGO is 2.5-4.5  $\mu\text{m}$ . The stylet length of second-stage juveniles (J2) is 12.0-13.5  $\mu\text{m}$  long and the DGO is 2.3-3.3  $\mu\text{m}$ . The J2 tail is conoid with round terminus and 40.0-48.5  $\mu\text{m}$  long. Biochemically, the esterase phenotype L3 (Rm: 1.05, 1.10, 1.25) is unique and is the most useful enzymatic character to differentiate this new species from all other species. Reproduction is by mitotic parthenogenesis,  $2n = 42-46$  chromosomes.

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**EUPHRESCO *Meloidogyne* project: a fruitful way of knowledge transfer**

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Both root-knot nematode species *Meloidogyne chitwoodi* and *M. fallax* are quarantine organisms in Europe. These nematode species have a very broad host range, high reproduction rate, can damage the host plants considerably, and are difficult to manage because of these broad host range and high reproduction rate combined with the lack of resistant cultivars. Awareness of these nematode species is of utmost importance, and finding out whether the nematode is present in a certain area or country is the first step towards a concerted management plan. Gathering information and combining knowledge on these nematodes with the aim of eradicating or controlling them is the second step. The aim of the EUPHRESCO project is to coordinate ongoing and future research and to validate and train new methods in a collaborative way. This umbrella project contains 5 elements: 1. ring testing of nematode extraction processes from soils, 2. ring testing molecular identification/detection methods, 3. organizing a workshop on sampling, detection, identification and management of *Meloidogyne*, 4. putting together a European *Meloidogyne* research agenda by gathering information from all joining countries, identifying gaps in the research and make proposals (calls) for filling in those gaps, and 5. gather information on treatments of waste (e.g. waste products of potato/vegetable processing industry) contaminated by nematodes in each joining country to prevent dissemination in the future. The first 3 topics were already carried out. An overview of their results will be given.

## Life cycle and damage of the root-knot nematode *Meloidogyne minor* on potato, *Solanum tuberosum* cv. Bintje

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*Meloidogyne minor* is a recently described root-knot nematode reported in Belgium, Ireland, the Netherlands and the United Kingdom. It was found in sport fields and golf courses where it causes the yellow patch disease. However, *M. minor* was also detected in potato fields in the Netherlands and the UK and may pose a threat for potato culture. The objective of this study was to examine the life cycle and damage of *M. minor* on potato cv. Bintje under controlled conditions. To assess the life cycle, the developmental stages were examined at weekly intervals after inoculation of young potato plants with freshly hatched second-stage juveniles (J2). One week after inoculation, only vermiform juveniles were found in the roots. After two weeks, swollen juveniles were detected but vermiform juveniles were still present. All juveniles were swollen after three weeks and the first adult females were observed. Four and five weeks after inoculation, a small proportion of swollen juveniles was still present, suggesting a longer life cycle than other *Meloidogyne* species. Damage development of *M. minor* on potato was examined in a pot experiment with inoculation densities of 1, 2, 5, 10, 50, 100 and 250 J2 per 100 cm<sup>3</sup> soil. Three months after inoculation, there was no difference in the number of newly formed tubers. At initial densities of 10 J2 per 100 cm<sup>3</sup> soil and more, tubers with root-knots were detected. The highest number of tubers with knots was detected at densities of 100 and 250 J2. At these densities, severely damaged potato tubers were observed. The results of this study shows that *M. minor* is able to develop on potato and cause severe damage at initial population densities of 100 J2 per 100 cm<sup>3</sup> soil. Therefore, further spread of this nematode in agricultural fields should be avoided.

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## Assessment of three PCR assays for *Meloidogyne chitwoodi* and *M. fallax* identification and specific reactions for *M. artiellia*, *M. minor* and *M. naasi*

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*Meloidogyne chitwoodi* and *M. fallax* are two root-knot nematode species causing important damage on many agricultural crops such as potatoes, carrots and tomatoes. They have already been detected in several regions worldwide and are both regulated in Europe so as to limit their dispersal over non-affected areas. Juveniles, females and males can be found in soil or roots, alone or in mixture with other root-knot species. Reliable identification of *M. chitwoodi* and *M. fallax* is highly important especially for their management and monitoring in cultivated fields. As several *Meloidogyne* species are morphologically similar, several published PCR tests allow their differentiation. This study consisted in evaluating performance criteria of three PCR tests recommended in the European consensus diagnostic protocol EPPO – PM7/41 (2). Evaluation has been carried out with DNA solutions from juveniles, males and also females. Sensitivity, specificity, repeatability and reproducibility have been determined according to EPPO recommendations (PM7/98). It was found that some PCR tests are highly specific (with regard to other nematodes species or genus) and detect only one juvenile alone or in mixture. Unpublished specific PCR products and RFLP profiles have also been obtained during this study for *M. artiellia*, *M. minor* and *M. naasi*.

## Widespread prevalence of root-knot nematodes in banana plantations in Fiji

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A survey of 110 semi-commercial and commercial banana farms from three different provinces in Viti Levu, Fiji revealed the widespread occurrence of root-knot nematodes (RKN), *Meloidogyne* species. Six varieties of banana were found cultivated on these farms and occasionally a variety of plantain within a farm. The incidence of root-knot nematodes was determined using soil, direct examination of roots, and bioassays using a susceptible host plant, tomato. Second-stage juveniles (J2) were found in varying numbers in all soil samples. Root samples were examined from a total of 76 farms. Prominent root galling was observed in approximately 50% of the samples, however, J2 and females were found inside the remaining root samples that did not show clear root symptoms. The results from bioassay tests showed that 100% of the farms surveyed were infested with *Meloidogyne* spp. All bioassay plants were infected with root-knot nematodes, with gall index ranging from 1-5. A relatively low gall index (1-3) was recorded in less than 20% of the bioassay plants. The highest incidence of root-knot nematodes was observed in the Naitasiri province, with approximately 57 % of the 30 surveyed farms infested. Even the recently released high yielding FHIA variety that is considered to be generally disease resistant was found to have prominent root galling. Common weeds such as *Ageratum conyzoides*, *Mikania micrantha*, and sedges that are potential reservoirs for RKN were found growing in the surveyed farms. Most banana plantations were intercropped with roots crops such as dalo, dalo-ni-tana, cassava, chillies, eggplant and other cash crops that can serve as alternative hosts for RKN. Such widespread occurrence has implications on banana yield and quality that needs thorough investigation.

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## Small lipophilic molecules of tomato and rice affected the behaviour of root-knot nematodes

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Plant chemicals in the rhizosphere originating from root exudates, or sites of previous nematode penetration, can influence nematode behaviour and a number of plant compounds. Some chemicals, present in root exudates, have been shown to either attract nematodes to the roots or result in repellence, motility inhibition or even death. Our data show that nematodes take the most direct route to their preferred hosts but often take the longest route towards poor hosts. We hypothesise that a complex of repellent and attractant chemicals influences the interaction between nematodes and their hosts. The present work was conducted to isolate small lipophilic molecules (SLMs) emitted by root exudates of *Solanum lycopersicum* and *Oryza sativa* to investigate their effect on root-knot nematodes (RKN). SLMs were extracted through solid phase extraction (SPE) from hydroponically collected root exudates of 40 tomato and rice plants, their chemical nature elucidated through GC-MS analysis. SLMs had an inhibitory impact on the motility of *Meloidogyne incognita* and *M. graminicola* J2 and showed a nematotoxic, or nematostatic (upon dilution) effect on both species. The semiochemicals present in the SLMs do not stimulate nematode stylet thrusting but a small amount of salivary secretion was observed around the stylet tip. A significant decrease of nematode head movement was observed and they negatively influenced behaviour of these RKN by strongly affecting their mobility. Therefore, it is proposed that SLMs present in both tomato and rice root exudates play important roles during the interaction of RKN with their host plant, and that they might exert a repellent, or allelopathic effect on these nematodes.



## **Sterile *Arabidopsis thaliana* root exudates induce changes in root-knot nematode behaviour and gene expression**

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The root-knot nematode *Meloidogyne incognita* is an obligate parasite that can infect up to 1700 plant species. These nematodes penetrate the root at the elongation zone and then migrate to the zone of differentiation where they establish their feeding site. Although physiological and molecular changes inside the root leading to the feeding site formation have been widely studied, very little is known about the molecular events preceding root penetration by nematodes. Root exudates have been for some time suspected to trigger nematode infective behaviour, especially by inducing stylet thrusting and secretions, and tomato root exudates have been previously used to study root-knot nematode behaviour. In order to investigate the influence of root exudates on nematode behaviour before plant invasion, sterile root exudates from the model plant *Arabidopsis thaliana* have been produced and used to treat pre-parasitic second-stage juveniles. Here we will present data showing how *Arabidopsis* root exudates (ARE) are able to induce *M. incognita* stylet thrusting and changes in nematode gene expression. Using a cDNA-AFLP approach, the transcriptome of water- and ARE-treated nematodes were compared. Preliminary results suggest that ARE are able to regulate the expression of genes coding for putative avirulence proteins as well as proteins involved in nematode metabolism. In addition, ARE has been fractionated using reverse phase-HPLC and ion exchange columns, and the fractions tested on nematode stylet thrusting in an attempt to identify compounds in the ARE that induce nematode infective behaviour.

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## A mode for *in planta* localization of *Meloidogyne incognita* secreted proteins

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The fine localization of a set of *Meloidogyne incognita* candidate effectors secreted *in planta* and throughout parasitism produced from diverse secretory tissues will be presented. So far, *in situ* localization of proteins within secretory glands and functional predictions was the main method applied for identification of candidate effectors. The actual validation by localization of these candidate effectors secreted in planta brings us a step further. The same technique was adapted and applied to localize proteins within juvenile and parasitic nematodes. The method presented here has a major advantage since it preserves tissues from the plant host as well as the pathogen. Moreover, it can be useful to validate candidate effectors of other pathogens secreted during parasitism within their hosts. In order to better comprehend the roles throughout parasitism, we analyzed the secretion of CBM2-bearing proteins and pectate lyases during migration, giant cell formation and nematode development. We also analyzed the spatio-temporal secretion of a new aspartyl protease-like effector produced in the subventral oesophageal glands, and MAP-1 produced within the amphids. Lastly, we localized the effector 6D4 that is profusely produced by the dorsal glands throughout infection. Overall, our study showed the apoplasm as a significant destination compartment for nematode effectors during migration and feeding cell formation.

## Molecular and functional analysis of hormonal and other plant defense pathways in rice-nematode interactions

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Complex defence signaling pathways, controlled by different hormones, are known to be involved in the reaction of plants to a wide range of biotic and abiotic stress factors. We have studied the ability of salicylic acid (SA), jasmonate (JA) and ethylene (ET) to induce systemic defense in rice (*Oryza sativa*) against the root knot nematode (RKN) *Meloidogyne graminicola*. Exogenous ET (ethephon) and JA (MeJA) supplied on the shoots induced a strong systemic defense response in the roots, exemplified by a major upregulation of OsPR1a and OsPR1b, while the SA-analogue BTH was a less potent systemic defense inducer from shoot to root. Experiments with *JA-biosynthesis* mutants and ET-insensitive transgenics showed that ET induced defense requires an intact JA-pathway while JA-induced defense was still functional when ET-signaling is impaired. Similar experiments are currently being performed with the migratory root rot nematode *Hirschmanniella oryzae*. To get a more comprehensive overview of the plant response to nematode infection, RNA sequencing was performed on rice after nematode infection. Local infected tissue and systemic tissue after infection (3 and 7 dpi) by *M. graminicola* or *H. oryzae* were compared with control tissue of the same developmental stage. Analysis of the obtained data is currently ongoing and results will be presented at the conference.

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## Early host relationship of *Meloidogyne incognita* infecting resistant and susceptible alfalfa cultivars

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Plant parasitic nematodes cause billions of dollars in annual crop losses. One of the most damaging is the root-knot nematode, *Meloidogyne incognita* which is known to attack more than 3000 plants. This research will contribute to the understanding of host plant resistance through characterization of the early infection processes of *M. incognita* race 3 in susceptible and resistant alfalfa cultivars by light microscopy and transmission electron microscopy. Neither differential penetration of *M. incognita* J2 into Lahontan, nor migration of J2 from Moapa, played a significant role in the resistance mechanism(s). Coiled nematodes in the cortex were observed in greater numbers in the Moapa 48 hours after inoculation. This was interpreted as a sign of disorientation and starvation. By 96 hours after inoculation, no coiled nematodes were observed in Lahontan. In Moapa, resistance probably depends not only on the failure of the J2 to identify a suitable feeding site and initiate giant cells, but also on its inability to maintain the giant cells, once they are initiated. At the ultrastructural level, 48 and 96 hours after inoculation, the most evident change in both cultivars was the appearance of a uniform interstitial material (IM) between the nematode cuticle and the root cell wall. Due to clear differences between both cultivars I propose that the IM plays a role in the resistance of Moapa to *M. incognita*.



**Do cover crops modify the structure of soil nematodes under banana fields?**

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New sustainable cropping systems using cover crops in banana field are developing rapidly in Martinique. To assess changes in nematodes community, we compared the association of banana plant with grass (*Paspalum notatum*), different Fabaceae (*Pueraria Phaseoloides*, *Neonotonia wightii* and *Stylosanthes guyanensis*), or spontaneous grassing with banana agroecosystem without cover crop (bare soil) in 2-years experiment. All nematodes extracted from soil were identified at genus or family level and classified in six trophic groups: bacterivores, fungivore, plant-parasitic, root-hair feeding, omnivore and carnivore. Plant Parasitic Index (PPI) was calculated and soil food web was analyzed using the Structure and Enrichment Indices. Plant-parasitic nematodes that numerically dominate the nematodes community do not change significantly according to cover crop system. However PPI was significantly lower in grass cover than in others systems. Bacterivores nematodes were more abundant in cover crop systems than on bare soil, indicating a higher biological activity. Omnivores were favored by *P. notatum* cover, while Fabaceae covers promote the carnivores group. Nematode faunal analysis showed that soil food webs of all system were structured; however, cropping system using cover crops have a higher primary productivity compared to bare soil. These results show that if the use of cover crops of Poaceae and Fabaceae under banana fields can modify the structure of nematode community, it did not change the maturity of soil food web 2 years after. They indicate that soil nematodes could be useful indicators of sustainability of cultural practices and therefore could be used to monitor new banana cropping system in Martinique.

## Distribution of plant parasitic nematodes on banana in Fiji

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Bananas are a significant crop for Fiji's subsistence and commercial growers. Banana farms were sampled in three provinces on the main island, Viti Levu of Fiji Islands to determine the nematode prevalence and diversity. Soil and banana root tissues were processed to study the nematode associations. Commonly cultivated varieties were Cavendish dwarf, Cavendish tall, Veimama, and Blue java while Lady's finger, Pacific plantain and FHIA were less common. *Meloidogyne*, *Helicotylenchus*, *Radopholus* and *Pratylenchus* were the most common genera encountered in that order. In Naitasiri province, *Radopholus* and *Meloidogyne* coexisted in all farms but *Radopholus* was the predominant nematode generally. *Helicotylenchus* and *Meloidogyne* were predominant in Tailevu and Nadroga provinces while no *Radopholus* was found on Nadroga farms. About 52% of the 44 root tissue samples from Nadroga and Naitasiri had high incidence of *Helicotylenchus* and low of *Meloidogyne*, however, ~16 % had high incidence of both *Meloidogyne* and *Helicotylenchus*. High percentage of toppling was observed in Nadroga where *Helicotylenchus* was very common, however correlation is yet to be established. Half of the 56 farms surveyed here showed toppling. Necrosis of roots was observed where *Radopholus* and *Helicotylenchus* incidence was high. Impact of these nematodes on banana yield and quality needs to be investigated further.

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## Diversity and connectance of soil food web channels along environmental gradients in an agricultural landscape

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Soil food webs respond to environmental variables and gradients. We studied abundance, connectance, and diversity in three different channels of the soil food web in which nematodes were involved: the microbivore channel (microbes and their nematode grazers), the plant-herbivore channel (plants and plant-feeding nematodes), and the predator-prey channel (predatory nematodes and their nematode prey) in intensively cropped soils and in grazed non-irrigated grasslands. Besides, samples were taken at three positions in relation to water channels across the landscape. Nematode communities, phospholipid fatty acid (PLFA) biomarkers, and soil properties were measured, riparian health ratings were scored, and macro-environmental variables were recorded. The abundance and richness in most food web components were higher in grazed grasslands than in intensive agricultural fields. The association between richness and abundance for each component was strongest for the lowest trophic links and weakest for the highest. The trophic interactions for the predator-prey and plant-herbivore channels were greater in the grassland than in the cropland. Fields for crops or grazing supported more interactions than the water's edge in the plant-herbivore and microbivore channels. Connectance increased with the total richness of each community. Higher connectance within the microbivore and predator-prey soil food web channels were associated with soil NO<sub>3</sub>-N and elevation respectively, which served as surrogate indicators of high and low agricultural intensification.



## Entomopathogenic nematodes in soil food webs: bioassays, molecular methods and changing perspectives

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Citrus orchards in Florida support rich, abundant communities of entomopathogenic nematodes (EPNs). There is evidence that native EPNs regulate a citrus root weevil *Diaprepes abbreviatus* to greater or lesser degrees in different soils with the result that the insect is a minor pest in some regions, while causing serious economic loss in others. Weevil larvae reside in the soil for several months and suffer EPN predation at rates as high as 70-80% per week; thus, small differences in predation should have large effects on weevil population size. Discovery of abiotic and biotic factors that affect either the population size or predation efficacy of EPNs could reveal ways to modify soil and profitably increase biological control. We used real-time PCR (qPCR) and a suite of species-specific primers and probes to directly measure EPNs and their natural enemies in monthly samples of nematodes from soil during two years. The abundance of *Paenibacillus* sp., a bacterial ectoparasite specific to *Steinernema diaprepesi*, was directly related to that of the nematode. However, *Paenibacillus* sp. abundance was inversely related to population growth of *S. diaprepesi* and explained significant variation in the population changes of that nematode for up to four months. Daily fertigation of trees in a field experiment increased soil pH to 7.2 (from 5.6 in conventionally irrigated plots) and increased the abundance of *Paenibacillus* sp., while reducing that of *S. diaprepesi*. Laboratory experiments confirmed that *Paenibacillus* sp. spores detach from *S. diaprepesi* increasingly at pH below 7.0. Thus, pH modification might be a means of conserving an important native entomopathogen in Florida orchards.

## The evolutive persistence of virus transmission: why introduce an extra parasite?

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Transmission mode is a key factor in the evolution of viruses and other pathogens. Nematode transmission of plant viruses, however, raises a question related to the vector evolutive advantage provided by such a strategy. Transmitted viruses reduce the health of infected plants, in fact rendering them less suitable for feeding. Differing from insect vectors, Longidoridae and Trichodoridae are not alternative hosts for the Nepovirus and Tobravirus they, respectively, transmit since these viruses multiply only inside the infected plants. On the virus side, the evolutive advantage of a vector-based transmission appears mainly related to constraints imposed by vectors on the rates of RNA mutation. As other arthropod transmitted viruses, nematode transmitted viruses also have RNA genomes, whose rates of nucleotide mutation are significantly higher than that of DNA. The mechanisms of adhesion/adsorption, which are also involved in the virus spreading within the host, may allow only a low number of suitable RNA substitutions in the coat proteins sequences for the virus to remain transmissible. The adaptive bottleneck of this constraint is considered to act as a genetic selective filter, thus stabilizing the virus genome. But the other side of the question still remains: given the damage induced by transmitted viruses on the host plants, why didn't evolution produce a negative selection on vectoring nematodes, favouring non-vectoring individuals feeding on healthy plants? One possible hypothesis is that such an evolutive puzzle may hinder, on the nematode side, cryptic but profitable changes induced by transmission, thus improving feeding fitness and/or balancing the plant damage and losses due to viral infection.

## Insights from *Pasteuria* endospore attachment and infection

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A collaboration between the United Kingdom and India has looked at A) *Pasteuria* infection between *Heterodera* and *Globodera* spp., and B) the role of mucins on the nematode cuticle of *Meloidogyne* spp. A) *Pasteuria* isolated from the pigeon pea cyst nematode, *H. cajani*, from India multiplied equally well on the potato cyst nematode *G. pallida* from the UK. Although the number of spores adhering to second-stage juveniles (J2) showed no statistical difference between species, the orientation of endospore attachment differed; unusually endospores attached to the cuticle by their convex surface. The inverted endospores germinating on the juveniles of *H. cajani* were significantly fewer in number (13%) than those germinating on the juveniles of *G. pallida* (22%). Both the inverted and the conventionally attached endospores produced bacillus-like rods that completed their life cycle on *G. pallida*. The endospore size and a 16S rRNA gene sequence of HcP suggested phylogenetic similarity to *P. nishizawae*. B) Mucins belong to a family of glycosylated proteins are present in the cuticle surface coat. Mucin-like proteins have also been identified in *Caenorhabditis elegans* and RNAi knockdown of these genes has been associated with changes of lectin staining of the cuticle surface. Orthologs to mucin-like genes from *C. elegans* were identified in *M. incognita* amplified, cloned, sequenced and dsRNA synthesised by *in vitro* transcription. RNAi knockdown was performed by oral feeding, and *Pasteuria* endospore attachment assays performed to test the hypothesis that mucin-like genes of the cuticle surface of infective juveniles have a role in endospore attachment. The results will be discussed in the context of multitrophic interactions in the rhizosphere.

