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ORAL PRESENTATIONS / PRESENTACIONES ORALES

PERSPECTIVES IN NEMATOLOGY RESEARCH, CROP PROTECTION AND FOOD SECURITY [PERSPECTIVAS EN LA INVESTIGACIÓN NEMATOLOGICA, PROTECCIÓN DE CULTIVOS Y LA SEGURIDAD ALIMENTARIA]

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Nematology originated as a specific monophyletic science stemming from more general disciplines like zoology and natural history, and early studies concerned descriptions and taxonomy. Research work was intensified in the 50s, when monocultures of industrial cropping systems required effective nematode management tools. Actual research is part of advanced endeavours, largely multidisciplinary, integrating approaches ranging from ecology to molecular biology and genome studies. As defined by FAO, food security means that all people have the right to sufficient and safe food. A fundamental ethic commitment of research in agriculture is then how to satisfy, in space and time, any dietary need and preference for a healthy life. A key issue concerns the transfer and application of main achievements and knowledge in other regions of the world, with different needs. Challenges include facing the consequences of demography and climate changes or other threats present. We have to recognize that the magnitude of the problems is much more demanding than ever in the history of agriculture, in terms of research and technology transfer. Given the social, political or economic roots of many food security threats, nematology may only partially contribute to reach this goal. Actual advances of scientific knowledge, spanning from genomes to plant biology and ecosystem services are, however, very significant. To solve or at least alleviate some of the food security problems affecting many rural systems and communities, nematology may contribute to reach specific milestones through the international cooperation. Fundamental are freedom and right for access to education and scientific information for everyone, and the support of global efforts aiming at an independent production of knowledge.

NEMATODE COMMUNITIES AS ECOLOGICAL INDICATORS OF ECOSYSTEM HEALTH [COMUNIDADES DE NEMATODOS COMO INDICADORES ECOLÓGICOS DE SALUD DEL ECOSISTEMA]

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Nematodes have attributes that make them useful as ecological indicators. Various kinds of perturbations to soils, such as addition of mineral or organic nitrogen fertilizer, cultivation, and accumulation of heavy metals affect the species richness, trophic structure and successional status of nematode communities. Through a series of experiments on sampling and experimental design at various spatial scales, it is concluded that maturity and trophic diversity indices are capable of differentiating among sampling sites better and more efficiently than measures based on populations or ratios of individual trophic groups. Maturity and trophic diversity indices measure different aspects of soil communities and are complementary when used together. 'Maturity' is a measure of successional status and trophic diversity measures food web structure. There are two major impediments to implement nematode communities in large-scale environmental monitoring programs, i.e., ecological interpretation and accessibility to non-specialists capable of identifying a multitude of free-living nematode taxa. Molecular probes are one way to expedite identification and enumeration of nematodes within whole community samples. Although this approach is available to non-taxonomists, it does not address concerns about interpretation and potential errors in assignments. Alternatively, nematode community indices would be more cost-effective and interpretable if ambiguous genera were removed and indices reduced to include sentinel taxa with known sensitivity or response to specific types of disturbance. Sentinel taxa will represent a subset of nematode communities but require knowledge of species assemblage patterns under different scenarios of management practices, which often represent a complicated mixture of abiotic and biotic factors. Once sentinel taxa are identified, subsequent studies are needed to verify their sentinel status to determine the geographic or ecological range of their utility.

INTEGRATED TAXONOMIC STUDIES HIGHLIGHT THE VIRUS VECTOR FAMILY TRICHODORIDAE AS A SOURCE OF MANY UNKNOWN CRYPTIC SPECIES IN SPAIN [ESTUDIOS TAXONÓMICOS INTEGRADOS DESTACAN A LA FAMILIA VECTORA DE VIRUS TRICHODORIDAE COMO FUENTE DE MUCHAS ESPECIES CRÍPTICAS DESCONOCIDAS EN ESPAÑA]

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The polyphagous root ectoparasitic family Trichodoridae occurs worldwide. Its major pest status is as vector of Tobraviruses, especially in the didelphic genera *Paratrichodorus* and *Nanidorus*, with 27% and 28.6% of vector species, respectively. Current study focused on the biodiversity of *Paratrichodorus* from southern Spain. Identification of *Paratrichodorus* species is hampered even more than in *Trichodorus*, by their largely conserved morphology and restricted number of diagnostic morphological features, overlap of morphometrics, difficulty to fix specimens properly and co-occurrence of at least two species in the same soil sample, often with a restricted number of specimens. Molecular analyses based on nuclear ribosomal RNA genes (D2-D3 expansion segments of 28S and partial 18S gene) clearly directs the comparative morphological study while the morphology and morphometrics helps the molecular research to interpret the results when dealing with co-occurrence of several species. Surveys for trichodorids were carried out in cultivated and natural habitats in southern Spain. The integrative taxonomic approach revealed several new species belonging to two main clades: (1) a *P. hispanus* related group, characterized in male by large sperm cells with sausage-shaped nucleus and spicules with undulating outline of anterior blade part, and (2) a *P. allius* related group with small sperm and nucleus and about straight spicules with finely striated blade. The first group is common within the Iberian Peninsula, while the second group of species occurs in general in warmer (Mediterranean) to subtropical climates. This study strengthens the need for integrative taxonomy in this group of nematodes because of their high molecular biodiversity and similar morphology and morphometrics with examples of cryptic diversity.