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## ***Pochonia chlamydosporia* as a biocontrol agent of nematodes – research progress, challenges and future prospects**

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Over the last few decades, the fungus *Pochonia chlamydosporia* has been extensively studied to assess its potential as biocontrol agent of plant parasitic nematodes such as *Heterodera*, *Globodera*, *Meloidogyne*, *Nacobbus* and *Rotylenchulus*. The efficacy of selected isolates has been demonstrated in several studies however, according to some reports, control was not achieved consistently. As a facultative parasite, the life cycle of *P. chlamydosporia* includes saprophytic and parasitic stages. Recent studies suggest that changes in the trophic phase may be related to nutrients that are either released by plants or available in nematode eggs. The gene expression of VCP1, a serine protease known to be involved in parasitism, was shown to increase in culture medium where nematode eggs were added and to be repressed in presence of certain nutrients, such as glucose. In a different study, VCP1 was expressed in roots of plants colonised endophytically by *P. chlamydosporia* in absence of a nematode host, thus suggesting that proteolytic enzymes may also play a role in protecting plants from nematode attack. Control of abiotic and biotic stress factors may be only a subset of fungal activities that benefit plants. Although the saprophytic behaviour of the fungus is seen as an advantage for establishment and survival in absence of nematodes and plant, it can also become an issue if the cues triggering the saprophyte to parasite trophic “switch” are not well understood. Unravelling the factors affecting this “switch” is therefore a key challenge for the successful development of the fungus as a biocontrol agent. The importance of these findings for future research will be discussed.

## Evaluation of biological alternatives for the control of *Nacobbus aberrans* populations

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In the last years there has been increasing interest in biological alternatives for the management of plant-parasitic nematodes that are harmful to agriculture. *Nacobbus aberrans* is a sedentary endoparasite native to the Americas, characterized by generating galls in the roots of the parasitized plant. In Argentina, it is widely distributed and the host range comprises numerous weeds and crops. To date, no biological control strategies have been implemented in the country to tackle the problems generated by this nematode, especially in horticultural crops. We have recently initiated research works in tomato plants grown under greenhouse conditions to evaluate the use of: a) arbuscular endomycorrhizas (*Glomus intraradices*), b) *Pseudomonas fluorescens* (CHA0 strain), and c) juveniles of entomopathogenic nematodes (Families Steinernematidae and Heterorhabditidae), as potential agents of control of local *N. aberrans* populations. The results obtained show that the application of the organisms mentioned significantly reduced multiplication of the nematode on plants (about 40-60%). These biological alternatives might represent a very good tool for the management of *N. aberrans*, mainly in crops grown under greenhouse conditions.

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## Top-down control in a natural ecosystem: interactions between plant-parasitic nematodes and their microbial enemies

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Large populations of plant-parasitic nematodes cause the die-back of marram grass, the pioneer plant species in primary succession in coastal sand dunes. These ecosystems have received much attention in the search for 'natural' nematode control mechanisms that could be relevant to nematode management in agriculture. Our aim was to investigate the potential top-down control processes of plant-parasitic nematodes in coastal sand dunes. A range of sand dunes across Europe was sampled seasonally and data were collected on the diversity as well as spatial and temporal variations of both the nematode and their microbial enemy communities. We identified microbial enemy traits that contribute to control success in sand dunes. Obligate parasitism was favoured in these systems, and the more frequent/abundant microbial enemies were either able to parasitise different hosts or they had the ability to form an environmentally resistant structure. A functional quantitative relationship was found between *Pasteuria penetrans* and the abundance of the most aggressive nematodes, *Meloidogyne* spp. Furthermore, the effects of *P. penetrans* on *Meloidogyne* spp. acted through the food-web to indirectly facilitate the most abundant *Pratylenchus* spp. Overall, we found a highly diverse and dynamic nematode and microbial enemy community, where top-down control mechanisms take place, producing direct and indirect effects on key plant-parasitic nematode populations.

## The use of *in vitro* produced *Pasteuria* spp. for the control of *Meloidogyne* spp.

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Innovations within the last decade have allowed for practical and economical in-vitro production of *Pasteuria* spp. spores for biological control of plant parasitic nematodes. A product utilizing *Pasteuria* spp. known to parasitize *Belonolaimus longicaudatus* has received USA EPA registration and has been commercially available for use on turfgrass in the USA since January 2010. Furthermore, EPA has allowed an exemption from the requirement of a tolerance for residues when applied pre-harvest, supporting the use and safety of *Pasteuria*-based products in food production. Root-knot nematodes, *Meloidogyne* spp., continue to be a major pest in food production globally. Preliminary greenhouse studies using *Meloidogyne* spp.-specific *Pasteuria* have demonstrated significant pesticidal efficacy. Subsequent greenhouse and field studies evaluating *Pasteuria* applied to cucumber and tomato crops are under evaluation. Further development and registration of *Pasteuria*-based products will offer growers novel biological products for *Meloidogyne* spp. control.

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## In vitro nematocidal activity on *Xiphinema index* of rhizobacteria isolated from grapevine soil

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*Xiphinema index*, the most aggressive root parasite affecting vines in Chile, is controlled mainly chemically. In this study, rhizobacteria previously isolated from grapevine roots growing in soils with low density nematode populations, were evaluated to determine the effect of bacterial supernatants on nematodes collected from vineyards in naturally infested soils. Rhizobacteria (38 isolates) were cultivated on half-strength TSB (15 g/l), into Erlenmeyer flasks, with a cell suspension concentration adjusted to 10<sup>6</sup> CFU/ml. Approx. 50 *X. index* were placed into glass Petri plates along with 1.5 ml of culture supernatant. Plates were maintained at 22-23 °C and the number of dead nematodes was counted under a dissecting microscope at 16-18 h. Subsequently, dead nematodes were transferred to sterile tap water for 48 h, to observe whether recovery occurred. The experiment was performed twice and means were compared with Dunnet's test at P<0.05 and P<0.01. Results showed that on immersion of nematodes in the culture filtrate, most of the bacteria metabolites caused nematodes death. Mortality determined in the filtrates occurred with almost all bacterial isolates, as 30 and 35 isolates gave results that differed from the control (TSB) in test 1 and 2, respectively. The most nematocidal effects were produced by isolates of *Bacillus mycoides*, *Pseudomonas putida* and *Stenotrophomonas* sp. These rhizobacteria appeared to be able to suppress nematode populations and may be useful in biological control programmes in vineyards.



## Phylogeny, systematics and biogeography of the phylum Nematoda

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The systematics of the phylum Nematoda have been unstable for decades. Depending on the structure(s) that was / were considered to be of primary importance – the buccal morphology (Micoletzky, 1922), the phasmids (Chitwood and Chitwood, 1933), or e.g. the pharyngeal structure and excretory system (Maggenti, 1963) – fundamentally different phylogenetic relationships were proposed. Lorenzen (1981) concluded that the number of informative morphological characters is just too small for a robust classification. The main advantage of switching to DNA characters is related to sheer numbers; it is relatively straightforward to generate hundreds if not thousands of DNA characters from an individual nematode. Over the last ten years, we have seen a steep increase in the number of sequences generated for groups of nematodes. Depending on taxonomic focus, nuclear or mitochondrial genes have been used. The small subunit of the ribosomal DNA (SSU rDNA,  $\approx$  1,700 bp) appeared to be highly informative with regard to the overall topology of the phylum. Currently, the SSU rDNA database encompasses about 2,300 sequences covering a substantial part of the terrestrial and freshwater nematode biodiversity. Phylogenetic trees based on a single gene should be considered as gene trees, not as organismal trees. Fortunately, more and more other genes such as LSU rDNA, RNA polymerase II and the mitochondrial genes COI and COII are used to clarify nematode interrelationships. Hence, we are gradually moving towards a stable framework for nematodes, and the practical implications of this for areas such DNA barcode-based pathogen detection and soil ecology will be discussed.

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## The taxonomy of the long-tailed dorylaims

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The so called long-tailed dorylaims are members of the Dorylaimidae de Man, 1876, a rather homogeneous taxon of the Dorylaimoidea characterized by a good number of morphological characters, among them the presence of an elongated tail in females or in both sexes. Still, the classification of this family, as far as the taxa of lower rank are concerned, has undergone many changes in relatively few years. Grouping the genera of Dorylaimidae in subfamilies on the basis of synapomorphies is no easy task since their identities, due to the often incomplete descriptions of the old species and to the great morphological convergence of most characters, are mainly speculative. The subfamily identities are generally based on character combinations rather than on autapomorphies, combinations which are themselves often the result of subjective judgments. In some cases, even the identities of genera are difficult to define due to the fact that intraspecific variability is not well defined. An analysis of the diagnostic value of the main morphological characters of Dorylaimidae will be made and the most critical aspects will be pointed out. In this context, the identity of *Paradorylaimus* Andrassy, 1969, considered a junior synonym of *Laimydorus* Siddiqi, 1969 by Loof and Coomans (1986), will be discussed.

## Is a biogeography of freshwater nematodes possible?

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An attempt was made to detect some possible biogeographic patterns in the distribution of freshwater nematodes. The literature concerning 14 geographic areas (in Eurasia, Africa and America) and 8 well studied lakes, was examined, i.e. many papers published from 1905 to 2011. After some taxonomic corrections, a total of 1030 nominal species were listed: the richest area was Germany+Australia (301 valid freshwater nematode species) whereas the richest lake was Balaton (143 species). Notwithstanding the ubiquity of most freshwater nematodes, some distributional patterns emerge from a multidimensional analysis: e.g. the nematode communities of the European regions are more similar inter se in comparison with the other continents. Examining the lakes only, Baikal nematodes appear more different from the other lacustrine nematodes from other parts of the world. It is possible to conclude: (1) that freshwater nematode communities show some difference at the continental level only, and (2) that the freshwater nematode biota is divided into two parts with the Lake Baikal community on one side, and the rest of the world community on the other.

## Nematode composition and distribution on a south Atlantic region (Campos Basin, Brazil)

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In the Campos Basin, nematodes are used for ecological and taxonomic studies in continental margins, including submarine canyons, as part of a research program: "Spatial Heterogeneity of Campos Basin". The aim of this work is to characterize the nematofauna from the Campos Basin, identifying the relations between the main genera and the different environments, in two different seasons (winter and summer). In total, nine profiles and twelve isobaths were prospected, five isobaths being at the continental shelf (25m, 50m, 75m, 100m and 150m) and seven isobaths at the continental slope (400m, 700m, 1300m, 1000m, 1900m, 2500m and 3000m). Samples were taken using a Box corer modified to sample 0.25m<sup>2</sup> of the sediment. At each station, three replicates were examined. The nematofauna from different environments were composed of 309 genera. Genera richness and abundance varied according to depth and season. The SIMPER (Similarity Percentage) analysis showed that *Sabatieria*, *Pselionema*, *Thalassomonhystera*, *Desmoscolex*, *Acantolaimus*, *Desmodorella*, *Cervonema*, *Pomponema*, *Tricoma*, *Microlaimus* presented high individual contributions to the depth similarities. The nematode composition, richness and the correlations between nematodes and sedimentological variables are totally unknown for the Brazilian continental margin. Therefore, these results will contribute to the knowledge of the Brazilian biodiversity in the deep sea as well as to an increase in the biodiversity of this ecosystem as a whole.

## Free living nematodes as indicators of sediment quality at Bacalar Lake, Mexico

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In order to analyze distribution, abundance and nematode community structure at Lake Bacalar and to determine quality of sediments, samples were collected during May 2010 at 15 sampling stations using a 2 liter capacity Eckman grab. For identification, nematodes were mounted in glass slides in anhydrous glycerin. Abundance values were transformed ( $\log x + 1$ ) and used to calculate diversity indices, and the maturity index. The nematode community was represented by 31 species, belonging to six orders, 18 families and 27 genera. Chromadorida had the highest number of species (9), followed by Monhysterida (7) and Enoplida (6 species). The more abundant species in terms of number of organisms were: *Dichromadora* (105), *Daptonema* (100), *Noetobrilus* (97), *Microlaimus* (60) and *Ironus* (55). Species richness was higher at stations: S2, S4, S6, S14, S18 and S24, with high Simpson index values (3.96 to 4.6) and they were consistent with Shannon index ( $>3$  bits.ind<sup>-1</sup>). The Maturity index varied from 2.42 at S10, to 3.0 at S6. Cluster analysis showed three groups; the first characterized by sampling stations with more of 25 organisms. The second group of stations had abundances between 7 to 20 nematodes and the third group had low abundance stations. The species number was similar to those reported in other sub-tropical fresh water environments. The diversity index was high and, even when the maturity index was slightly low, the quality of sediment was good, and there were no pollution conditions at Lake Bacalar.

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## Contribution to the knowledge of the genus *Tripylina* (Nematoda: Enoplida)

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Three species of the genus *Tripylina* have been found in Mexico. One species was found around roots of sugar cane in Zacatepec, Morelos State, Mexico, a second species was collected from moss in San Pablo Isayo, and a third species from moss in the archaeological site at Tlaminca, both in Mexico State. The *Tripylina* species that was found in sugar cane has a slender body with a smooth cuticle; the mouth opening is shifted dorsally and the stomal cavity is dorsally thickened. The well developed outer labial setae, 13-20 ( $17\pm 1.0$ )  $\mu\text{m}$  long, are arranged in a single whorl with the thinner and smaller, 5-8 ( $6.8\pm 0.6$ )  $\mu\text{m}$  long, cephalic setae. There are two ventromedian setae in the cervical region in males that are absent in females. The oesophageal-intestinal junction has large cardiac glands with two gland-like bodies. Females have a post-uterine sac and slightly protruding vulval lips with two sclerotized plates. The *Tripylina* species from San Pablo Isayo does not have body pores or sclerotized pieces in the vagina. The small dorsal tooth is anterior to the subventral teeth. There is one cervical setae, and the cardia is 15-20 ( $17\pm 0.7$ )  $\mu\text{m}$  long and 28-35 ( $31\pm 0.8$ )  $\mu\text{m}$  wide. The *Tripylina* species from the archaeological site at Tlaminca, has outer labial setae 10-15 ( $12.5\pm 0.36$ )  $\mu\text{m}$  long, two cervical setae and the subventral teeth anterior to the dorsal tooth. The dorsal wall of the stomatal chamber is not sclerotized, the cardia is 7-2 ( $9.5\pm 0.3$ )  $\mu\text{m}$  long and 20-27 ( $24\pm 0.4$ )  $\mu\text{m}$  wide and with very small sclerotized pieces in the vulva.

## The pinewood nematode problem: current status of research in Europe

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a major threat to European forests, following its establishment in continental Portugal in 1999, and recent spread to Spain (2008) and Madeira Island (2009). Because of the direct damages caused to pine trees, along with environmental and social impacts, there is an urgent need to develop the most effective pest management strategies to deal both with established outbreaks and, particularly, for early detection and eradication of incipient infestations. In this context, the EU-funded collaborative research project REPHRAME (KBBE.2010.1.4-09) has been launched in 2011, with the objective to develop improved methods for detection, control and eradication of PWN in support of EU Plant Health policy. REPHRAME has a consortium of participants from 7 European countries, reflecting the EU-wide nature of the project and of the problem it addresses. It is expected that REPHRAME will deliver impacts of: i) improving the knowledge base on the critical aspects of pine wood nematode biology and its interactions with vectors and host trees; and ii) synthesizing this knowledge base into a user-friendly interface to enable a wide range of end-users to consult the data and customize solutions and advice. The talk will present an overview of the scientific topics covered by the consortium, with a focus on the molecular aspects of nematode detection and diversity. In particular, current research aimed at determining the risk of non-vector spread of PWN to healthy forests and molecular tracking of the invasion routes of the PWN in Europe will be presented.

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## Pinewood nematode *Bursaphelenchus xylophilus* under northern skies – forest resources, perspectives and strategies

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In the Nordic countries the total forest area is 67 million hectares, with conifers covering 38 million hectares. The Nordic standing crop of conifer wood is about 5 million m<sup>3</sup>, with an almost equal share of pine (*Pinus sylvestris*) and spruce (*Picea abies*). Because of this large and precious forest resource, the recent spread of pinewood nematode (PWN) *Bursaphelenchus xylophilus* in Europe is of great concern to Nordic countries. The expression of pine wilt disease requires summer mean temperatures above 20 °C, which is higher than normal for Scandinavia. However, in the area of the recent outbreak in Galicia, Spain, temperatures seem modest, with mean values for July and August at or just above 20 °C. Climate change may push the Nordic area into warmer conditions. Available models suggest that damage to Nordic forests is expected to be small in a 50 years perspective. Long-term models are scarce, but in view of pine tree production cycles of up to 120 years, mortality may occur more frequently within one or a few forest generations. Since PWN may exist in trees free of wilt symptoms, its distribution in Europe becomes unclear, and an introduction into the Nordic area may be imminent. Monitoring of the pest in Nordic forests, where symptoms are not expected, requires sampling of cutting waste with signs of activity from vector insect in the genus *Monochamus*. In the Nordic area more than 9000 samples have so far been analyzed from risk areas and risk commodities. A recent simulation of PWN spread in Norwegian forests indicates that 14 years may elapse before detection by the present level of 400 samples annually. We are convinced that large sampling volumes and strict import regulations are vital strategies for keeping the Nordic area free from PWN.



## Prevention, control and eradication of the pinewood nematode in Spain

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After the pinewood nematode (PWN) outbreak in Portugal (1999) and the declaration of most of the country as affected area (2008), Spain set up a special surveillance plan, early warning and control, in order to avoid its dispersal. A first outbreak of PWN was detected in Extremadura in October 2008, 35 km from the Portuguese border. A prompt Action Plan (AP) was implemented, based upon: eradication of the outbreak, intensive and continued surveillance of the surrounding area and systematic plus random monitoring at national level. The plan followed in the beginning the general directives about control and eradication of quarantine pests and the suggestions of EPPO. Now it has been adapted to the actual circumstances of the territory and to the new pest risk analysis developed within the framework of the Forest Quarantine Panel of EPPO and within the related Working Group of the European Commission. A second outbreak was detected in Galicia (November 2010), 5.6 km from the Portuguese border. The new implemented Action Plan focuses on targeted clear-cut, more surveillance and the use of new tools such as kairo-pheromonal vector baits, early detection of positives, and dynamic and intensive monitoring in sensitive areas. General strategy needs to be revised in order to find a good usefulness-cost balance. Eradication, Containment and Coexistence are the keywords in future scenarios.

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## Portuguese national action plan for pinewood nematode control: strategy, actions and results

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Since the detection in 1999 of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, in Portugal, in declining maritime pines, *Pinus pinaster*, a set of actions aiming to control and eradicate the PWN and to control its vector, *Monochamus galloprovincialis*, have been implemented and have been consubstantiated in the National Eradication Programme to Control the Pinewood Nematode (PROLUNP). Diverse strategic action plans were prepared and implemented focusing on diverse actions namely surveying, eradication, monitoring, insect-vector control and inspection and control of forest operations, particularly the ones related with coniferous trees forestry operations. Given the detection in 2008 of PWN in new pine forest areas, located in the Central Region, considered before as 'Free Zone', the continental territory surveying actions were intensified and the territorial coverage amplified. The results of this monitoring plan pointed to the presence of PWN in several municipalities. A new strategy was adopted and a new Action Plan was set up, in line with the EU emergency measures against the PWN, adapted to the characteristics of the new affected areas and taking into account the up to date related scientific knowledge to define adequate phytosanitary measures. The current scientific knowledge supports the current control strategy employed in Portugal. A brief overview of the results will be presented.

## Pine wilt disease in Portugal: control strategies

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The pinewood nematode (PWN) *Bursaphelenchus xylophilus* is the causal agent of pine wilt disease (PWD), a quarantine organism within Europe. This pathogen was detected in Portugal in 1999 associated with maritime pine (*Pinus pinaster*) and its vector, the native insect *Monochamus galloprovincialis*. Between 1999 and 2008 the nematode was restricted to the Setúbal Peninsula. Subsequently, new PWD foci were detected in Central Continental Portugal and Madeira Island. Over the years, management and control of both the nematode and the vector has been implemented. The management strategies are focused on three key points: (i) identification of affected areas based on periodic surveys in pine forest, during the autumn and winter periods, and on the collection of wood samples for PWN lab identification; (ii) prevention of wilt dispersal to new locations is based on mandatory regulations that make wood treatment mandatory when transported between infested and non-infested locations during the flight season of the vector, namely by procedures such as heat-treatment; other strategies, such as preventive trunk-injection with insecticides in healthy pines and an intensive monitoring system, are also being employed; (iii) reduction of PWD incidence in affected areas by harvesting of wilted trees and cut waste from November to April, when the insect vector is inactive. In the May-October period, an active complementary management approach against other declining agents such as bark beetles is also being implemented, consisting on the immediate cut of affected trees and the installation of a trap network baited with pheromones and host kairomones to monitor and diminish both the bark beetle and the *Monochamus* populations.

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**PURSUE®: Long lasting prevention of pine wilt disease****Rui Delgado**Corresponding author: [rui.delgado@syngenta.com](mailto:rui.delgado@syngenta.com)

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PURSUE® is a crop protection product based on the active substance emamectin benzoate, used as both insecticide and as nematicide to control different pests. In field trials carried out in Portugal, which started in March 2009, maritime pine (*Pinus pinaster*) treated by micro-injection with PURSUE® at three different rates, did not exhibit wilting disease caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, contrary to the control trees which were not treated with PURSUE®. Within these two-year trial periods, 21 non-treated trees died from PWN, which represents around 28% of the non-treated trees. Furthermore, analysis of emamectin benzoate carried out in pine tissues, collected from treated trees, successfully detected the substance in the branches of pines, the quantity of emamectin benzoate recovered increasing accordingly to the injection dose rate. The active substance recovered was well above the inhibitory concentration of  $IC_{95} = 0.031 \mu\text{g/g}$  needed for propagation inhibition of *B. xylophilus*. The effect of emamectin benzoate on the adult vector *Monochamus galloprovincialis* was also studied under laboratory trials. Emamectin showed a marked effect on longevity and feeding activity of the insects with reductions of 44% and 47% respectively at the recommended dose rate. Thus, PURSUE® showed a long last prevention of pine wilt disease during the two-year trial period.

## Composting for wood bark heat treatment to control the pinewood nematode

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The ISPM No. 15 standard, established by FAO/ IPPC, defines the general requirements for heat treatment of wood for elimination of pests of forest products. This standard establishes the temperature of 56 °C during a period of at least 30 minutes for wood treatment. Several studies have confirmed that during composting natural processes can develop temperatures that exceed the internationally established temperature throughout a bark heap. The first phase of the composting process (aerobic thermophilic activity) is characterized by natural heating and can be controlled to achieve temperatures (>60 °C) that lead to the elimination of the pinewood nematode (PWN). Therefore, the Portuguese authority for agriculture and forests protection (DGADR) has defined a procedure, "Technical Requirements for Heat Treatment of Isolated bark of Conifers", establishing the rules for the elimination of PWN and treatment of bark by composting. Each bark heap must be overturned three times and submitted to four heating cycles after a minimum period of six days. During treatment, the temperature is monitored by six temperature probes and should be 60 °C in all probes during six hours, except in the last treatment where the minimum duration is reduced to three hours. The required temperatures are easily achieved and in some zones rapidly exceed 85 °C while in others hardly reach 45 °C. Studies are being conducted to improve the turning and heating processes of bark heaps and to understand the role of other parameters such as bark moisture, air humidity, degree of compression and degree of mixture of fine and coarse particles. Results of the temperature profile in different compost heaps will be presented and discussed.

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## Socio-economic and environmental aspects of the pine wilt disease problem in Portugal

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is one of the most serious threats to pine forests worldwide. In 1999, the PWN was reported for the first time in Portugal and in Europe, south-east of Lisbon (Setúbal Peninsula), associated with dead trees of maritime pine, *Pinus pinaster*. Following detection, this nematode has spread quickly to the Central Region of Portugal and in 2008 all continental Portugal was declared as affected area. The PWN constitutes a serious problem for the forest economy such as annual loss of pine timber and consequent disturbance of forest industry, based on use of pinewood as a raw material. The total negative impact of the PWN and of pine wilt disease (PWD) is more complex and involves different socio-economic and environmental aspects. The objective of this research is to understand how anatomic changes of wood due to nematode attack and biochemical incidences of tree defense reaction affect the technological aptitude of PWN infected wood biomass and to analyze the interrelationship between economic criteria and disease control activities. A comparative analysis of the gross calorific value (GCV), ignitability, moisture content variation and chemical composition of infected wood samples and non-infected wood samples was performed. The GCV was determined using a Parr 6300 automatic isoperibol calorimeter, the ignitability by cone calorimeter and the moisture content using a climatic chamber. Some data which demonstrate economic and environmental impact of the PWN were assessed.

## Thermal imaging, a new approach to detect pine wilt disease in *Pinus pinaster*

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, forced the felling of thousands of *Pinus Pinaster* Ait since it was detected in 1999, in Portugal. It is urgent to find new methods to identify infected trees in the field as well as to increase our knowledge about environmental conditions that help pathogen distribution. In this study, we evaluated the suitability of thermal imaging (TI) for the early and indirect detection of the infection caused by the PWN. TI detects temperature increases at the canopy level, as a consequence of the reduction in water transport and transpiration resulting from the infection. It can also be useful to identify relationships between plant water status and infection. In a preliminary experiment, watered and non-watered *P. pinaster* seedlings were grown in a growth chamber under controlled temperature, PAR and relative humidity. Plant transpiration was measured with an open path IRGA and TI images of the canopies were taken. In the field, canopy temperature of adult *P. pinaster* was registered with thermal images at known temperature and air humidity conditions. At the same time, the temperature of wet and dry branches was used as reference. To assess PWN presence in the trees, wood samples (about 60 g) were collected at 1.5 m from the base of the trunk, using a low-speed drill, nematodes extracted by the tray method and *B. xylophilus* identification made, based on diagnostic morphological characters. Our results showed that TI can be usefully applied to detect trees infected by PWN.

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**Direct detection of the pinewood nematode, *Bursaphelenchus xylophilus*, from the insect vector, *Monochamus galloprovincialis* and *Pinus pinaster***

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Pine wilt disease (PWD), caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, has caused serious losses to pine forestry worldwide. Transmission of this nematode to non-native areas is carried out by insects, belonging mainly to the genus *Monochamus*, and by wood products carrying the nematode and/or the insect vector, used in international trade. The economic impact of the introduction of the PWN into new areas has highlighted the need for the development of accurate and reliable detection methods. In most cases, PWN is identified based on morphological characters after nematode extraction from the insect vector or from wood samples, requiring several time-consuming steps and a high level of expertise in taxonomy. In the present study, a molecular method has been developed for direct detection of the PWN by conventional PCR assay, using a species-specific set of primers based on satellite DNA, which allows the rapid and direct detection of one single nematode present in one entire insect and in 100 mg of *Pinus pinaster* wood and bark without the need of prior nematode extraction. The methodology involves direct homogenisation of insects, wood and bark using liquid nitrogen, total DNA extraction and one or two PCR amplification steps. A second PCR can be performed in order to increase the sensitivity of the test. This methodology is species-specific, easy to perform, not very expensive, not time consuming and represents a new tool for PWN diagnosis.



## The pinewood nematode, *Bursaphelenchus xylophilus*, in Portugal: possible introductions and spread routes of a serious biological invasion revealed by molecular methods

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Pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease is a major worldwide pathogen and pest of pine, with impacts on forest health, natural ecosystem stability and international trade. In Portugal, the first diagnosis of PWN was in 1999, a first occurrence also for Europe of this major biological invader. The disease was recently detected in Madeira Island, in the center of Continental Portugal and in the Northern Spain. In an attempt to search for more reliable and robust molecular markers, that enable the study of intraspecific variability of *B. xylophilus*, from different geographic locations, intergenic spacer (IGS) region of the 5.8S rDNA gene was used, combined with inter-simple sequence repeats (ISSR), to determine the genetic relationships among 45 *B. xylophilus* isolates from Portugal, China, Japan, South Korea and USA. IGS sequences revealed that this region can only be used to establish interspecific correlations. IGS is not a good marker to establish genetic diversity of *B. xylophilus*, since no differences were detected among Portuguese isolates from different geographic locations. Fingerprints obtained with ISSR detected a low genetic variability among Portuguese isolates, except for the ones isolated after 2008. ISSR dendrograms suggest the possibility of multiple introductions in Madeira Island, from Asia and/or North America. Recently, collected isolates, from the center of the country, showed a greater similarity with Asian or USA isolates, suggesting multiple introductions from different origins, contrary to previous information which suggests a single introduction from Asia in the initial affected area.

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## Genetic diversity within the mitochondrial genome of *Bursaphelenchus xylophilus*

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Pine wilt disease, a major threat to conifer forests worldwide, is caused by the pinewood nematode, *Bursaphelenchus xylophilus*. Although native to North America, *B. xylophilus* was introduced in Japan, China, Taiwan, Korea and, more recently, in Portugal through timber trade. Morphological plasticity and overlap of phenotype within the genus *Bursaphelenchus* makes species classification difficult and therefore inter- and intra-specific genetic surveys are mandatory. In this context, the variability of mitochondrial DNA (mtDNA) sequences has been successfully used as a tool for species identification and to study evolutionary relationships among many eukaryotic lineages. Here we report the partial sequence of the *B. xylophilus* mitochondrial genome. A comparative analysis using the complete mitochondrial genome of 32 nematode species shows that *B. xylophilus* mtDNA has a unique gene order in the phylum Nematoda that resulted from, at least, two major rearrangement events involving the AT-rich region and the Asparagine tRNA gene. We also confirm that *B. xylophilus* and *B. mucronatus* are distinct phylogenetic groups, that *B. xylophilus* retains higher genetic diversity in its geographical origin, in North America, and that it was recently introduced in the Iberian Peninsula by a unique long-distance dispersal of a small pioneer population from Asia. The catalogue of new *B. xylophilus* mtDNA sequences provides the necessary information to carry out taxonomic, ecological and population studies.

## Transcriptomic approach to understand the pathogenicity of *Bursaphelenchus xylophilus*, the pinewood nematode

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a devastating pathogen of pine forests, causing pine wilt disease. Aiming to unravel the molecular mechanism of pathogenicity, we have pyrosequenced the transcriptome of seven PWN isolates from distinct locations: Portugal (4); China (1); Japan (1); and USA (1). This process generated a total of 679,559 reads and 39,623 transcripts, 17,772 of which had recognized protein domains and 11,209 were recognized in Gene Ontology, thus significantly increasing the knowledge of the nematode available in the public domain. The transcriptome and its corresponding protein information were organized in a database, where targeted searches can be made on each individual population. Additionally, by assembling together the set of contigs from all populations, we were able to generate a digital transcriptome of the species *B. xylophilus*. Genes characteristic of pathogenicity in this nematode were identified in the transcriptome, namely cellulases, chitinases, expansins or venom allergen proteins. The analysis of the transcripts and the corresponding sequences from each population revealed the presence of diversity at the nucleotide level (SNP). Restraining the analysis to the genes involved in pathogenicity, we were able to detect intra- and inter-population diversity, with some SNPs identified causing alteration of the codified aminoacid. We found discriminative SNPs between Portuguese and Chinese isolates, between Portuguese and USA and Japan isolates and within the Portuguese nematodes. These data were used to perform a phylogenetic analysis, which revealed the proximity of the Portuguese isolates with the Chinese isolates.

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## Searching for resistance genes to the pinewood nematode, *Bursaphelenchus xylophilus*, in pine using subtractive suppression hybridization and high throughput sequencing

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The pinewood nematode, *Bursaphelenchus xylophilus*, constitutes a severe problem for pine producers in different regions of the world. *Pinus pinaster* is a very susceptible species, and no resistant genotypes have yet been identified. By contrast, *Pinus pinea* seems to have less susceptibility to the nematode, and the reasons for this have not yet been clear. Also, the molecular ethiology of this disease has not been elucidated to this date. In order to devise a biotechnological strategy to create resistant *P. pinaster* trees, and to better understand the molecular responses of the plant to infection by the pathogen, we utilized two non-target techniques: suppressive subtraction hybridization and high throughput sequencing. From the former we were able to clone and identify genes that are differentially expressed in infected and non-infected *P. pinaster* and *P. pinea*. From the latter, four normalized cDNA libraries from stem tissue were sequenced in a full 454 GS-FLX run, producing 2,086,030 sequencing reads with an average length of 414 bp. Assembly of the high quality reads resulted in 106,242 contigs of which 6,086 were singletons. A total of 93,597 unigenes were conceptually translated into amino-sequences of which matched known proteins in the NCBI non-redundant protein database, 52,018 revealed conserved protein domains through InterPro functional classification and 39,425 were assigned with Gene Ontology terms. Queries conducted within the database enabled the identification of genes putatively involved in disease response and resistance reactions which had not been previously evidenced in *P. pinaster* or *P. pinea*.

## Are bacteria positive or negative for *Bursaphelenchus xylophilus*? Nematicidal activity found in bacteria associated with the pinewood nematode

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*Bursaphelenchus xylophilus*, the pinewood nematode, is one of the most interesting nematodes since it is a pathogen of trees and is the causal agent of pine wilt disease. Recent work published on bacteria associated to the nematode showed that in recently invaded areas by *B. xylophilus*, healthy pine trees of the species *Pinus pinaster* have a diverse endophytic microbial community. From these areas, nematodes isolated from infected trees carried associated bacteria that belonged to different species according to the area where the nematodes were isolated. Consequently, the possibility of a protective role to the tree, by the endophytic microbial community, against the nematode has become a hypothesis. In order to test it, isolated bacteria associated to the nematodes infecting *P. pinaster* were screened for their potential to kill *B. xylophilus*, *in vitro*. Bacterial strains more active in killing nematodes were selected and the bacterial products, produced during growth, studied in order to determine their nematicidal activity. This nematicidal activity was analyzed for 46 strains. Only seven bacterial strains did not show toxicity against *B. xylophilus* and the only genus with all the strains being non-toxic was *Burkholderia*. All strains of the genus *Pseudomonas*, except one strain of *P. putida*, showed toxicity against the nematodes. The genus *Serratia* included the more toxic strains to the nematodes: all except one showed the highest toxicity level. *Serratia* strains were screened for the different products that could interact with the nematode. Major products identified with potential biotechnological use were Serrawettin and proteases.

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# ***POSTER***

*Abstracts*

## Preliminary evaluations of conventional and glyphosate-tolerant soybean genotypes related to their susceptibility to soybean cyst nematode-race 10

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The soybean cyst nematode (SCN) is one of the main factors limiting the soybean production in the world. The number of resistant soybean cultivars available in Brazil nowadays is still low. This work aimed to select *Heterodera glycines* (race 10)-resistant soybeans genotypes. It was done in the 2009/2010 agricultural season under field conditions in Uberaba, MG, in a naturally infested area. Sowing was done manually into the nematode's attack surrounding (reboleira) in a non-tillage growth. The experimental design adopted was the randomized blocks, with 4 replications of each strain. Each parcel was composed of a 2m line with about 40 plants. The evaluation was done 30 days after soybean sowing. In each parcel, 10 plants were taken carefully using a hoe. After counting, grades from 0 to 4 were given following the Hartwig (1985) criteria. BRSMG 810 C and MGBR 46 Conquista cultivars were used as resistance and susceptibility patterns, respectively. From the 65 conventional soybean genotypes tested, 14 were considered resistant to SCN-race 10, being 10 of them already considered resistant to SCN-race 3 in another experiment done before. None of the 37 glyphosate-tolerant soybean genotypes tested were considered resistant. This information combined to the agricultural characteristics obtained in other experiments will be useful as a criterion to select promising genotypes that will go on into the selection process and eventually will be able to become a cultivar. Support: FAPEMIG.

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## On the relevance of freshwater nematodes for ecological assessment in transitional waters

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Nematodes are the most common group of organisms in the meiobenthic assemblages of freshwater and marine systems and, in Portugal, its distribution has been studied along the estuarine gradient of two estuaries. Nematodes are recognized as good bioindicators of anthropogenic impacts in aquatic ecosystems and, together with the taxonomic data, the inclusion of functional diversity can create a powerful tool, providing information on the functioning of ecosystems. Most estuarine studies do not encompass information to genus/species level of freshwater nematodes or reduce the information to family level, impoverishing thus information on diversity. The main aim of this study was to evaluate the information loss effect on the assessment of the ecological quality status of the upstream area of the Mondego estuary, caused by the non identification of freshwater nematodes—both taxonomic and functional approaches of nematode estuarine communities. Results showed that nematode assemblages reflected the salinity gradient, with increasing densities and diversity from oligohaline to euhaline areas. Freshwater nematodes contributed with a small percentage (3.5% of total density), but were very abundant in the upstream areas (0.5-47.4%). Without freshwater nematodes, the separation of salinity stretches was maintained (with lower distinction of the stretches) and the performance of the Index of Trophic Diversity and the Maturity Index revealed a worse condition of the ecological status of oligohaline and mesohaline stretches. This study showed that taxonomic and biological characteristics of freshwater. Nematodes should be evaluated when assessing the ecological status of an estuary in order to correctly classify the uppermost sections of estuaries.



## Intelligent Teaching Assistant supporting nematode learning

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Nematology learning has been a challenge to teachers who have detected several difficulties during the educational process, with emphasis on the fragilities of knowledge and the use of taxonomy. The need of taxon memorization, their characteristics and identification limits, observing morphological variability and biology, plus the life cycle, understanding the combined use of the different data to characterize diverse nematode groups and instability in their classification systems, which are present in the majority of undergraduate and postgraduate nematological students. Because of this situation in nematological graduate studies, some lecturers have been searching for technological resources, to accommodate the individual characteristics of each student and their minimum requirements for graduation. This work refers to Intelligent Teaching Assistant to propose a methodological organization about the context that is mentioned in classroom and by the artificial assistant during the out of class period. This artificial assistant interaction with each student and taking into account their difficulties, promoting the study with virtual resources to overcome their difficulties, supplying adequate pedagogic orientation to the reality discovered by the interactions. All the recovered data are available to the teachers with the purpose of working in an appropriated way with each student during the semester. In that way, we enthusiastically desire one apprenticeship – teaching more efficiently and to synchronize with the necessities of each student. (Support by CNPq).

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## Information and communication technology use for nematological research, education and extension projects in Costa Rica

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A new collaborative project funded by CRUSA (Foundation Costa Rica-USA) and the CSIC (Consejo Superior de Investigaciones Científicas, Spain) is implemented by three research centers, the Institute for Sustainable Agriculture (IAS-CSIC) from Córdoba, Spain, and the Nematology and Molecular Biology Laboratories at Universidad Nacional and Instituto Tecnológico from Costa Rica. The project is focus on *Meloidogyne* species using polyphasic approaches for their identification. Morphological and morphometrical studies, protein electrophoresis (isozyme patterns) and molecular markers expected to provide an accurate identification of species present in Costa Rica. Taking advantage of the new technologies, one of the goals of the project is to design a web page for an educational and extension purposes, using the information and communication technology, to share the main methods and outcomes of the project. This information resource is going to be design using a free and open source content management system for publishing content on the World Wide Web and intranets. *Joomla!* was the software selected because it is written in PHP (Hypertext Pre-processor), uses object-oriented programming (OOP) techniques and software design patterns, stores data in a MySQL database, and includes features such as page caching, RSS feeds, printable versions of pages, news flashes, blogs, polls, search, and support for language internationalization. The project purpose is to strengthen the ability to transfer the results to the students, growers and technicians. The program outcomes can contribute to a shift from broad taxonomy-based nematology research to deep, yet focused investigations of nematodes, especially for the control of agricultural pests.

## A new species of nematode, *Sclerorhabditis neotropicalis* sp. n. (Rhabditida), associated with Azteca ants in Cecropia trees

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*Sclerorhabditis neotropicalis* sp. n. is described and illustrated. The new species was found in close association with an ant colony (*Azteca constructor* Emery, 1876) inside a tree (*Cecropia obtusifolia* Bertol.) in a tropical rain forest in Costa Rica. Certain species of Azteca ants live in a mutualistic association with Cecropia trees and preliminary evidence suggests that the new nematode species is invariably present in the colonies of at least some of these Azteca species. It is therefore possible that the new nematode species represents a third partner in the mutualism, although its role is still unknown. *S. neotropicalis* sp. n. can be distinguished from *S. tridentata* by its body length (515-560 µm in females and 363-455 in males), stoma 15-22 µm long or 1.0-1.6 times lip region diameter long, stegostoma lacking glottoid apparatus and denticles, pharynx 107-135 µm long, large hemizonid located at 21-22% from the anterior end. Female reproductive system is didelphic-amphidelphic, with ovaries straight or reflexed, and the vulva is a transverse slit, 55-61% from the anterior end, female tail conoid-elongated, 62-76 µm long, with pointed tip. Male reproductive system monorquic, testis ventrally reflexed, spicules 36-47 µm long or 1.6-1.8 times longer than anal body width, gubernaculum 15-18 µm long or 38-42% of spicule length, and bursa peloderan, anteriorly open and bearing eight pairs of genital papillae (1+1/2+2+2f), and male tail conoid-spicate, 23-30 µm long.

## Molecular techniques and scanning electron microscopy for elucidating the taxonomic status of Brazilian populations of *Pratylenchus*

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The coffee root-lesion nematode, *Pratylenchus coffeae*, is an important pathogen of this crop on several countries. However, numbers of authors have questioned the taxonomic status of this group, suggesting the existence of different races or species in the taxonomic group currently named as *P. coffeae*. Thus, the main objective of the present study was to characterize three *Pratylenchus* populations (K5, IB04P, and IB13P), collected on coffee and citrus in São Paulo state, Brazil, through sequencing D2 and D3 expansion fragments of the 28S rDNA, and study morphological characteristics using scanning electron microscopy. Additionally, there was used the DNA barcode technology, with the aim of demonstrating the utility of this technique for the diagnosis of three *Pratylenchus* populations studied. Based on morphological taxonomic studies, were observed the following characteristics: labial region with two distinct annuli, female tail terminus truncate or hemispherical, oval and functional spermatheca, and the presence of males. According with the molecular analyses, it was concluded that both K5 and IB04P populations are *P. jaehni*, since they showed high homology level based on D2-D3 expansion fragments (respectively 99 and 98%) with an isolate of this species deposited in the GenBank. Similarly, the identification of IB13P population was confirmed as *P. jaehni* (99% of homology), validating the application of DNA barcode technology in the identification of *Pratylenchus* species.

## Effects of trichodorids and TRV on potato growth and yield

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Potato production is highly conditioned by some viruses, such as Tobacco rattle virus (TRV). In some cultivars TRV causes severe tuber symptoms, rendering them unmarketable. Some TRV strains are specifically transmitted by soil-inhabiting trichodorids. The virus, in association with its vector nematode species is worldwide spread, and causes economically important diseases in agriculture. Vector trichodorids are widespread throughout Portugal, however little or no information is available on TRV effects in the country. The aim of the present research is to determine the effect of soil-born TRV isolates on yield and some quality attributes of eight potato cultivars in potato growing areas in northern and central Portugal. The study was initiated with a field survey for the association of TRV-trichodorids in potato fields. Sampling was planned based on the results of an inquiry for TRV-like symptoms in potato tuber flesh. TRV isolates were recovered from viruliferous trichodorids in soil samples using virus transmission tests. TRV was molecularly confirmed in 5% of the sampled sites and the vector specimens identified. Potato field trials were carried out on the patches naturally infested with viruliferous trichodorids. Results of putative effects of the association on commercial yield attributes (total yield, tuber numbers within size grades, tuber yield within size grades, tuber appearance of TRV like symptoms and secondary growth) are presented. Potato production was severely affected due to quantitative and qualitative yield losses that could be attributed to TRV isolates. The history of the sites with long persistence of TRV-like symptom expression indicates that TRV and its vector find conditions for survival in these soils.

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## Proper use of chemical nematicides and benefits for the plant, in grapevines cv. Chardonnay in Peralillo, Chile, over two seasons

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During the 2009-2010 and 2010-2011 agricultural seasons, a strategy of nematicide management and application was tested varying concentration of the solution and dose in a grapevine cv. Chardonnay field from Peralillo, Chile. The field had furrow irrigation and was strongly attacked by *Meloidogyne* spp. Before starting the study, soil was decompacted with a subsoiler until 0.5 m deep. Ten nematicide treatments were applied in a furrow beside the plants, plus two control treatments. The nematicide dose was doubled and tripled in some treatments. In both seasons, populations of plant-parasitic nematodes decreased and remained low in chemical treatments. In all chemical treatments, quality of superficial roots (0.3 m deep) improved significantly in the second season. Four treatments had yields higher than control in the first season, whereas six treatments did in the second season. *Meloidogyne* spp. and *Mesocriconema xenoplax* were the most important nematodes damaging grapevines roots in the study field. These results highlight the importance of proper application of nematicides considering concentration and dose of the applied solution.

## Proper use of chemical nematicides and benefits for the plant, in table grapes cv. Flame seedless in Copiapó, Chile over two seasons

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At the beginning of the 2009-2010 agricultural season, a strategy was applied to recover plants and their productivity in a table grape field cv. Flame seedless from Copiapó, Chile. This field has historically suffered from a number of problems strongly affecting plant roots, including high populations of plant-parasitic nematodes, associated to a high electric conductivity of soil, high compactation, and an inadequate irrigation management with excessive frequency which caused asphyxia and stopping the growth of the root system that was reduced, at that time, to the line of planting. The field had no commercial yield during the two previous seasons (2007-2008 and 2008-2009). Work begins with soil decompactation using a subsoiler to penetrate soil and moved until to 0.5 m deep, in the entire surface, which meant 5000 m<sup>3</sup>/ha of aerated soil. The irrigation frequency was spaced in time and with increased amounts of water per irrigation. Nematicides were applied in two ways, through a drip irrigation system or an injection device that place the product directly under the soil surface; taking care of product concentration in both methods and applying the nematicides in the direction of the irrigation lines. The nematicide dose was doubled and tripled in some treatments. The soil volume that was defended for root growth increased in proportion to the nematicide dose. The 2010-2011 season yield increased to 13,000 kg/ha in average, and the previous season fruit size was significantly improved. *Meloidogyne* spp. and *Mesocriconema xenoplax* were the nematodes damaging grapevines roots in the study field.

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## Initial behaviour of coffee tree genotypes in area infested by *Meloidogyne paranaensis*

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Genetic resources represent a reservoir of natural genetic variability and a potential indispensable for genetic improvement programs of cultivated species. Aiming to identify resistant genetic materials, this work evaluates the behaviour of *Coffea* sp. genotypes in a coffee area infested by *M. paranaensis* in Southwestern Minas Gerais, Brazil. Forty-four *Coffea* sp. genotypes were planted in a completely randomized block design with three replicates of seven plants. A biotest was carried out in a green house with plantings of Santa Clara cv. tomato seedlings, in pots containing soil taken from field plots, one month after the planting. The stem diameter, number of plagiotropic branches, height of the plant and development of the aerial part of the plants, according to the nematode parasitism symptoms, were evaluated 12 and 18 months after the planting. In the selection, based on the individual additive genetic effect, some individuals of the 16 and 29 genotypes were selected as superiors and promising for an area infested by *M. paranaensis* with a behaviour similar to the material 33, recommended for areas infested by this nematode.



## First record of *Steinernema diaprepesi* (Steinernematidae) in Argentina

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To date, six *Steinernema* spp. have been reported in Argentina: *S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. rarum*, *S. ritteri*, and *S. scapterisci*. In a recent study, soil samples were collected from a carrot field in the locality of Santa Rosa de Calchines (Department of Garay, Santa Fe province). The insect-baiting technique was used to recover entomopathogenic nematodes. One *Steinernema* isolate was recovered. This isolate was first diagnosed by morphological traits and was placed in the “*glaseri* group”. Morphological and morphometric studies were supplemented with the molecular methods. Specifically sequence data of the ITS and 28S r DNA genes was considered. Generated sequences were compared with those known for other species of the *glaseri*-group. This is the first report of *S. diaprepesi* in Argentina.

01

02

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## ATR/FTIR Characterization of *Steinernema glaseri* and *Heterorhabditis indica*

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The use of Fourier transform mid-infrared spectroscopy with attenuated total reflection for characterizing entomopathogenic nematodes is evaluated for the first time. This technique allows the understanding the molecular composition of complex samples. The resulting spectra of two well known representatives of entomopathogenic nematodes; *Steinernema glaseri* and *Heterorhabditis indica* were compared with the spectrum of *Caenorhabditis elegans*. In the absorption spectra generated by the nematodes samples, the most important variations can be recognized observing the band around 1745 cm<sup>-1</sup> (assigned to C=O of phosphodiester), which is characteristic of triglycerides and in the region of 1200 to 900 cm<sup>-1</sup> which contains many bands assigned to lipids and carbohydrates. A band around 992 cm<sup>-1</sup> was present in *S. glaseri* and *H. indica*, but in different levels represents trehalose. Glycogen representative bands were around 1032, 1081 and 1155 cm<sup>-1</sup>. These compounds have shown correlation with infectivity failure when they are in low levels. Knowledge generated by the FTIR spectra of entomopathogenic nematodes aid other studies such as quality control for mass production processes, and also to assess the role of these components in nematode behaviour and physiology.

## Nematicidal activity of Spanish aromatic plants against the root-knot nematode *Meloidogyne javanica*

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Essentials oils (EOs) are complex mixtures of volatile organic compounds produced as secondary metabolites in plants and have been suggested as alternative sources for control products of plant parasitic nematodes because are easily extractable and environmentally friendly. Their chemical composition may vary considerably between aromatic plant species and varieties, and within the same variety from different geographic areas. In this study we have evaluated the nematicidal activities of EOs obtained from fifteen Spanish medicinal aromatic plants experimentally cultivated in the field, belonging to the genera *Hisopo*, *Lavandula*, *Mentha*, *Rosmarinus*, *Satureja*, *Thymus*, and *Origanum*. The target specie was the root-knot nematode *Meloidogyne javanica*. The EOs were tested in vitro against second stage juveniles (J2) and their motility was evaluated. Seven of the fifteen EOs paralyzed more than 95% *M. javanica* J2 at a concentration of 20µg/µl for three immersion periods (24, 48, and 72 h). The EO from *Satureja* spp. showed the highest toxicity. For the active EOs, dose response experiments were carried out and their nematicidal activity has been correlated with their chemical composition. The results confirm that some EOs are potential nematicidal agents against root-knot nematodes.

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## Nematicidal activity of *Eugenia winzerlingii* against the root-knot nematode *Meloidogyne javanica*

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Environmentally friendly control measures against phytoparasitic nematodes need to be developed. Plants produce a broad spectrum of active compounds which have been shown to have nematicidal effects. *Eugenia winzerlingii* Standl. (Myrtaceae) is a woody shrub endemic to Mexico, Guatemala and Belize growing in an altitude range of 0-400 m. In a previous screening, this plant demonstrated nematicidal properties against *Meloidogyne incognita*. The aim of this study was to test the nematicidal activity of *E. winzerlingii* against the root-knot nematode *M. javanica* along with the isolation and elucidation of the bioactive compounds present in the organic extract of *E. winzerlingii*. The leaves of *E. winzerlingii* were collected, dried and extracted with ethanol. The bioassay-guided fractionation of this extract was carried out by solvent partition and chromatography. The in vitro assays revealed the high toxicity of the ethanolic (100% mortality) and partition extracts from *E. winzerlingii* to J2 *M. javanica* (20 µg/µL). High mortality was observed for several fractions when tested at different concentrations. The chemical analysis (RMN1H, GC-MS, HPLC-MS) of the active fractions showed the presence of different compounds, including fatty acids, terpenoids and flavonoids that could be responsible for the nematicidal effects. All these fractions are being studied. In general the results of this study indicate that *E. winzerlingii* can be considered as a source of bioactive compounds with great potential on control of root-knot nematodes.

## Soil nematodes associated with *Brassica rapa* under organic farming

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A vegetable garden is maintained under organic farming in northern Portugal. Plant-parasitic nematodes are generally associated with intensive agriculture resulting in reduced yields. Their control is not always easy, often being based on the application of chemical pesticides, a practice not permitted in certified organic farming. In the garden only natural organic fertilization is applied using locally produced compost. The present study aimed to determine the nematofauna associated with *Brassica rapa* grown in such production system. Soil samples were collected from the rhizosphere, at 10 to 30 cm deep, along the cultivated plot. The soil was subsequently processed in the laboratory by the tray incubation method. Aqueous suspensions were observed and the nematodes were quantified and identified at the family level considering various trophic groups (bacteriophagous, fungivorous, omnivorous, predators, and plant parasites). Thirteen families were registered: six were plant parasitic nematodes, four bacteriophagous, two predators and one of fungivorous nematodes. Although the incidence of phytonematodes was 41%, populations of free-living nematodes principally bacteriophagous, was the dominant group (52.9%). The Rhabditidae were the most common nematodes and Tylenchidae the most numerous plant parasitic species. Results indicate that the natural organic fertilization used may be a healthy practice for the organic farming system used; however, for long-term sustainability, it is important that populations of plant parasitic and beneficial nematodes be regularly monitored.

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## Evaluation of plant extracts and powders to control *Mesocriconema xenoplax* related to the peach tree short life disease in Morelos state, Mexico

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Peach tree short life disease (PTSL) appears in three to seven year-old trees and is caused by the combination of different factors. One factor that predisposes to PTSL is the nematode *Mesocriconema xenoplax* and *Pseudomonas syringae* pv. *syringae*. Plant species with nematicidal properties might be a potential alternative control of PTSL. Our objective was to use aqueous extracts and powders of 12 plant species from the peach-producing region of Morelos, Mexico to evaluate their action against *M. xenoplax* *in vitro* and in nursery. For the *in vitro* assays, we used Petri dishes with moistened soil with 1% extract of each plant (four repetitions/treatment). After 24 hours, live and dead nematode populations were compared through the centrifugation-flotation method, the control being soil without extracts. Seven plant species were selected for the nursery experiment. Peach trees were planted in 10 kg nursery bags, to which 50 g of powder of each species was previously added, the control being no added powder (10 repetitions/treatment). Trees were maintained for six months under nursery conditions and height data was taken, number of leaves and percentage nematodes at the beginning and end. Trees were subsequently removed from the bag and fresh and dry weight of foliage and root was measured. Both studies used naturally infested soil with *M. xenoplax*. *In vitro* results showed that extracts of *Rosmarinus officinalis*, *Cyperus rotundus*, *Argemone mexicana* and *Tagetes lucida* caused a mortality of *M. xenoplax* greater than 80%. The nursery experiment showed that powders from *Chenopodium ambrosioides* and *Jatropha curcas* caused the highest values in number of leaves and tree height and fresh and dry weight of foliage and roots. Powders of *A. mexicana* caused phytotoxicity to peach trees.

## Exploring the nematocidal effect of *Colocasia esculenta* on *Meloidogyne megadora*

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In previous work we reported that *Colocasia esculenta* (colocasia, taro), an *Araceae*, did not allow *Meloidogyne megadora* reproduction and no plant galls were observed. An aqueous rhizome extract of colocasia caused 100% inhibition of second stage juveniles (J2) hatching and 100% J2 mortality of *M. megadora*, after 168 hours exposure. Therefore, we studied in more detail the rhizome extract of colocasia. Boiling, trypsin digestion, and dialysis through a cut off membrane of 10 kDa, all abolished the effect of the water rhizome extract on J2 mortality, indicating the active constituent(s) as proteinaceous. Column chromatography of the extract, using Sephacryl-200-HR, elicited two major fractions (designated as samples), one white and the other yellow. Each sample (100 mg dry weight sample/ml distilled water) caused 100% mortality of *M. megadora* J2 (360 hours exposure). The protein concentrations of white and yellow samples were 6.875 and 2.637 mg protein/ml sample (Bradford), respectively. These results indicate that more additional research on active proteins from colocasia against *M. megadora* is in order.

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## Development of *Coffea arabica* cv. Catuaí Vermelho infected with *Pratylenchus brachyurus* in microplots

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Greenhouse trials have showed that coffee is intolerant host of *Pratylenchus brachyurus*, since seedling growth can be reduced by the nematode, although reproduction was very low. However, there is a paucity of information under field conditions. Thus, this study aimed to investigate the development of *Coffea arabica* cv. Catuaí Vermelho infected with *P. brachyurus* (Pb) in field microplots (0.5 m<sup>3</sup> box filled with sterilized soil inoculated with 24,000 Pb per microplot). Additionally, it was assessed the effects of intercropping *Brachiaria decumbens* (Bd), *Mucuna deeringiana* (Md) and *Crotalaria spectabilis* (Cs) with coffee plants on *P. brachyurus* population. The experiment was carried out in a randomized complete block design with six replicates. The treatments applied were: 1. Coffee without nematodes and any intercrop; 2. Coffee intercropped with Bd infested with Pb; 3. Coffee intercropped with Cs infested with Pb; 4. Coffee intercropped with Md infested with Pb; 5. Coffee infested with Pb without intercrop. There was a significant ( $P = 0.05$ ) effect of Pb on height of the coffee plants at 164, 225 and 318 DAI when intercropped with Bd and Md. Conversely there was not statically difference between the check and the coffee plants intercropped with Cs or without intercropping. Although Pb reproduce poorly on coffee plants, high nematode populations were found in plants intercropped with Bd and Md (246 DAI), which are often used as cover crop by coffee growers. Low Pb population was detected when coffee was intercropped with Cs suggesting that this plant could be a good option on Pb management.



## Microbiologization of 'Prata-Anã' banana explants with rhizobacteria for *Meloidogyne javanica* control and plant growth

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Micropropagated banana plantlets are used in plantations for their genetic uniformity and pest-and disease-free condition; however there are reports that they are very susceptible to soil-borne pathogens. The study aimed to evaluate in vitro colonization by rhizobacteria [RB] of roots and rhizomes from RB-treated 'Prata-Anã' banana (*Musa* spp.) explants, and the effects of such treatment on infection by *Meloidogyne javanica* and plantlet development in a greenhouse experiment. Ten rhizobacteria isolates were evaluated in the in vitro assay. The experiment was arranged in a completely randomized design with eight replicates. The explants were soaked in the bacterial suspension (control: saline solution) for 15 minutes and were transferred to medium (Phytigel). Colonization of roots and rhizome was evaluated after 30 days for the presence of whitish halo over or around them. For the greenhouse test, the explants treated with the isolates were cultured on MS medium. After 30 days the plantlets were transferred to tubettes and acclimated for 30 days when they were transplanted to 25-cm diameter pots. The trial was conducted within a randomized block design with four rhizobacteria isolates and eight replicates. After 24 hours the soil was infested with 3,000 eggs of *M. javanica*. After 60 days the plants and soil were evaluated for: number of galls, egg masses and eggs per root, juveniles/100g soil, reproduction factor, plant height, leaf number, fresh weight of root, shoot, and dry shoot matter. None of the rhizobacteria colonized roots. *Bacillus pumilus*-1, *B. pumilus*-3, *B. pumilus*-10 and *Bacillus* sp.-36 colonized the rhizomes. The other isolates caused rhizome and pseudostem's necrosis and plant death. *B. pumilus*-76 increased shoot dry weight. *B. pumilus*-1, *B. pumilus*-3 and 76 reduced the nematode population in roots. Support: FAPEMIG

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## Effects on soil nematode community structure associated with swine manure application techniques

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Organic amendments can be used as a means for restoring biodiversity in agricultural soil and reducing or eliminating the negative effects of chemicals used commonly in agriculture. The aim of this study was to analyze the effect of dehydrated swine manure application on soil nematode trophic structure in an agricultural soil under conventional tillage. A 5x5 factorial experiment in a randomized complete block design with five replications was established. Treatments in the experiment were: dehydrated swine manure at 1, 2, and 4 t.ha<sup>-1</sup>, and two controls. Abundance, diversity and variability of the different nematode trophic groups in the soil, as well as their evolution were evaluated. The treatments applied and sampling periods significantly influenced nematode communities. The trophic structure of the nematode community was composed of five groups: bacterivorous, fungivorous, plant parasites, omnivorous and predators. An increase in the number of individuals in the course of experiment was observed. This agroecosystem presented low-diversity indicating the dominance of a trophic group over the rest. The variability in the abundance of plant parasitic nematodes was closely related to the presence of a suitable host enabling its establishment and development, and population increase. Application of organic waste can improve soil nematodes diversity, while acting as regulator of pathogenic organisms and increasing crop production. Incorporation of organic residues into soil can result in increased soil health and improved general environmental quality. It represents a practical alternative for the disposal of agroindustrial waste.

## Evaluation of the reaction of soybean to root lesion nematode

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Among the nematode control measures in soybeans, the resistant cultivars utilization is the most economic and the most acceptable by the farmers. To *Pratylenchus brachyurus* soybeans germplasm resistant sources are not known. This work aimed identify in the soybeans germplasm resistant source to use in the breeding programs, and or if they adapt, to use in infested areas. The essay was done in greenhouse at Aprosmat, Rondonópolis, MT, from September to November 2010. De design used was the completely randomized with 33 treatments and 6 replications. The genotypes were sowed in sand and two days after emerging seedlings were transplanted (one in each pot) to clay pots filled with soil: sand sterile substrate (1:3). One week after transplanting each seedling was inoculated with 600 specimens of nematodes. The evaluation was 90 days after inoculation, evaluating the nematode Reproduction Factor (RF) in each genotype. None of the genotypes evaluated presented RF lower than one. However there was a big variability among them, RF varying from 2,5 to 28,3. Several recommended cultivars to the main country regions as M-SOY 7211RR (2,5), M-SOY 6101 (2,7), GB881RR(3,1), MG/BR 46 Conquista (3,3), FMT TUCUNARÉ (3,4), M-SOY 8914 (5,1), M-SOY 7578 RR (5,4), M-SOY 7908RR (5,7), FMT PERDIZ (5,9) e M-SOY 8360 RR (5,9) presented good tolerance levels. Lower RF genotypes must be preferred to be included in breeding programs aiming the resistant cultivar development or to planting in infested areas.

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## Integrative diagnosis of *Meloidogyne* species infecting tropical and subtropical crops in Costa Rica

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Costa Rica is a predominantly agricultural country with approximately 12 % of the national territory suitable for intensive agriculture. Root-knot nematodes, *Meloidogyne* spp., are considered one of the most important plant-parasitic nematodes in tropical and subtropical agriculture. The accurate and timely identification of *Meloidogyne* spp. infecting crops in Costa Rica is essential for designing effective crop management strategies. The objective of this study was to identify the *Meloidogyne* spp. infecting several ornamental and food crops in three regions of Costa Rica (Huetar Norte, Atlántica and Central) using esterase phenotypes, mitochondrial and ribosomal DNA markers, and morphology (female perineal patterns). Nematodes were collected from infected roots and rhizosphere of banana (*Musa acuminata*), chayote (*Sechium edule*), coffee (*Coffea arabica*), coriander (*Coriandrum sativum*), guava (*Psidium guajava*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), papaya (*Carica papaya*), pepper (*Capsicum annuum*), rain tree (*Samanea saman*), rice (*Oryza sativa*), strawberry (*Fragaria* × *ananassa*), tomato (*Solanum lycopersicum*), vegetable sponge (*Luffa cylindrica*) and several ornamental plants (*Agapanthus africanus*, *Begonia* sp., *Caladium* sp., *Calathea makoyana*, *Chamedoria metalica*, *Dianthus* sp., *Impatiens walleriana*, *Rosa* sp.). The nematode species identified were *M. arenaria*, *M. exigua*, *M. hapla*, *M. hispanica*, *M. incognita*, *M. javanica*, and *M. salasi*. Further analyses, including DNA sequencing should be carried out to determine the unidentified nematode species found in this study.

## Evaluation and improvement of an extraction method for *Meloidogyne* sedentary life-stages in potato tubers using enzymatic digestion technique

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The species of the genus *Meloidogyne* parasitize many crops, such as carrot, potato, tomato and salsify, suppressing their yield and affecting adversely their marketability. The symptoms they cause consist of typical galls on roots or on the surface of tubers. According to EU regulations, seed potatoes imported from abroad and traded internationally have to be found free from *Meloidogyne chitwoodi* and *M. fallax*, either by visual inspection or laboratory tests. As external symptoms are not always visible, the improvement of a reliable laboratory detection method is important in order to avoid the spread of these quarantine organisms by infested seed potatoes. The efficacy of enzymatic digestion followed by sieving and centrifugation was evaluated for the extraction of *Meloidogyne* females from potato tubers. This evaluation focused on critical steps that contribute to nematode losses during the sieving and centrifugation processes. Other scrutinized factors that affect nematode detection included the distribution of the sedentary-life stages in the tuber tissues, and the thickness and location of the peels sampled from the tubers. The findings obtained in this study are very useful to improve this extraction method.

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## Molecular diagnosis of *Meloidogyne hispanica* using mtDNA PCR RFLP

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An accurate identification of *Meloidogyne* spp. is essential to define efficient and sustainable integrated pest management programmes. *Meloidogyne hispanica* originally appeared to be restricted to the Southern of the Iberian Peninsula causing important losses to *Prunus* spp. However, it has now been reported to also cause damage to other economically important crops worldwide. The purpose of this work was to identify molecular markers to differentiate *M. hispanica* from other *Meloidogyne* species by PCR-RFLP of mtDNA region between *COII* and 16S rRNA genes, using primers C2F3 (5' GGTC AATGTT CAGAAATTTGTGG 3') and MRH106 (5' AATTTCTAAAGACTTTTCTTAGT 3'). The amplified products of the Portuguese *M. hispanica* isolates resulted in a fragment of ca 1800 bp similar to *M. javanica*, *M. ethiopica* and *M. incognita*, and could be discriminated from *M. hapla* (650 bp), *M. chitwoodi* (650 bp), *M. mayaguensis* (850 bp) and *M. arenaria* (1300 bp). The enzyme *Hin*I produced two fragments in *M. incognita* and the restriction patterns produced by enzyme *Dr*III discriminated *M. hispanica* from *M. ethiopica* and *M. javanica*. No digestion occurred with *Dr*III to *M. hispanica*, while *M. ethiopica* and *M. javanica* presented a restriction site to *Dr*III (1000 and 800 bp). *Meloidogyne ethiopica* (1240 and 580 bp) and *M. javanica* (1000, 580 and 240 bp) were differentiated by *Al*I. The restriction endonuclease *B*fal differentiated *M. hapla* from *M. chitwoodi*. *Meloidogyne hispanica* can be separated from the other species using the *Hin*I and *Dr*III.

## Adaptation of Instant FAME analysis for *Meloidogyne* species identification

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Studies have indicated that *Meloidogyne* spp. can be identified within root tissue using the standard fatty acid methyl ester (FAME) analysis. Instant FAME extraction and Rapid analysis techniques have recently been developed, decreasing sample processing time by 90%. Two experiments were carried out to evaluate the efficacy of the Instant FAME extraction and Rapid analysis with that of the standard FAME extraction for identification of two *Meloidogyne* spp. The first experiment compared roots of *Solanum lycopersicum* 'Rutgers' infected with either *M. incognita* or *M. javanica*. Samples equivalent to the recommended amount of tissue for each extraction (3 mg for Instant FAME and 40 mg for standard FAME) were ground before undergoing their representative analysis. Results indicated that the Rapid analysis was significantly different ( $P < 0.0001$ ) from the standard analysis, and that using the standard FAME extraction method coupled with the Rapid analysis may yield more fatty acids on average (74) than the Instant FAME extraction in conjunction with the Rapid analysis (39). In a second experiment, 40 mg tissue samples of turmeric (*Curcuma longa*) roots infected with *M. incognita* or *M. javanica* were subjected to the standard FAME or Instant FAME extraction followed by Rapid analysis. Statistical analysis using stepwise and canonical methods found that the Instant FAME extraction was significantly different from the standard FAME extraction ( $P=0$ ) while still maintaining the ability to separate roots infected with *M. incognita* from *M. javanica*. The results of this study indicate that using the Instant FAME extraction coupled with the Rapid analysis can produce FAME profiles for differentiating these two *Meloidogyne* spp. that are more accurate and faster than the standard FAME methods.

## ***Solanum sisymbriifolium*: a potential alternative to agrochemical control of potato cyst and root-knot nematodes**

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Potato cyst nematodes (PCN), *Globodera* spp., are a serious problem in potato growing areas. Several measures are available to control PCN: crop rotations; resistant cultivars; trap crops; antagonistic plants; biological control using fungi; GM potato plants; and soil disinfestations with agrochemicals. Moreover, due to EU imposed restrictions, the use of soil disinfectants in Europe must be reduced. Despite the use of all those control measures and Integrated Management Systems, the area of land infested with PCN has increased. There is a need for other control measures to be developed such as the growth of an effective trap crop. Recently it was demonstrated that trap cropping can reduce PCN populations by 80% or more and is effective on a commercial scale on a range of soil types. After extensive screening of non-tuber-bearing Solanaceae, a resistant trap crop that produces a high level of hatching agents, *Solanum sisymbriifolium*, seemed an ideal control method for PCN. This plant stimulates hatching but is totally resistant to PCN. Recently, root-knot nematodes (RKN), *Meloidogyne* spp., have been found coexisting with PCN in some potato fields, in Portugal. The presence of *M. chitwoodi*, a species with quarantine status, was detected and may be an additional problem for farmers and potato growers. In view of the aggressiveness demonstrated by some *M. hispanica* isolates to potato, management of this RKN species should also be considered. A trap crop that can reduce RKN densities as well as PCN could be an alternative to other methods. This study aims to improve crop protection systems based on trap crops (i.e. *S. sisymbriifolium* cultivars) for the control of PCN and RKN populations.



## Nematodes associated with cork oak, *Quercus suber*, in Montado ecosystems

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The largest stands of cork oaks, *Quercus suber*, are present in the semi-natural Montado ecosystems, usually with pastures or agricultural crops as understory. Changes in land management have led to Montado degradation, accompanied by cork oak decline and sudden death. This multi-factor event is being studied by the RESCOE Project that aims to integrate research on the various interacting components in these ecosystems. The aim of this study was to investigate the nematode community associated with healthy and declining cork oaks in the Montado. Trees were sampled seasonally and nematodes were extracted from rhizosphere soil and wood collected from the tree crown. Nematodes were classified into trophic groups and, whenever possible, to genus level through the observation of their morphology using a microscope; ecological indices were calculated. We have compiled inventories of nematodes associated with healthy and declining cork oaks both below- and above- ground. Bacterial-feeders, fungal-feeders, omnivores, predators and over 12 genera of plant parasitic nematodes with different life strategies (endo-, semiendo- and ectoparasites, including potential virus-vectors) were detected. Cork oak seems to be the preferred host of *Rotylenchus*, *Tylenchorhynchus* and *Xiphinema*, whereas many nematode populations seem to associate with the herbaceous layer. The Nematode Channel Ratio was significantly higher in healthy areas. Our results form the basis for further investigation on the role of the nematode community in cork oak decline in Montado ecosystems.

## Host suitability of sugarcane varieties to *Meloidogyne javanica*

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In Brazil, *Meloidogyne javanica* is one of the most important nematode species for sugarcane. Nowadays, nematode control is based on nematicides, but there is great interest in the use of resistant varieties. This study was carried out with the objective of evaluating the reaction of new sugarcane varieties to *M. javanica*. Two experiments were conducted, in 65 L pots containing sandy soil, kept in open area, according to completely randomized experimental design, with six replicates. In each experiment, 13 varieties were evaluated. The SP81-3250 variety was used as a susceptible standard in both experiments. Single buds of each variety were planted in 0.5 L pots. One month later, when the buds had germinated, the plants were replanted in 64 L pots (one plant per pot). On this occasion, a suspension of 100 000 eggs and juveniles of a population of *M. javanica* was applied to each pot. Five months later, the plants were uprooted to determine the amount of nematodes in the roots and the reproduction factor was calculated ( $RF = Pf/Pi$ , PF being the final population and Pi the initial population that was inoculated on each plant). The SP81-3250 variety presented  $RF > 1$  in both experiment. Only IACSP93-3046, IACSP94-2101 and IACSP94-4004 presented  $RF < 1$  and were considered not hosts to *M. javanica*. The others varieties presented  $RF > 1$  and were considered good hosts of the nematode.

## Molecular characterization of the venon allergen-like protein gene in *Meloidogyne hispanica*

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The root-knot nematode (RKN), *Meloidogyne hispanica*, detected for the first time in Seville, Spain, has been found in all continents associated with a wide range of plant hosts. Secretory proteins encoded by genes expressed in the oesophageal glands of plant-parasitic nematodes have key roles in nematode parasitism of plants. The venon allergen-like protein gene was isolated and cloned from *M. hispanica* and designated Mh-vap-1. In situ hybridization analysis revealed that Mh-vap-1 transcripts were accumulated exclusively within the subventral oesophageal glands. In order to determine whether this gene is differentially expressed during nematode development, cDNA was amplified from RNA isolated from the eggs, second-stage juveniles (J2) and females. Preliminary studies revealed that Mh-vap-1 is expressed in all the developmental stages tested and has higher levels of expression in the J2. The potential role(s) of this protein in the plant-nematode interactions is discussed.

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## Reaction of the olive cultivars to *Meloidogyne ethiopica*

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The resistance of three olive cultivars (Arberquina, Koroneiki and Manzanilla) to *Meloidogyne ethiopica* was studied. Plantlets of the distinct genotypes were inoculated with 10.000 eggs + second stage juveniles/plant with a pure *M. ethiopica* population. Tomato plants 'Santa Cruz' similarly inoculated with the same nematode level served as control. The experiment was conducted under a randomized design with six replicates per treatment in a greenhouse at  $25 \pm 2$  °C. Six months after the inoculation, roots of each plant were evaluated for gall number and nematode reproduction factor (RF). The host reaction of each cultivar was rated according to RF (Pf/Pi), as immune RF=0.00, resistant RF<1.00 and susceptible RF>1.00. All the olive cultivars behaved as immune whereas the control presented the RF=38.85.

## Effect of irrigation on roots and *Meloidogyne ethiopica* parasitism, in *Vitis vinifera* cv. Chardonnay

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Drip irrigation accentuates root health problems causing an unusual distribution of the root system which search for more oxygen than water. Nematodes are added to the root-soil-water system, being *Meloidogyne* of great viticulture importance. This study evaluated the effects of irrigation on quality and weight of roots, vegetative growth and parasitism of *Meloidogyne ethiopica*. *Vitis vinifera* cv. Chardonnay Rootstock SO4's roots were divided into two bags of soil and received different irrigation treatments for 6 months. Quality of roots, weight of pruning and living and dead roots were evaluated. Non irrigated roots were maintained by the roots of higher quality and weight through hydraulic redistribution. Roots with high frequencies of irrigation helped to maintain roots without irrigation. The increase in irrigation frequency increased the pathogenicity of *M. ethiopica*. Pruning was larger when the root had better quality, diminishing when half the roots were without irrigation. Irrigation which allows a high concentration of oxygen and adequate soil moisture was an important factor which contributed for a good condition of the root system allowing the root to defend itself against nematodes.

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## Controlling water frequency can reduce infection of *Meloidogyne ethiopica* to roots of tomato plants growing under greenhouse conditions

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Two trials were conducted in greenhouse conditions to evaluate the effect of watering frequency on the damage to the roots of tomato plants inoculated with eggs and juveniles of *Meloidogyne ethiopica*. It was expected that a lower frequency of irrigation would reduce nematode infection as they would move into the pores of the soil. The experiment was set up in 0.9 L pots with sandy loam soil, and plants were inoculated with 200 eggs and juveniles of *M. ethiopica*. Nematode infection was scored separately, in the roots growing inside the pots and also in the roots that grew out or on the surface of the pots. The soil was subjected to higher saturation in the high frequency irrigation which causes an increase of root knots, these were reduced when the frequency of irrigation was low. The quality of the roots that grew into the soil improved the watering frequency was reduced and the quality of the roots that grew out of the ground improved by increasing the frequency of watering. The weight of the roots was strongly influenced by the weight of the galls. The decrease in the number of galls was evident when the frequency of irrigation decreased. The J2 of *M. ethiopica* were able to infect roots that grew outside the ground, due to the higher humidity. The quality of the roots without nematodes was better than those with nematodes and the roots of tomato grew better when the pots were irrigated every 4 days. In field conditions, the frequency of irrigation should be determined to help reduce the damage caused by nematodes.

## How does *Solanum sisymbriifolium* affect *Globodera pallida* behaviour?

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Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are commercially important pests of potato (*Solanum tuberosum*) crops in the UK and mainland Europe. The non-tuber-bearing *Solanum* species, *S. sisymbriifolium*, has been used as an effective trap crop for PCN in the UK and the Netherlands, but its mode of action is unknown. The interaction between *G. pallida* and *S. sisymbriifolium* has been investigated through in vitro bioassays. *Globodera pallida* second-stage juveniles (J2) were equally attracted to the roots of *S. sisymbriifolium* and to those of *S. tuberosum*, with the invasion per-gram-root exceeding that for *S. tuberosum*. However, time course of infection indicated that the nematodes in *S. sisymbriifolium* roots failed to follow the normal life cycle seen in *S. tuberosum*. Hatching assays with high-performance liquid chromatography fractions of root exudate extracts suggest that hatching factor components differ between the two *Solanum* species.

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## Histopathological analysis of peanut roots (*Arachis hypogaea*) attacked by *Meloidogyne arenaria*

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Peanut plants cv. Granoleico severely attacked by *Meloidogyne arenaria* were recently detected at the Módulo Experimental FMA (Fundación Maní Argentino)-INTA General Cabrera (province of Córdoba, Argentina). Root samples (galled and non-galled) were fixed in FAA, processed and analyzed using conventional optical microscopy techniques. Galls were round, less than 3 mm in diameter, and had numerous egg masses on them. Some galls had several lateral roots. In the central cylinder, feeding sites (FS) were observed embedded in vascular tissues, which were reduced, displaced and showing atypical arrangement. In galls containing more than one female (up to four were found, each associated with its FS), these alterations were more severe. The body of females occupied part of the cortex, damaging surrounding cells. FS were composed of a variable number of giant cells (up to six) that developed from cells of vascular tissues. Cells showed marked hypertrophy (up to 200  $\mu\text{m}$  measured at the major axis), dense cytoplasm, nucleus division (several nuclei arranged in clusters per cell) and development of internal cell wall ingrowths in sectors adjacent to the xylem. Juveniles of the nematode were observed in diverse zones of the galls. The peanut cultivar showed to be very susceptible to *M. arenaria*.



## The use of plant elicitors to induce plant defence against the root-knot nematode *Meloidogyne chitwoodi*

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There is an increase demand for sustainable strategies to reduce dependence on chemical pesticides. Plants respond rapidly to the presence of plant pathogens and it has been demonstrated that foliar application of various compounds can induce the systemic defence response to reduce nematode infection. The aim of this study was to further expand this work and to assess the effects of benzothiadizole (Bion®), DL-β-amino-n butyric acid (BABA), jasmonates (cis-jasmone and methyl-jasmonate) and salicylic acid (SA), in the development and reproduction of the root-knot nematode, *Meloidogyne chitwoodi*. Three-week old tomato plants cv. Tiny Tim were treated twice with foliar sprays and inoculated with 300 second-stage juveniles/pot. Nematode development and reproduction were assessed 21 and 45 days post-inoculation. Treatments with cis-jasmonate and SA had a negative effect on the development of the nematode but had no effect on reproduction. Among the plant elicitors tested, BABA and methyl-jasmonate were the most effective in reducing nematode penetration (by 49 and 30% respectively) and the reproduction factors (Rf) were significantly lower (4.1 and 5.2, respectively) when compared to the control (Rf=13). The use of plant elicitors to strengthen the endogenous defence of plants may lead to a practical alternative to current methods of nematode control.

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## Effects of 1,3-dichloropropene and chloropicrin on soil diversity and functioning in a strawberry field and its surroundings in Southern Spain

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Since the phase out of methyl-bromide (MeBr) by the Montreal Protocol due to the concerns regarding ozone depletion in the stratosphere, multiple efforts have been carried out to develop alternatives less aggressive to the environment. The use of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) has increased in recent years, and has become essential to maintain strawberry (*Fragaria ananassa* Duch.) production in Southern Spain, the main producer within the EU. However, both pesticides have uncertain effects on the environment. Nematodes play important roles in the soil system and can be used as indicators of soil diversity and functioning. The main objective of this study was to evaluate the effects of 1,3-D and Pic on soil biota and functioning in a commercial strawberry crop in Southern Spain. Soil was sampled before treatment and 5, 17, 28, and 35 weeks after soil treatment (WAT). Soil nematodes, soil physical-chemical properties, soil pesticide residues and plant growth were analyzed within the treated field, at the field edges and at an adjacent natural vegetation area. 5 WAT low amounts of 1,3-D and Pic were detected in the soil, which had disappeared 17 WAT. 49 nematode taxa were found in the study area. Of them, 30 showed significantly different abundances among habitats after treatments were applied. Nematode diversity was always lower within the treated area than in adjacent habitats. The effects of soil fumigants after treatment were significant for all soil food web and diversity indices. Some seasonal effects were also detected. Soil pH, phosphorous, carbon, and NO<sub>3</sub><sup>-</sup> were the soil properties most strongly related to soil food web and nematode diversity.

## New records of longidorid nematodes from Central Peru

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Nematological surveys in the central region of Peru showed the occurrence of three longidorid species. Adult and juvenile *Longidorus laevicapitatus* and *Xiphinema imambaksi* were recovered from soil collected in replicated samples from the rhizosphere of *Citrus* spp. and grasses in a research farm at Marona Baja (Tingo Maria, Huanuco). The *X. imambaksi* population contained four juvenile stages. Specimens of *X. ensiculiferum* were also found in samples collected from grasses on the Mazan river bank (Iquitos, Loreto) and at Acomayo (Huancavelica) in the rhizosphere of potato. All species recovered represent new nematological records for the country. Research funded by EU STD contract CI1.CT94.0041.

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## Observations on root-knot nematodes from asparagus crops in Peru and associated antagonists

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A study of root-knot nematode (RKN) parasites of asparagus and associated biological antagonists was carried out in Northern Peru. RKN were identified by means of standard procedures and ITS sequencing. Nematode attacking fungi were isolated and cultured in vitro and maintained in a culture collection for further characterization. The RKN species recovered included *Meloidogyne incognita* and *M. ethiopica*. Antagonists included the diplogasterid predatory nematode *Koerneria sudhausi*, that multiplied in petri plates inoculated with asparagus roots and soil. Nematode attacking fungi identified through standard morphological methods as well as by sequencing of the ITS regions included *Drechslerella brochopaga*, *Lecanicillium psalliotae* and an unidentified *Monacrosporium* sp. *Meloidogyne ethiopica* and the antagonist species recovered represent new records for the country. Research funded by CNR-CONCYTEC Bilateral Agreement.

## Assessment of sensitivity of soil nematode assemblage to heavy metal pollution

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Changes in soil nematode community structure are interesting biological tools to assess disturbances in the soil ecosystem. Mine workings gravely affect the environment, whose discharges and waste are a continuous source of risk for heavy metal pollution. This work aims to assess the spatial dispersion at a metallic pollution site and to study the effects of lead/zinc pollution and soil uses on a soil nematode population. The study area is the Sierra Minera abandoned mine (Murcia, Spain) and its surroundings. Total and bioavailable lead and zinc were determined in 153 composite soil samples, along with Shannon's, Simpson's, maturity, plant parasite, basal, structure and channel indices, and the enrichment, structure, functional herbivore and composite metabolic footprints from the nematode community study. Geostatistical techniques were used to analyse spatial variations. The effects of two factors on these chemical and biological variables are discussed. The grid factor (as a distance of mine workings) only significantly affected heavy metal content. The soil uses factor (with 6 levels: crop fields, unproductive crop land, mine bare residues, mine residues with vegetation, mine area with natural vegetation, and mine area unproductive) significantly affected both variables, as did the grid  $\times$  soil uses interaction. The amount of bioavailable lead exceeded the generic reference values in the three grids; this occurred only in grid 1 and with some treatments in grid 2. Functional indices were better indicators of soil disturbances than diversity ones, and the bacterial channel metabolic footprint improved the separation among significant differences.

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## Soil nematode assemblages in post-mining areas of Sierra Minera, Murcia (Spain)

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Soil nematode assemblage, the food web and footprint were assessed in a 200 km<sup>2</sup> area of post-mining works and surroundings in the "Sierra Minera", Murcia (Spain). One hundred eighty-four composite samples were collected. Grids of 3x3 km in non-mining areas, 1x1 km in intermediate zones and 0.5x0.5 km in areas with more mining points were established, mainly in bare soils of mining waste, fallow lands, crop lands, untilled habitats and scrublands. A total of 81 genera were discriminated, five of which were dominant (5% relative abundance): *Acrobelloides*, *Aphelenchus*, *Eudorylaimus*, *Filenchus* and *Helicotylenchus*. The mean nematode density ranged from no individuals in bare soils of mining waste to more than two thousand individuals in natural scrublands. Nematode assemblages were dominated by bacterial feeders, mainly in crops, untilled and fallow lands. Fungal feeders appeared frequently in untilled lands and scrubland, omnivores and plant feeders in crops and fallow lands, while carnivores emerged only in scrublands. When comparing sampled habitats, measurable differences were found in soil nematode abundance, diversity, and enrichment indices. No significant differences were observed in MI and related ratios. Differences appeared only in PPI between scrubland and bare soils of mining waste. The means in the enrichment footprint did not significantly differ, although the values obtained were very low in bare soils and mine afforested residues. The structure footprint values differed significantly.

## Suppression of *Meloidogyne arenaria* race 1 by *Pasteuria penetrans* on vegetables

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*Pasteuria penetrans* causes suppression of root-knot nematodes in agricultural fields. Generally speaking at least three years are required since *Pasteuria* is added to soil until its densities can induce suppression. In 2001, a *Pasteuria* specific to *Meloidogyne arenaria* race 1 was transferred from a suppressive field to a site free from this beneficial organism. High densities of *M. arenaria* race 1 were added. Peanut was grown in spring-summer months followed by common hairy vetch as a winter cover crop. Peanut had been grown over the site each summer for the past nine years. In spring 2011, the field was arranged in a split plot design with eight replicates. Four vegetable crops grown on the site included tomato cv. Bella Rosa, cucumber cv. Cobra, okra cv. Clemson Spineless and squash cv. Golden Summer. The objective was to determine whether the soil suppressiveness would be maintained on vegetables or whether the site had become infested with other root-knot nematode species. The vegetables served as the main crop and the split plot was fumigated with 1,3-dichloropropene applied broadcast at 112 L/ha. Each plot was covered with aluminized metallic polyethylene film and irrigation and fertilizer were applied via drip tubes. Plot size was 6 m long rows arranged on 1.8 m centers. Data collected included root-knot nematode galling on each crop, number of juveniles recovered from each plot, and the number of endospores attached per juvenile. At the end of this 10-year experiment, the field site remains highly suppressive to *M. arenaria*. No other species of root-knot nematode were detected in the site.

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## Nematodes associated with different arthropod species in Córdoba, Argentina

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There is no information available about nematodes parasitizing diplopods for the province of Córdoba. Specimens of *Argentocricus nodulipes* (n=10) (Rhinocricidae), *Orthoporus americanus* (n=5) (Spirostreptidae), and *Platinodesmus cordobensis* (n=10) (Chelodesmidae) were collected from Córdoba city. The arthropods were dissected, the hindgut was excised and the nematodes present were removed. Nematodes were identified based on the analysis of morphological characters. Individuals of *A. nodulipes* were parasitized by juveniles and adults of the genera *Rhigonema* (Family Rhigonematidae), *Heth* (Hethidae), and representatives of the family Carnoyidae. All specimens exhibited 100% prevalence (P). Females of *Thelastoma* sp. (Thelastomatidae) were detected in *O. americanus*, with a P value of 40%. Males and females of *Thelastoma* sp. were found in the intestine of *P. cordobensis* (P=10%), whereas juveniles of *Oscheius myriophila* (Rhabditidae) (P=30%) were found in the host's hemocele. The high prevalence of the nematodes detected in *A. nodulipes* is noticeable. Except for the genus *Thelastoma*, the remaining nematodes observed are reported for the first time in Argentina.



## Factors associated with young peach trees death in Morelos, Mexico

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Morelos is one of the ten main states of Mexico producing peach fruits. However, peach production in Morelos has been limited by a disease of unknown etiology that caused severe losses. Aim of this work was to determine the possible factors associated with young peach tree death. In 23 orchards of the principal municipalities (Tetela del Volcán, Ocuituco, Zacualpan) samplings of soil, roots and other diseased tree parts were carried out to detect fungi, nematodes or bacteria and to determine soil texture and pH. At the same time in each orchard the owners were interviewed to get informations about cultural practices and other abiotic factors. In all sampled diseased trees parts the bacterium *Pseudomonas syringae* pv. *syringae* was found, but no significant fungal species was detected in roots. Furthermore, in most orchards, nematodes of the family Criconematidae were found. The main species found were *Criconemoides xenoplax* and *Criconema mutabile*, the first associated with the peach tree short life disease and the second with the bacterial canker complex. Soils were sandy loam, and pH fluctuated between 4.1 and 6.4. Records of frosts did not exist in the producing zone but prunings were made when plants were more susceptible to *P. syringae*. Criconematids, *P. syringae*, pH and pruning time were hence considered to originate a complex of factors involved in the young peach trees death observed.

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## Selected aspects on the morphology and parasitism biology of the nematophagous fungus *Pochonia chlamydosporia* var. *chlamydosporia*

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The nematophagous fungus *Pochonia chlamydosporia* (Clavicipitaceae) can parasitize eggs of plant-endoparasitic nematodes such as cyst (*Globodera* spp., *Heterodera* spp.), root-knot (*Meloidogyne* spp.), false-root knot (*Nacobbus* spp.) and reniform nematode (*Rotylenchulus reniformis*). Its potential as a biological control agent has been the subject of numerous studies to understand the micro-ecological conditions and host-parasite relationships that allow the fungus to thrive in the soil, the rhizosphere environment and in soils suppressive to nematodes. However, some aspects such as the morphology and parasitism biology of the fungus have not been fully examined. In order to exploit the fungus effectively in regulating plant endo-parasitic nematodes, a careful selection of fungal isolates (biotypes) appropriate for both host plant and nematode is essential. Ongoing studies on the biology and physiology of *P. chlamydosporia* parasitism using light and cryo-scanning electron microscopy have provided new information on morphological structures including chlamydospores, as well as aerial and submerged conidia. The potential to interchange genetic material between fungal isolates has started to be explored. Selected isolates of different nematode biotypes assessed both microscopically and molecularly for anastomosis have shown differences in mating groups. It is expected that the combination of classical and molecular approaches to study the biology and physiology of parasitism will contribute to increase the effectiveness of this microorganism as a biopesticide of plant-parasitic nematodes in different agroecosystems.

## Native isolates of entomopathogenic nematodes in Spain and Egypt and development of *Meloidogyne javanica* on tomato

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Two native isolates of entomopathogenic nematodes (EPN) were isolated in Spain and Egypt, respectively, and tested against *Meloidogyne javanica* on tomato, in controlled conditions. The EPN populations were isolated from vegetable and fruit tree crops in each country by the *Galleria mellonella* soil trap method. The EPN population from Spain belonged to the genus *Steinernema*, while the isolate from Egypt was identified as *Heterorhabditis bacteriophora*. Two trials were then carried out in Barcelona (Spain) and Cairo (Egypt) with the local EPN populations, and different EPN inocula: 0, 1, 5 and 10 *H. bacteriophora* infective juveniles per cm<sup>3</sup> of soil (Egypt), and 0, 1, 500, 1000, 5000 and 10 000 infective juveniles of *Steinernema* spp. per cm<sup>3</sup> of soil (Spain). Commercial isolates of *S. feltiae* and *H. bacteriophora* were also included for comparison. Pots of 1000 cm<sup>3</sup> capacity with a sterilized sand substrate were inoculated with the EPN and juveniles of a population of *M. javanica*. Susceptible tomato plants were transplanted and grown until *M. javanica* completed one generation, counting the number of egg masses (Egypt) or eggs (Spain) per plant at the end of the trial. The population development of *M. javanica* in soil inoculated with the EPN was similar to uninoculated soil, showing that the densities were not affected by the isolates of EPN used in the test, nor by their origin (native or commercial), or levels of infestation. This work was funded by AECID-PCI-Mediterranean A/017988/08-023072/09.

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## ***Pasteuria penetrans* - a better understanding**

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*Pasteuria penetrans* has been studied for many years with the hope of deploying this hyperparasite for the biological control of root-knot nematodes. From under the microscope to field deployment, we explore the complexities of *P. penetrans* biology and discuss how this knowledge can be used by researchers wishing to find and utilize *Pasteuria* spp. as a biological control agent. Using schematic diagrams, the relationship between the life cycle of *P. penetrans* and temperature are illustrated and why infection in the female nematodes can often be overlooked. An alternative way of calculating the different spore densities required to achieve attachment in different soil types will be discussed.

## Nematicidal effect of BCPBIO in banana parasitic nematodes

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The banana culture is of great economic importance to Madeira Island, but phytoparasitic nematodes are very common in plantations. The implementation of control strategies against the major nematodes that parasitize banana roots is therefore of utmost importance. With this goal in mind, an assay was developed in order to evaluate the efficacy of the biological product BCPBIO to control nematodes like *Pratylenchus goodeyi*, *Meloidogyne* sp. and *Helicotylenchus multicinctus*, among others. The treatments chosen were as follows: BCPBIO at 3 kg/ha; BCPBIO at 4 kg/ha; BCPBIO at 4 kg/ha with organic matter; BCPBIO 4 kg/ha with adjuvant TSF 0.2% v/v; organic matter by adding a product based on 100% vegetal organic matter; SCRO 135 B WP 1.5 kg/ha; the nematicide oxamyl at 3 to 4 kg/ha and the untreated control, in four replications. Soil and root samples were collected at three different times, before products application, four months after the first application and four months after the second application. The main phytoparasitic nematodes present were identified and quantified, as well as the free-living species. Statistical analysis by ANOVA (GLM) showed significant differences over time and treatments only for some nematode species, as the application of BCPBIO 4 kg/ha and BCPBIO 4 kg/ha with adjuvant was more effective mainly for *P. goodeyi* and free-living nematodes present in roots and soil. Therefore, the application of BCPBIO helped reduce some nematode populations present in the soil and promoted biological balance of the nematological fauna. However, further studies are needed to determine the direct effect of these products on nematodes, the most efficient doses and possibly their mode of action.

## Parasitism of *Meloidogyne incognita* eggs from *Pochonia chlamydosporia* in the presence of plant extracts

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*Meloidogyne* spp. are important plant-parasitic nematodes all over the world. There are several alternatives to control these pests without inducing a collateral damage to the environment. They include use of a great number of nematode antagonistic plants that affect several plant-parasitic species. Other control alternatives consider the use of the fungus *Pochonia chlamydosporia*, an egg parasite of cyst and root-knot nematodes that can reduce the damage they cause. Since both methods could be complementary in nematode control, aim of this study was to select antagonistic plants that do not affect the parasitism capacity of *P. chlamydosporia* (when used in combination) on *Meloidogyne* spp. eggs. A bioassay was made adding 5% aqueous extracts to water-agar medium, for each of 22 plant species assayed. After sterilization and pouring of media, a chlamydospore suspension ( $5 \times 10^6$ ) was added per Petri dish and then incubated for 48 h. Subsequently, a suspension of *Meloidogyne* eggs ( $\pm 500$ ) was added. Finally, all treatments were incubated at 27 °C for 48 h, with 72 h for the control without extracts. Egg parasitism (%) was measured and analyzed by ANOVA and Tukey means separation ( $P=0.05$ ). The best treatments were *Chenopodium album*, *Nerium oleander* and *Tagetes lucida*, showing 80% parasitism by the fungus and similar to control. These results offer the possibility to combine plant extracts with *P. chlamydosporia* as part of an integrated control management of *Meloidogyne* spp.

## Two rare species nematodes from lichen and moss (Nematoda: Cephalobida) from America

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Two genera (*Geraldus* and *Diastolaimus*) from the family Chambersiellidae Thorne, 1937 were collected in tropical (Ecuador) and temperate (Mexico) forest environments. The *Geraldus* species has the head separated from the body by a conspicuous constriction; oesophagus 141-207 ( $179\pm 4.5$ )  $\mu\text{m}$  long, isthmus short, 32-45 ( $37\pm 1.7$ )  $\mu\text{m}$  long, and the vulva in gravid females is placed on a prominent vulval cone and covered with a gelatinous-like material. There is a pair of vulval glands. The male is similar to the female, with a single testis extending anteriorly, not reflexed. Rectal glands present. Tail curved ventrally, 78-98 ( $98\pm 89$ )  $\mu\text{m}$  long, ending in a dorsally hooked tip. Pre-cloacal and post-anal papillae present. Two spicules slightly curved 44-59 ( $49\pm 2.0$ )  $\mu\text{m}$  long, conspicuous gubernaculi 20-25 ( $22\pm 0.7$ )  $\mu\text{m}$  long. The *Diastolaimus* is characterized by the oesophagus length of 189-236 ( $216\pm 216$ )  $\mu\text{m}$ , isthmus 60-92 ( $76\pm 7.6$ )  $\mu\text{m}$  long, female and male tail 70-88 ( $78\pm 5.2$ ) and 61-82 ( $71\pm 5.6$ )  $\mu\text{m}$  long, respectively, and gubernaculum 16-21  $\mu\text{m}$  long.

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## ***Belonolaimus* species (Tylenchida: Belonolaimidae) from tropical forest in Mexico**

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A new *Belonolaimus* species was collected in tropical forest at La Mancha, Experimental Ecological Station of the Ecology Institute, Veracruz State, Mexico. The female is characterized by a body 1.4-1.8 (1.6±1.6) mm in length; stylet 85-103 (96±4.6) µm long; excretory 146-195 (178±13.2) µm from the anterior end; vulval lips not protruding and vagina without sclerotized pieces; tail hemispherical, 68-96 (83±9.1) µm long. Spicules slightly curved, 25-34 (30±3.0) µm long, gubernaculum 10-15 (13±2.3) µm long, the anterior flexure 3-4 (3±0.5) µm long. Molecular characterisation of a new *Belonolaimus* species using the D2-D3 expansion fragments of 28S rRNA and ITS rRNA gene sequences is also provided. The phylogenetic relationships of new species with other representatives of the genus are presented and discussed.



## Occurrence of *Pratylenchus* species in different regions of Brazil

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*Pratylenchus* species are among the most harmful to world agriculture. In Brazil, some species of the nematode attack crops of great importance for the Brazilian economy. The aim of this study was to conduct a survey to identify which species of *Pratylenchus* are present on different crops in several states of Brazil. Species identification was based on morphology and morphometrics. At least ten specimens of each population were killed by heat, mounted on temporary slides in water, and observed with light microscopy. The measurements were made on scanned images using the software Image-Pro Plus® 4.1. A total of 75 *Pratylenchus* populations from 12 Brazilian states and Federal District were studied. The species of this genus that were identified as present in the different states and respective crops were: *Pratylenchus brachyurus* in coffee, pineapple, corn, cotton, *Sorghum*, sugarcane and soybeans; *P. coffeae* in banana and graviola (*Annona muricata*) - soursop; *P. jaehni* in *Citrus*; *P. penetrans* in *Amaryllis*, lily and soybeans; *P. vulnus* in rose and *P. zaeae* in sugarcane, corn, *Dracaena*, peanuts and soybeans.

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## Identification of Longidoridae (Dorylaimida) species from Minas Gerais state, Brazil

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There have been relatively few nematological investigations to determine the occurrence and distribution of longidorid nematodes in Minas Gerais State, Brazil. In this study data are presented based on 126 soil samples collected from different habitats of 12 cities of Minas Gerais State during February and March of 2010. Nematodes were extracted from a 1 kg sub-sample of soil by a modified decanting and sieving technique, heat-killed at 60 °C and processed to anhydrous glycerine using a slow method. Species identification and measurements were made using a high power microscope with the aid of a camera lucida. Seven *Xiphinema* species were identified (*X. brasiliense*, *X. diffusum*, *X. elongatum*, *X. ensiculiferum*, *X. krugi*, *X. variegatum* and *X. surinamense*) and also two *Xiphidorus* species (*X. cf. parthenus* and *X. amazonensis*). The most frequently occurring species were *X. krugi* (44.9% of all samples) and *X. variegatum* (43.9%). Populations of *X. krugi*, *X. elongatum*, *X. ensiculiferum* and *Xiphidorus cf. parthenus* showed high morphometrical variability. None of the recorded species has been reported as a virus-vector. *Xiphinema diffusum*, *X. variegatum*, *X. ensiculiferum* and both *Xiphidorus* species constitute new records for Minas Gerais.

## Morphometric variability in populations of *Xiphinema krugi* (Dorylaimida, Longidoridae) from Minas Gerais state, Brazil

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*Xiphinema krugi* is a widespread species of Longidoridae often found in tropical and subtropical regions of the world. Populations of this species exhibit large morphological and morphometric variability besides the well-known genotypic variation. Considering the recent discovery of 44 populations of this species in different municipalities in the State of Minas Gerais, the aim of this study was evaluate its morphometric variability. Nematodes were extracted from a 1 kg sub-sample of soil by a modified decanting and sieving technique, heat-killed at 60 °C and processed to anhydrous glycerine using a slow method. Measurements were made using a high power microscope with the aid of a camera lucida. The evaluated characters were body length, odontostylet and odontophore length, tail length, diameter of the body and diameter of the body in the region of the anus, and the value V. The average of these characters in the 44 populations plus the average of them in standard populations of four distinct genotypes, described in the literature (*i.e.*, A, B, C and D), were subjected to principal component analysis. Populations formed five distinct groups, but most of them formed a group with the type species and the standard population C. Two populations have clustered to standard A, eight to standard B and two did not cluster to any of the patterns, which indicates that these are a genotype of *X. krugi* not yet characterized. These results reinforce the need for re-examination of the taxonomic status of *X. krugi*.

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## Genetic variability of pathogenic isolates of *Meloidogyne incognita* to cotton as revealed by molecular markers

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*Meloidogyne incognita* is wide spread and recognized as a major pathogen of cotton (*Gossypium hirsutum* L.) and in cotton producing regions of Brazil. The objective of this study was to evaluate the genetic variability of *M. incognita* populations from cotton using RAPD (Random Amplification of Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter Simple Sequence Repeat) markers. Five isolates of *M. incognita* were obtained from different geographic regions in Brazil. For all isolates, isoenzyme analysis revealed the typical patterns of esterase Est I1 (Rm: 1.0) and Est I2 (Rm: 1.05 and 1.1) for *M. incognita*. One isolate of *M. enterolobii* was included as the outgroup. DNA amplifications were done using 22 RAPD; 9 ISSR and 13 AFLP primers. Cluster analyses were done for UPGMA (Unweighted Pair Group Method with Arithmetic Mean). Only one isolate from Luis Eduardo Magalhães – BA revealed relevant polymorphic fragments. Despite the existence of two races (race 3 and 4) and two esterase profiles (I1, I2), a low genetic variability among isolates was observed, which might be related to the mitotic parthenogenetic reproduction.

## Search for pinewood nematode control using essential oils isolated from Portuguese aromatic flora

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a major worldwide *Pinus* pathogen and pest, with impact on forest health, natural ecosystem stability and international trade. Essential oils (EOs) may be an alternative to the current control options. Seventy three EOs from 46 species from the Portuguese aromatic flora were screened for in vitro nematocidal activity against PWN. The suitability of Triton X-100 or acetone as EOs diluting agent was assessed; accuracy of results using the two solvents was compared and the latter found preferable. One hundred percent mortality was achieved with EOs from *Chamaespartium tridentatum*, *Origanum vulgare*, *Ruta graveolens*, *Satureja montana*, *Thymbra capitata* and *Thymus caespititius* at concentrations ranging between 0.5 and 2.0 mg/mL, and those oils analyzed by GC and GC-MS. The dominant components of the effective oils were 1 octen-3-ol (9%), n nonanal and linalool (both 7%) in *C. tridentatum*, carvacrol (36% and 39%),  $\gamma$ -terpinene (24% and 40%) in *O. vulgare* and *S. montana*, 2-undecanone (> 90%) in *R. graveolens* and carvacrol (75% and 65%) in *T. capitata* and *Th. caespititius*. Jorge Faria is grateful to the FCT for the PhD grant SFRH/BD/43738/2008. Partially funded by FCT under research contract PTDC/AGR-AAM/74579/2006.

## Intraspecific and intra-isolate variability in the ITS rDNA region sequence of *Bursaphelenchus xylophilus*

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Genetic diversity of the rDNA internal transcribed spacer (ITS) region, among and within pinewood nematode (PWN) *Bursaphelenchus xylophilus* isolates, was evaluated. The ITS region of North American, Portuguese and Japanese PWN isolates was amplified by PCR and directly sequenced. Multiple peaks were observed in sequencing chromatograms of the American and Japanese isolates, suggesting the presence of more than one ribosomal sequence for each isolate. PCR products were further cloned and 10 clones of each isolate were then sequenced. Phylogenetic analysis revealed that sequences obtained from a single isolate did not always cluster together. The 10 sequences of the American isolate formed a relatively homogeneous group while the sequences of the Japanese and Portuguese isolates were loosely distributed among the sequences of other PWN isolates. The intra-isolate ITS molecular variability detected in the American isolate was higher (21 variable sites) than in the Japanese (9) and Portuguese (8) isolates. One of the variable sites in the American isolate changed a HhaI recognition site, altering the predicted restriction pattern from virulent to avirulent isolates. Although this site was not found as a variable site among the 10 sequenced clones of the Japanese isolate, it corresponds to one of the multiple peaks observed in the direct PCR product sequencing, revealing that this isolate also contains both variants. In the Portuguese isolate, no variability was found in this nucleotide position. Further work should be conducted in order to better understand the genetic diversity and evolutionary history of this pathogenic species.

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## Nematicidal activity of essential oil-bearing plants, from Portuguese flora, against the pinewood nematode *Bursaphelenchus xylophilus*

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Most synthetic chemicals used to control phytoparasites are toxic to humans and animals, and can accumulate in the soil and in food plants. The bioactive potential of essential oils (EOs) has been recognized, albeit empirically, for centuries. In the present work, the nematicidal potential of EOs extracted from 16 *Eucalyptus* spp., six *Thymus* spp. and four *Mentha* spp., from the Portuguese flora, were assessed. EOs were extracted by hydrodistillation and identified by GC and GC-MS. EO/methanol stock-solutions were added to 50-100 mixed-stage *B. xylophilus* suspensions to attain 2 and 1 µl EO/ml of suspension final concentration. After 24h in darkness, dead and live nematodes were counted. Assays were repeated, at least, 10 times in 2 series. Of all *Eucalyptus* spp. EOs, only the citronellal-rich (36%) *E. citriodora* EO showed 97% mortality, at 2 µl/ml. *Thymus zygis*, *T. vulgaris* and one chemotype of *T. caespititius* EOs revealed 100% mortality at 2 and 1 µl/ml. *T. zygis* and *T. vulgaris* EOs were thymol-rich (≈50%) and *T. caespititius* EO was carvacrol-rich (58%). *Mentha arvensis* EO, dominated by piperitone oxide (56%), also revealed to be an effective nematicide, showing 100% mortality in both concentrations. Additional studies are being carried out to identify the most effective components from each oil. Acknowledgement: Jorge Faria is grateful to the Fundação para a Ciência e a Tecnologia (FCT) for the PhD grant SFRH/BD/43738/2008.

## **Axial distribution of the pinewood nematode, *Bursaphelenchus xylophilus*, in *Pinus pinaster* trees**

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Early detection of the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is of primary importance to enable rapid actions to prevent the spread and introduction of this nematode into new areas. Without efficient sampling methods, the results of surveys will not be adequately reliable. The main objective of this study is to understand the axial distribution of PWN in *Pinus pinaster*, in order to define the best sampling sites for an early PWN detection. *Pinus pinaster* trees were classified into six pine wilt disease (PWD) symptom classes based on general wilting of the needles: 0 - tree without symptoms, I - 10% reddish brown needles, II - 10-50% reddish brown needles, III - 50-80% reddish brown needles, IV - 80% reddish brown needles and V - dead tree without needles. Cross-sections of the trunk (1.5 m and 6-8 m from the base of the trunk) and crown branches of trees with different symptom classes were collected and nematodes extracted using the tray method. *Bursaphelenchus xylophilus* was identified on the basis of species-specific diagnostic morphological characters and quantified. The results revealed that the distribution of PWN in the host tree varied with the developmental stage of PWD. In trees exhibiting advanced symptoms, PWN was detected in higher numbers at the trunk and, in trees with early symptoms, PWN was detected in higher numbers at the branches of the crown. In order to perform a more effective sampling for the detection of PWN, it is recommended that wood samples should be obtained according to the developmental stage of the disease and from different parts of the tree.

01

02

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04

05

06

07

08

09

10

11

P  
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## Investigation of the nematocidal activity in *Serratia* sp. and characterization of its nematocidal extracellular proteases

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, has been thought to be the only causal agent of pine wilt disease (PWD), however lately bacteria have been suggested to play a role in PWD. Several studies have shown that bacteria from various genera, associated with *B. xylophilus*, could be isolated. In Portugal, healthy pine trees of the species *Pinus pinaster* contain a diverse endophytic microbial community. The nematocidal ability was analyzed for 46 strains isolated associated to nematodes from trees with PWD. Screening of these isolates revealed the presence of bacterial strains producing different extracellular products. Strains more toxic to the nematodes belonged to the genus *Serratia*. Strains from *S. marcescens* have already been isolated in association with *B. xylophilus* J2 from *P. densiflora* collected in Korea. The objectives of the present work were to identify and characterize the hydrolytic enzymes produced by the *Serratia* isolates and to assess their nematocidal activity. The toxicity assays revealed that only two fractions, separated by FPLC, showed nematocidal activity compared to the whole extract. These fractions showed higher toxicity rates and included different concentrations of two different proteases. The use of inhibitors selective to serine proteases or to metalloproteases demonstrated that serine protease was most responsible for the toxicity of the supernatant. Understanding the infection process and all the factors involved is important in order to develop strategies to control *B. xylophilus* dispersion.

## **Bacteria associated with *Bursaphelenchus xylophilus* and other nematodes isolated from *Pinus pinaster* trees affected by pine wilt disease**

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The importance and potentially devastating impact of tree diseases was recognized early in the 20th century with severe epidemics associated with the introduction of new pathogens to native forest ecosystems. The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, has been thought to be the only causal agent of pine wilt disease (PWD), however, since bacteria have been suggested to play a role in PWD, it is important to know the diversity of the microbial community associated to it. This study aimed to assess the microbial community associated with the PWN and with other nematodes isolated from pine trees, *Pinus pinaster*, with PWD from three different affected forest areas in Portugal. Bacterial strains (143) were isolated from nematodes collected from 14 *P. pinaster* trees. The bacterial strains were identified by 16S rRNA gene partial sequence. All, except one Gram-positive strain (Actinobacteria), belonged to the Gram-negative Beta and Gammaproteobacteria. Most predominant genera are *Pseudomonas*, *Burkholderia* belonging to the family Enterobacteriaceae. Species isolated in higher percentage were *P. lutea*, *Yersinia intermedia* and *B. tuberum*. The major bacterial population associated to the nematodes differed according to the forest area and none of the isolated bacterial species was found in all forest areas. The isolates produced 60 to 100% of siderophores and at least 40% produced lipases that enable these bacteria to play a role in plant physiological response. This research showed a high diversity of the microbial community associated with *B. xylophilus* and other nematodes isolated from *P. pinaster* with PWD.

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02

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04

05

06

07

08

09

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P  
12

## Developing an adaptive management system for predicting and mitigating damage caused by the pinewood nematode *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae) in Portugal

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Pine wilt disease (PWD) is complex and until now, after decades of intensive research, there has been no agreement on a single factor determining its development. The present project will work with different competing models that could explain the occurrence of PWD: a) Tree resistance: phytochemicals (e.g. stilbenoids); b) Climate: moisture, temperature, photosynthesis; c) Community interactions: Mutualistic and antagonistic fungi and bacteria as a determinant factor of pathogenicity and vector selection; c) Vector population dynamics: dispersion, reproduction and vector nematode load; d) Evolution of the epidemics at the landscape level. These models will be integrated in a general model for optimizing the management of the pinewood nematode (PWN). We will use a Bayesian Belief Network where the influence diagram establishes the relationship between the different competing models, allowing the inclusion of variables representing the experience of different consultants and forest managers, evaluating the best models and assisting decision-making. Thus, this general model will allow us to include risk and uncertainty, for optimizing the management of the PWN, and improve communication between scientists and forest managers, using the adaptive management concept. The model can be updated as new information is obtained through monitoring programmes. The concept of adaptive management provides tools to achieve these objectives in a changing decision-making environment, favouring advice to management, instead of simply testing scientific hypotheses.

## Extraction of *Bursaphelenchus xylophilus* from wood and bark using zonal centrifugation

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The pinewood nematode, *Bursaphelenchus xylophilus*, is a quarantine organism in the EU. Legislation requires that samples from surveys in standing trees and imported wood products are analyzed for the presence of this nematode. The extraction method recommended by EPPO is incubation on Baermann funnel. We use the automated zonal centrifuge, an apparatus designed at ILVO, for routine extraction of nematodes from soil and plants. Earlier research (EU project FAIR1-CT95-0034) showed that the extraction efficiency of *B. xylophilus* from wood with the zonal centrifuge is similar to that with the Baermann funnel. Lately, we have been using the zonal centrifuge to detect *B. xylophilus* in imported (ornamental) bark, a commodity inspected more frequently in recent years. Extraction of *B. xylophilus* from bark with the zonal centrifuge was optimized using "spiked" bark (soaked in water infested with cultured *B. xylophilus*). Blending bark prior to nematode extraction, as done for other plant materials, resulted in cut pieces of nematodes, making subsequent morphological identification impossible. Therefore, similar to wood, 50 g bark is chopped into small pieces and incubated for 24 h in water. This material is then passed over an 850- $\mu$ m sieve and nematodes are extracted from the resulting aqueous suspension, using zonal centrifugation. The extraction efficiency of *B. xylophilus* from spiked bark was > 85% for both the zonal centrifuge and the Baermann funnel in mistifier. A proficiency test with naturally infested bark and wood could tell us more about the detection efficiency of the zonal centrifuge compared with other techniques.

## A

<b>Abad, Pierre</b>	..... O4 <sup>(2X)</sup> .....	31, 33
<b>Aballay, Erwin O.</b>	..... O10 .....	76
<b>Abd-Elgawad, Mahfouz M. M.</b>	..... P10 .....	147
<b>Abolafia-Cobaleda, Joaquín</b>	..... O11 .....	81
	..... P2 .....	107
	..... P11 .....	156
<b>Abrantes, Isabel</b>	..... O10 .....	72
	..... O12 <sup>(6X)</sup> .. 92, 93, 94, 96, 97, 99	
	..... P7 .....	126
	..... P8 <sup>(4X)</sup> .....	128, 129, 131, 137
	..... P12 <sup>(4X)</sup> .....	161, 163, 164, 165
<b>Achard, Raphael</b>	..... O8 .....	66
<b>Adão, Helena</b>	..... P2 .....	104
<b>Agaras, Betina</b>	..... O10 .....	73
<b>Ahmad, Wasim</b>	..... P11 .....	157
<b>Ahumada Ortiz, Teresa</b>	..... P3 <sup>(2X)</sup> .....	110, 111
	..... P8 .....	134
<b>Akyazi, Faruk</b>	..... P10 .....	143
<b>Almeida, Maria R. A.</b>	..... O7 <sup>(2X)</sup> .....	53, 55
	..... P11 .....	158
<b>Almeida, Maria Teresa M.</b>	..... P3 .....	109
	..... P6 .....	117
	..... P8 .....	129
<b>Alonso-Prados, José Luis</b>	..... P9 .....	138
<b>Alvarado, Ysaías</b>	..... P5 .....	114
<b>Álvarez-Ortega, Sergio</b>	..... O11 .....	81
	..... P11 .....	156
<b>Alves, Ana S.</b>	..... P2 .....	104
<b>Alves, Raquel Campos</b>	..... O12 .....	92
<b>Amorim, António</b>	..... O12 .....	96
<b>Amorim, Regina M. S.</b>	..... O4 .....	34
<b>Andrés, Maria Fe</b>	..... P6 <sup>(2X)</sup> .....	115, 116

<b>Anthoine, Géraldine</b>	..... O7 <sup>(3X)</sup> .....	51, 56, 58
	..... P7 .....	125
<b>Arantes, Neylson E.</b>	..... P1 .....	103
<b>Arosa, Maria Luísa</b>	..... P8 .....	129
<b>Azevedo, Alexandra</b>	..... P10 .....	149
<b>Azevedo, Bruce</b>	..... P2 .....	105
<b>Azevedo, Luísa</b>	..... O12 .....	96

## B

<b>Bagshaw, John</b>	..... O6 .....	46
<b>Bakker, Jaap</b>	..... O11 .....	77
<b>Barbosa, Pedro</b>	..... O12 .....	95
	..... P12 <sup>(2X)</sup> .....	160, 162
<b>Bardgett, Richard D.</b>	..... O10 .....	74
<b>Barroso, Cristina</b>	..... O12 .....	97
<b>Barroso, José G.</b>	..... P12 .....	160
<b>Batista, Maria T.</b>	..... P8 .....	128
<b>Becerra, Alejandra</b>	..... O10 .....	73
<b>Belchior, Almerinda</b>	..... P3 .....	109
<b>Bello, Antonio</b>	..... O6 <sup>(2X)</sup> .....	41, 42
	..... P6 .....	122
<b>Benavides, Ingrid Varela</b>	..... P7 .....	124
<b>Beneventi, Magda A.</b>	..... O4 .....	34
<b>Bessi, Rosana</b>	..... P2 .....	108
<b>Bighiu, Anca</b>	..... O12 .....	168
<b>Birkett, Michael A.</b>	..... O8 .....	60
	..... P8 .....	135
<b>Blackshaw, Rod P.</b>	..... P8 .....	135
<b>Blok, Vivian</b>	..... O2 .....	18
<b>Bonifácio, Luis</b>	..... O12 .....	89

<b>Borges, José G.</b>			
..... P12 .....	166		
<b>Bork, Jusek</b>			
..... P10 .....	149		
<b>Borrero, Yusney</b>			
..... O5 .....	39		
<b>Botelho, Alessandra P.</b>			
..... O11 .....	82		
<b>Botelho, Cesar E.</b>			
..... P4 .....	112		
<b>Bravo-Luna, Leticia</b>			
..... P10 .....	145		
<b>Brito, Janete A.</b>			
..... O7 .....	54		
<b>Buena, Ana Piedra</b>			
..... O6 .....	42		
<b>Burillo, Jesus</b>			
..... P6 .....	115		
<b>Burns, Dennis</b>			
..... O6 .....	50		
<b>Buzo, Tom R.</b>			
..... O3 .....	29		
<hr style="width: 30%; margin: 10px auto;"/> C			
<b>Cabello, Marta</b>			
..... O10 .....	73		
<b>Caccia, Milena G.</b>			
..... O10 .....	73		
..... P5 .....	113		
<b>Campos-Herrera, Raquel</b>			
..... O9 .....	69		
<b>Cantalapiedra-Navarrete, Carolina</b>			
..... P7 .....	124		
<b>Cardoso, Joana M. S.</b>			
..... O12 .....	94		
..... P12 .....	161		
<b>Cares, Juvenil</b>			
..... P2 .....	105		
<b>Carmona, Wayner Montero</b>			
..... P7 .....	124		
<b>Carneiro, João</b>			
..... O12 .....	96		
<b>Carneiro, Regina M.D.G</b>			
..... O7 <sup>(2X)</sup> .....	53, 55		
..... P8 .....	132		
..... P11 .....	158		
<b>Carrascosa, Marina</b>			
..... P9 .....	138		
<b>Carvalho, Gladyston R.</b>			
..... P4 .....	112		
<b>Castagnone-Sereno, Philippe</b>			
..... O7 .....	53		
..... O8 .....	62		
..... O12 .....	85		
..... P11 .....	158		
<b>Castillo, Pablo</b>			
..... O2 .....	19		
..... P7 .....	124		
<b>Cerasoli, Sofia</b>			
..... O12 .....	93		
<b>Cerejeira, Maria José</b>			
..... P12 .....	167		
<b>Chabrier, Christian</b>			
..... O8 .....	66		
<b>Chaverri, Fabio</b>			
..... P2 .....	106		
<b>Ciancio, Aurelio</b>			
..... O10 .....	70		
..... P9 <sup>(2X)</sup> .....	139, 140		
<b>Cid del Prado Vera, Ignacio</b>			
..... O11 .....	84		
..... P11 <sup>(2X)</sup> .....	151, 152		
<b>Cobon, Jenny</b>			
..... O6 .....	46		
<b>Colagiero, Mariantonietta</b>			
..... P9 .....	140		
<b>Conceição, Luci</b>			
..... O12 <sup>(2X)</sup> .....	96, 97		
..... P8 .....	128		
<b>Costa, Maria J.</b>			
..... P2 .....	104		
<b>Costa, Sofia R.</b>			
..... O10 .....	74		
..... P8 .....	129		
<b>Costa, Viviane P. da</b>			
..... P8 .....	130		
<b>Crow, William T.</b>			
..... O6 .....	45		
..... P7 .....	127		
<b>Crozzoli, Renato</b>			
..... O5 .....	40		
<b>Cruz, Roy</b>			
..... P2 .....	106		
<b>Cubillán, Nestor</b>			
..... O2 .....	24		
..... P5 .....	114		
<b>Culman, Steven W.</b>			
..... O9 .....	68		
<b>Cunha, Maria José</b>			
..... P8 .....	128		
<b>Curtis, Rosane H. C.</b>			
..... O8 <sup>(2X)</sup> .....	60, 61		
..... P7 .....	126		
..... P8 <sup>(3X)</sup> .....	131, 135, 137		
<hr style="width: 30%; margin: 10px auto;"/> D			
<b>Damme, Nicole</b>			
..... P12 .....	168		
<b>Davies, Keith G.</b>			
..... O10 <sup>(2X)</sup> .....	71, 74		

de Almeida Engler, Janice	
..... O4.....	33
..... O8.....	62
De Luca, Francesca	
..... O2.....	19
De Veylder, Lieven	
..... O4.....	33
Decraemer, Wilfrida	
..... O2.....	20
Deimi, Abbas M.	
..... O7.....	55
Del Valle, Eleodoro	
..... P5.....	113
Delgado, Rui	
..... O12.....	90
den Nijs, Loes	
..... O7.....	56
Devonshire, Jean	
..... P10.....	146
Diane, Tânia F. S. Santos	
..... P6.....	123
Dias, Luís S.	
..... P12.....	160
Dias, Waldir Pereira	
..... P6.....	123
Dickson, Donald W.	
..... O3.....	25
..... O7.....	54
..... P10.....	143
Díez-Rojo, Miguel A.	
..... O6 <sup>(2X)</sup> .....	41, 42
..... P6.....	122
Dinardo-Miranda, Leila L.	
..... P8.....	130
Díz-Viruliche, Luisa	
..... O5.....	39
Djigal, Djibril	
..... O8.....	66
Doroh, Mark C.	
..... O10.....	75
Doucet, Marcelo E.	
..... O10.....	73
..... P5.....	113
..... P8.....	136
..... P10.....	144
Duarte, Aida	
..... P8.....	131
Duarte, Isabel M.	
..... P3.....	109
Duarte, Joana	
..... P6.....	117
Dueñas, Juan C. Rondan	
..... P5.....	113
..... P10.....	144
Duncan, Larry W.	
..... O9.....	69

Dutta, Tushar	
..... O8.....	60
Duyck, Pierre-François	
..... O8.....	66
Dybal, Katarzyna	
..... O8.....	61

## E

Egas, Conceição	
..... O12 <sup>(2X)</sup> .....	97, 98
..... P7.....	126
El-Borai, Fahiem E.	
..... O9.....	69
Engler, Gilbert	
..... O4.....	33
..... O8.....	62
Enrique, Roberto	
..... O5 <sup>(2X)</sup> .....	39, 40
Escuer, Miguel	
..... P9 <sup>(2X)</sup> .....	141, 142
Espada, Margarida	
..... O12.....	95
Esquivel-Hernández, Alejandro	
..... O11.....	81
..... P2 <sup>(2X)</sup> .....	106, 107
..... P7.....	124
Esteves, André M.	
..... O11.....	82
Esteves, Iván	
..... O2.....	24
..... P5.....	114
Esteves, Ivânia	
..... O10.....	72
Eulálio, Juliana	
..... P6.....	120

## F

Faria, Jorge M. S.	
..... P12 <sup>(2X)</sup> .....	160, 162
Favoreto, Luciany	
..... P1.....	103
Fenández-Herrera, Ernesto	
..... O1.....	16
Ferris, Howard	
..... O8.....	65
..... O9.....	68
..... O11.....	84
Figueiredo, Ana Cristina	
..... P12 <sup>(2X)</sup> .....	160, 162
Figueiredo, Joana	
..... O12.....	97
Finetti-Sialer, Mariella M.	
..... O10.....	70

..... P9 .....	140
..... P10 .....	146
<b>Firmino, Alexandre A. P.</b>	
..... O4 .....	34
<b>Flores-Lara, Yolanda</b>	
..... O8 .....	64
<b>Flores-Moctezuma, Hilda E.</b>	
..... P6 .....	118
..... P10 .....	150
<b>Fonseca, Luís</b>	
..... O12 <sup>(6X)</sup> ... 92, 93, 94, 96, 97, 99	
..... P12 <sup>(4X)</sup> ..... 161, 163, 164, 165	
<b>Fonseca-Genevois, Verônica da</b>	
..... O11 .....	82
<b>Fontes, Ana M.</b>	
..... O12 .....	89
<b>Fracasso, Juliano V.</b>	
..... P8 .....	130
<b>Francisco, Romeu</b>	
..... O12 .....	99
..... P12 .....	165
<b>Freitas, Helena</b>	
..... P8 .....	129
<b>Furlaneto, Cleber</b>	
..... P11 .....	158

## G

<b>Galhano, Cristina I. C.</b>	
..... P6 .....	119
<b>Gamboa-Angulo, M. Marcela</b>	
..... P6 .....	116
<b>Gamel, Sylvie</b>	
..... O7 <sup>(2X)</sup> .....	51, 58
..... P7 .....	125
<b>Gardenal, Cristina N.</b>	
..... P5 .....	113
<b>Gaur, Harish S.</b>	
..... O8 .....	60
..... O10 .....	71
<b>Gheysen, Godelieve</b>	
..... O8 .....	63
<b>Giblin-Davis, Robin M.</b>	
..... O2 .....	22
<b>Giora, Fabiana C.</b>	
..... P6 .....	120
<b>Gleason, Cynthia A.</b>	
..... O4 .....	32
<b>Godínez-Vidal, Damaris</b>	
..... O1 .....	16
<b>Gomes, Ana C. M. M.</b>	
..... O7 .....	55
<b>Gomes, Cesar B.</b>	
..... P8 .....	132
<b>Gomes, Paula</b>	
..... O12 .....	97

<b>Gómez, Lucila</b>	
..... O5 .....	39
<b>Gonzaga, Vilmar</b>	
..... P2 .....	105
..... P11 .....	153
<b>González Bernal, S.</b>	
..... P8 .....	134
<b>González, Esteban</b>	
..... O5 .....	39
<b>González-Carcedo, Salvador</b>	
..... P6 .....	122
<b>González-Coloma, Azucena</b>	
..... P6 <sup>(2X)</sup> .....	115, 116
<b>Gowen, Simon</b>	
..... P10 .....	148
<b>Grossi-de-Sá, Maria de Fátima</b>	
..... O4 .....	34
<b>Grove, Ivan G.</b>	
..... O3 .....	30
<b>Guerra, Mayamarú</b>	
..... O2 .....	24
..... P5 .....	114
<b>Guerrero, Pablo</b>	
..... O11 .....	81
<b>Guevara-Olvera, Lorenzo</b>	
..... O1 .....	16
<b>Guilherme, Rosa</b>	
..... P8 .....	128
<b>Gutiérrez, Carmen</b>	
..... P9 <sup>(2X)</sup> .....	141, 142
<b>Gutiérrez-Yáñez, Flor D.</b>	
..... P6 .....	118

## H

<b>Hanson-Snortum, Paul</b>	
..... P2 .....	107
<b>Harakava, Ricardo</b>	
..... P2 .....	108
<b>Haydock, Patrick P.J.</b>	
..... O3 .....	30
<b>Hedden, Peter</b>	
..... O8 .....	61
<b>Helder, Johannes</b>	
..... O11 .....	77
<b>Henriques, Joana</b>	
..... O12 .....	89
<b>Hernández, Mario</b>	
..... O5 .....	39
<b>Hernández, Miguel A.</b>	
..... O5 .....	39
<b>Hernández-Ochandía, Dainé</b>	
..... O5 .....	39
<b>Hernández-Villalobos, Silvia</b>	
..... P7 .....	124



<b>Hewlett, Thomas</b>	
..... O10 .....	75
<b>Hilje, Irena</b>	
..... P2 .....	106
<b>Hockland, Sue H.</b>	
..... O2 <sup>(2X)</sup> .....	20, 23
<b>Hongli, Ji</b>	
..... O8 .....	63
<b>Huchet, Emilie</b>	
..... O7 <sup>(2X)</sup> .....	51, 58
..... P7 .....	125

---

<b>Inácio, Maria L.</b>	
..... O12 .....	89
<b>Insera, Renato N.</b>	
..... O2 .....	19

---

<b>Jackson, Louise E.</b>	
..... O9 .....	68
<b>Jesus, Dalila S.</b>	
..... P11 <sup>(2X)</sup> .....	154, 155
<b>Jesús-Navarrete, Alberto</b>	
..... O11 .....	83
<b>Jovicich, Elio</b>	
..... O6 .....	46

## K

---

<b>Kakaire, Stephen</b>	
..... O3 .....	30
<b>Kaku, Stephanie R.</b>	
..... O3 .....	29
<b>Kanzaki, Natsumi</b>	
..... O2 .....	22
<b>Karegar, Akbar</b>	
..... O11 .....	77
<b>Karsen, Gerrit</b>	
..... O7 .....	55
..... O11 .....	77
<b>Kenyon, David</b>	
..... O2 .....	19
<b>Kerry, Brian R.</b>	
..... O8 .....	61
..... O10 .....	74
<b>Khan, Junaid A.</b>	
..... O10 .....	71
<b>Khurma, Uma R.</b>	
..... O7 .....	59
..... O8 .....	67

<b>Klapp, Alexander K.</b>	
..... O6 .....	47
<b>Knapič, Matej</b>	
..... O7 .....	52
<b>Köhler Solís, A.</b>	
..... P8 .....	134
<b>Kolombia, Yao</b>	
..... P11 .....	156
<b>Kubo, Roberto K.</b>	
..... P2 .....	108
..... P6 .....	120
<b>Kukulies, Tegan</b>	
..... O6 .....	46
<b>Kyndt, Tina</b>	
..... O8 .....	63

---

<b>Lamoreaux, Mary</b>	
..... O5 .....	37
<b>Landell, Marcos G. A.</b>	
..... P8 .....	130
<b>Lax, Paola</b>	
..... O10 .....	73
..... P5 .....	113
..... P8 .....	136
..... P10 .....	144
<b>Leroux-Nio, Anne-Claire</b>	
..... O7 <sup>(2X)</sup> .....	51, 58
..... P7 .....	125
<b>Liébanas, Gracia</b>	
..... O11 .....	81
..... P11 <sup>(2X)</sup> .....	156, 157
<b>Lima, Rita de C. C.</b>	
..... O11 .....	82
<b>Lira, Viviane F.</b>	
..... O11 .....	82
<b>Liu, Qingli L.</b>	
..... O4 .....	32
<b>Lopes, André</b>	
..... P12 .....	163
<b>Lopes, Pollyanna S.</b>	
..... P6 .....	121
<b>López, Lidia</b>	
..... O5 .....	39
<b>López-Cepero, Javier</b>	
..... O6 .....	42
<b>López-Martínez, Noé</b>	
..... O1 .....	16
<b>López-Pérez, Antonio</b>	
..... O6 <sup>(2X)</sup> .....	41, 42
..... P6 .....	122
<b>López-Robles, Javier</b>	
..... O6 <sup>(2X)</sup> .....	41, 42
..... P6 .....	122

Lousada, José L.	
..... O12 .....	92
Luna-Guerrero, Assael Y.	
..... P10 .....	145
Lundstedt, Jorge	
..... P3 <sup>(2X)</sup> .....	110, 111

---

## M

Machado, Andressa C. Z.	
..... P2 .....	108
Machado, Óscar C.	
..... P8 .....	128
Magnusson, Christer	
..... O12 .....	86
Magunacelaya, Juan Carlos	
..... O3 .....	26
..... O6 .....	44
..... P3 <sup>(2X)</sup> .....	110, 111
..... P8 <sup>(2X)</sup> .....	133, 134
Maleita, Carla M. N.	
..... P7 .....	126
..... P8 .....	129
Mancilla, Roberto	
..... P3 <sup>(2X)</sup> .....	110, 110
Manzanilla-López, Rosa H.	
..... O10 .....	72
..... P10 <sup>(2X)</sup> .....	146, 150
Marques, João C.	
..... P2 .....	104
Marro, Nicolás	
..... O10 .....	73
Mårtensson, Anna	
..... O10 .....	76
Martins, Ana F.	
..... P8 .....	129
Maselli, Anna	
..... O5 .....	40
Mattos, Vanessa S.	
..... O7 .....	53
..... P11 .....	158
Mauchline, Tim H.	
..... O10 .....	71
McClure, Michael	
..... O8 .....	64
McGawley, Edward C.	
..... O6 .....	50
McKenry, Michael V.	
..... O3 .....	29
Melo, Filipe	
..... P8 .....	128
Mendes, Marta D.	
..... P12 .....	160
Miller, Anthony J.	
..... O8 .....	61
Miranda, Victoria	
..... O5 .....	36

Mizobutsi, Edson	
..... P6 .....	121
Moens, Maurice	
..... O7 .....	57
..... P12 .....	168
Mohan, Sharad	
..... O10 .....	71
Molina, Ramón	
..... P2 .....	106
Molinari, Sergio	
..... O1 .....	17
Montes-Belmont, Roberto	
..... P10 <sup>(2X)</sup> .....	145, 150
Mooyman, Paul	
..... O11 .....	77
Morais, Paula V.	
..... O12 .....	99
..... P12 <sup>(2X)</sup> .....	164, 165
Moreira, Cláudia	
..... O12 .....	96
Mota, Manuel	
..... O4 .....	33
..... O12 <sup>(2X)</sup> .....	95, 96
..... P12 <sup>(2X)</sup> .....	160, 162
Moura, Glaucia	
..... P1 .....	103
Murga-Gutiérrez, Santos Nélide	
..... P9 .....	140

---

## N

Nadler, Steven A.	
..... O11 .....	84
Nahar, Kamrun	
..... O8 .....	63
Navarro, Patricia D.	
..... O5 .....	37
Naves, Pedro	
..... O12 .....	89
Neher, Deborah A.	
..... OPENING LECTURE .....	11
Neres, Patricia F.	
..... O11 .....	82
Nguyen, Duong	
..... P11 .....	159
Nietsche, Silvia	
..... P6 .....	121
Nyczepir, Andrew P.	
..... O7 .....	54

---

## O

Oka, Yuji	
..... O1 .....	15

<b>Olalla, Casilda</b>	
..... O6.....	41
..... P6.....	122
<b>Oliva-Rivera, José Juan</b>	
..... O11.....	83
<b>Oliveira, Antonio Carlos B.</b>	
..... P4.....	122
<b>Oliveira, Cláudio M. G.</b>	
..... P2.....	108
..... P6.....	120
..... P11 <sup>(2X)</sup> .....	154, 155
<b>Oliveira, Rosângela D. L.</b>	
..... P11 <sup>(2X)</sup> .....	154, 155
<b>Oliveira, Solange</b>	
..... O12.....	95
<b>Ollivier, Fabrice</b>	
..... P7.....	125
<b>Ornat, Cesar</b>	
..... P10.....	147
<b>Overstreet, Charles</b>	
..... O6.....	50

---

**P**

<b>Paiva, Gabriel</b>	
..... O12.....	99
..... P12.....	164
<b>Palomares-Rius, Juan E.</b>	
..... P7.....	124
<b>Papadopoulos, Apostolos</b>	
..... P8.....	135
<b>Pappas Jr, Georgios J.</b>	
..... O4.....	34
<b>Pathak, Ekta</b>	
..... O9.....	69
<b>Pattison, Anthony B.</b>	
..... O6.....	46
<b>Pedelini, Ricardo</b>	
..... P8.....	136
<b>Pedro, Luís G.</b>	
..... P12.....	160
<b>Pembroke, Barbara</b>	
..... P10.....	148
<b>Peña-Santiago, Reyes</b>	
..... O2.....	21
..... O11 <sup>(2X)</sup> .....	79, 81
..... P11 <sup>(3X)</sup> .....	156, 157, 159
<b>Peraza-Padilla, Walter</b>	
..... P7.....	124
<b>Peraza-Sánchez, Sergio R.</b>	
..... P6.....	116
<b>Pereira, Antonio Alves</b>	
..... P4.....	112
<b>Pereira, Celeste</b>	
..... O12.....	92

<b>Pereira, Filipe</b>	
..... O12.....	96
<b>Pereira, Gabriel H.</b>	
..... P1.....	103
<b>Pereira, João S.</b>	
..... O12.....	93
<b>Pereira, Thamiris B.</b>	
..... P4.....	112
<b>Pereira-Castro, Isabel</b>	
..... O12.....	96
<b>Persson, Paula</b>	
..... O10.....	76
<b>Pestana, Margarida</b>	
..... P10.....	149
<b>Peteira, Belkis</b>	
..... O5 <sup>(2X)</sup> .....	39, 40
<b>Pickett, John A.</b>	
..... P8.....	135
<b>Pickup, Jon</b>	
..... O2.....	18
<b>Pimenta, Livia</b>	
..... P6.....	121
<b>Pimentel, Carla S.</b>	
..... P12.....	166
<b>Pinheiro, Miguel</b>	
..... O12 <sup>(2X)</sup> .....	97, 98
<b>Pinto-Tomas, Adrian</b>	
..... P2.....	107
<b>Pita, Pedro</b>	
..... P10.....	149
<b>Portillo, Edgar</b>	
..... O2.....	24
..... P5.....	114
<b>Powers, Stephen J.</b>	
..... O8.....	60
..... P8.....	135
<b>Prior, Thomas J.</b>	
..... O2 <sup>(2X)</sup> .....	20, 23
<b>Proença, Diogo N.</b>	
..... O12.....	99
..... P12 <sup>(2X)</sup> .....	164, 165
<b>Puente, Liliana</b>	
..... O5.....	40

---

**Q**

<b>Quénéhervé, Patrick</b>	
..... O3.....	27
..... O8.....	66

---

**R**

<b>Rad, Carlos</b>	
..... O6.....	41

Ramirez, Cecilia	
..... P8 .....	133
Rao, Uma	
..... O10 .....	71
Reid, Alex	
..... O2 .....	18
Reis, Carlos	
..... P10 .....	149
Reva, Valeria	
..... O12 .....	92
Rezende, Juliana C.	
..... P4 .....	112
Ribeiro, Neucimara R.	
..... P6 .....	123
Ribeiro, Regina C. F.	
..... P6 .....	121
Ricardo, Alexandra C.	
..... P12 .....	166
Rissoli, Vandor	
..... P2 .....	105
Roca, Francesco	
..... P9 .....	139
Rocha, Leandro S.	
..... P6 .....	121
Rocha-Sosa, Mario	
..... O1 .....	16
Rodrigues, José Manuel	
..... O12 .....	88
Rodríguez, Jose A.	
..... P9 <sup>(2X)</sup> .....	141, 142
Rodríguez, Mayra G.	
..... O5 <sup>(2X)</sup> .....	39, 40
Rodríguez-Kabana, Rodrigo	
..... O6 <sup>(4X)</sup> .....	43, 47, 48, 49
Rojas-Martínez, R. I.	
..... O1 .....	16
Rosales, Ligia C.	
..... O5 <sup>(2X)</sup> .....	39, 40
Rosso, Laura Cristina	
..... P9 .....	140
Rosso, Marie-Noëlle	
..... O8 .....	62
Ruiz Rojas, D.	
..... P8 .....	134
Ryan, Michael F.	
..... P6 .....	119
<b>S</b>	
Sá, Maria Eugénia L. de	
..... O4 .....	34
Sainz, Paula	
..... P6 .....	115
Salazar, Efrain	
..... O5 .....	40
Salgado, Sonia M. L.	
..... P4 .....	112
San-Blas, Ernesto	
..... O2 .....	24
..... O5 .....	38
..... P5 .....	114
Sanches, João	
..... P12 .....	162
Sánchez, Gerardo	
..... O12 .....	87
Sánchez-Moreno, Sara	
..... O9 .....	68
..... P9 .....	138
Santos, Carla S.	
..... O12 .....	98
Santos, Jaime M.	
..... P11 .....	153
Santos, José António dos	
..... O12 .....	91
Santos, M. Susana N. de A.	
..... P6 .....	119
Santos, Márcia	
..... O12 .....	93
Santos, Marcilene M. F. A.	
..... O7 <sup>(2X)</sup> .....	53, 55
Santos, Susana S.	
..... O12 .....	99
..... P12 .....	164
Sanz, Jesus	
..... P6 .....	115
Sasaki-Crawley, Ayano	
..... P8 .....	135
Sayadi, Samir	
..... O3 .....	28
Sekora, Nicholas S.	
..... P7 .....	127
Signorini, Chaiane B.	
..... P8 .....	132
Silva, Felipe L.	
..... P4 .....	112
Silva, Maria Cristina da	
..... O11 .....	82
Silva Jr, Orzenil B.	
..... O4 .....	39
Simmons, Lee J.	
..... O6 <sup>(3X)</sup> .....	47, 48, 49
..... O10 .....	75
Simões, Maria José	
..... O12 .....	97
..... P7 .....	126
Singh, Richard R.	
..... O7 .....	59
..... O8 .....	67
Širca, Saša	
..... O7 .....	52
Somavilla, Lúcia	
..... P8 .....	132

<b>Sorribas, Francisco J.</b>	
..... P10.....	147
<b>Sousa, Edmundo</b>	
..... O12.....	89
<b>Stamler, Rio A.</b>	
..... O7.....	54
<b>Stanciola, André R.</b>	
..... P11.....	154
<b>Stare, Barbara Geric</b>	
..... O7.....	55
<b>Stefanoski, C.</b>	
..... P6.....	123
<b>Stock, S. Patricia</b>	
..... O5 <sup>(2X)</sup> .....	36, 37
<b>Strajnar, Polona</b>	
..... O7.....	52
<b>Stuart, Robin J.</b>	
..... O9.....	69
<b>Suárez, Susana A.</b>	
..... P8.....	136
<b>Suárez, Zoraida</b>	
..... O5.....	40
<b>Subbotin, Sergey A.</b>	
..... P11.....	152

## T

<b>Talavera-Rubia, Miguel F.</b>	
..... O3.....	28
..... O4.....	35
..... P10.....	145
<b>Tanning, Lirette M.</b>	
..... O7.....	57
<b>Taylor, C. Robert</b>	
..... O6 <sup>(3X)</sup> .....	47, 48, 49
<b>Teillet, Alice</b>	
..... O8.....	61
..... P8.....	131
<b>Tenente, Renata C. V.</b>	
..... P2.....	105
..... P11.....	153
<b>Thomas, Stephen H.</b>	
..... O7.....	54
<b>Tietz, Aline T.</b>	
..... P8.....	132
<b>Tigano, Myrian S.</b>	
..... O7.....	53
<b>Tixier, Philippe</b>	
..... O8.....	66
<b>Togawa, Roberto C.</b>	
..... O4.....	34
<b>Tordable, María del C.</b>	
..... P8.....	136
<b>Torres, Elsa Carbonell</b>	
..... P9.....	139

<b>Trindade, Manuel</b>	
..... P12.....	167
<b>Troccoli, Alberto</b>	
..... O2.....	19

## U

<b>Urek, Gregor</b>	
..... O7.....	52

## V

<b>Vagelas, Ioannis</b>	
..... P10.....	148
<b>Valadas, Vera</b>	
..... O12.....	95
<b>Vale, Raquel</b>	
..... O12.....	93
<b>Valverde, Claudio</b>	
..... O10.....	73
<b>van Asch, Barbara</b>	
..... O12.....	96
<b>van den Elsen, Sven</b>	
..... O11.....	77
<b>van Megen, Hanny</b>	
..... O11.....	77
<b>Vasconcelos, Marta W.</b>	
..... O12.....	98
<b>Velásquez, Liliana</b>	
..... O5.....	90
<b>Verdejo-Lucas, Soledad</b>	
..... O3.....	28
..... O4.....	35
<b>Verissimo, Paula</b>	
..... O12.....	99
..... P12.....	164
<b>Viaene, Nicole</b>	
..... O7 <sup>(2X)</sup> .....	56, 57
..... P12.....	168
<b>Viegas, Domingos X.</b>	
..... O12.....	92
<b>Vieira Santos, M. Clara</b>	
..... O10.....	72
..... P8.....	137
..... P12.....	165
<b>Vieira, Margarida</b>	
..... O12.....	89
<b>Vieira, Paulo</b>	
..... O4.....	33
..... O8.....	62
<b>Vilchis-Martínez, Kathia</b>	
..... P6.....	118
..... P10.....	150
<b>Villar-Luna, Edgar</b>	
..... O1.....	16

<b>Vinciguerra, Maria Teresa</b>	
..... O11 .....	78
<b>Vovlas, Nicola</b>	
..... O2.....	19
<b>Vu, Tam T.</b>	
..... P11 .....	159

---

**W**

<b>Walker, Andrew</b>	
..... O8.....	65
<b>Wesemael, Wim M. L.</b>	
..... O7.....	57
<b>Williamson, Valerie M.</b>	
..... O4.....	32

---

**X**

<b>Xavier, Adelica A.</b>	
..... P6 .....	121

---

**Y**

<b>Young-Mathews, Anna</b>	
..... O9.....	68

---

**Z**

<b>Zavaleta-Mejía, Emma</b>	
..... O1.....	16
<b>Zheng, Liang</b>	
..... O8.....	65
<b>Zullini, Aldo</b>	
..... O11 .....	80

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