REVERSE TAXONOMY, PHYLOGENY, AND DNA BARCODING TO ILLUMINATE THE DIVERSITY OF APHELENCHOIDES [TAXONOMÍA INVERSA, FILOGENIA, Y CÓDIGO DE BARRAS GENÉTICO PARA ILUSTRAR LA DIVERSIDAD DE APHELENCHOIDES]

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Aphelenchoidea comprises nematodes with a high variety of feeding types and ecology roles; mycophagous, predators as well as insect and plant parasites are represented in this superfamily. Among plant-parasites, species of *Bursaphelenchus* and *Aphelenchoides* have an important impact on several plant species. Selected taxa of both genera have been characterized and the resulting molecular analyses revealed a monophyletic origin of *Bursaphelenchus* but the phylogeny and the evolution of the plant-parasitism within *Aphelenchoides* not yet well understood. Moreover, species of the genera *Laimaphelenchus* and *Schistonchus* are found within the clade of *Aphelenchoides*, confirming its origin is not monophyletic and that the family needs major revision. Little is known about the phylogenetic relationship depicted by molecular data in relation to the morphology-based classification. For example, four groups of *Aphelenchoides* (based on the tail shape) are commonly referred in literature, but such grouping lacks molecular evidence despite its taxonomical importance. Furthermore, some molecular data of *Aphelenchoides* spp. in GenBank appear to be completely wrong, but these data cannot be checked because morphological vouchers are absent. To tackle these problems, we will record the detailed morphology of several *Aphelenchoides* specimens from different locations and several substrates and unequivocally link these morphological data with molecular data. With this information it will be possible to plot morphological and ecological data on a molecular phylogenetic tree, providing a better insight in the evolution of plant-parasitism as well as the taxonomic status of the genus. By this integrative work and by reverse taxonomy, unknown molecular clades that are supported with morphological information can serve as a well-founded starting point for species descriptions.

ON THE VARIABILITY OF THE STOMATAL PROTRUSIBLE STRUCTURE IN THE GENUS *SECTONEMA* THORNE, 1930 (DORYLAIMIDA: APORCELAIMIDAE) AND ITS TAXONOMICAL CONSEQUENCES [SOBRE LA VARIABILIDAD DE LA ESTRUCTURA EVAGINABLE DEL ESTOMA EN EL GÉNERO *SECTONEMA* THORNE, 1930 (DORYLAIMIDA: APORCELAIMIDAE) Y SUS CONSECUENCIAS TAXONÓMICAS]

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The genus *Sectonema* is a remarkable and widely distributed dorylaimid nematode taxon. It has been reported from five continents (it is apparently absent in Antarctica) and nowadays it contains 24 valid species. Originally classified under Dorylaimidae and nowadays under Aporcelaimidae, the taxonomy of *Sectonema* has suffered relevant changes after its proposal. Some of these taxonomical decisions deserve further analyses. The study of abundant material, belonging to the genus *Sectonema*, mainly from Spain and Vietnam as well as the re-examination of the type material of some species of the genus has allowed a deeper analysis of the variability observed in the stomatal protrusible structure on the different members of the genus. Taking into account the new data now available, four tentative patterns may be identified. The type species of the genus, *S. ventrale*, shows a special odontostyle rather than a mural tooth. The species before belonging to the genus *Aporcelaimoides*, now regarded as identical to *Sectonema*, has a mural protruding structure bearing dorsal aperture. A few species present a true, typical mural tooth resembling that found in nygolaims (Nygolaimina). And a small group of species show a reduced odontostyle.

SPECIES OF THE CYST-FORMING NEMATODE CACTODERA (NEMATODA: HETERODERIDAE) FROM MEXICO [ESPECIES DEL NEMATODO FORMADOR DE QUISTE CACTODERA (NEMATODA: HETERODERIDAE) DE MÉXICO]

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Different populations of species in the genus *Cactodera*: *C. galinsogae*, *C. evansi*, *C. rosae*, *C. salina*, and *C. torreyanae* have been found in different host plants in México: in carnation (*Dianthus caryophyllus*), barley (*Hordeum vulgare*), Amaranthus (*Amaranthus hybridus*), and two populations from salty soils in *Saliconia bigelovii* (Chenopodiaceae), and *Suaeda edulis*. The objective of this study was to compare the morphology of the species described from México using light and scanning microscope. One of the key morphological characters to separate the species is the size of the vulva cone; in *Cactodera rosae* and *C. torreyanae* it is very conspicuous and in *C. salina*, *C. galinsogae*, and *C. evansi* it is small and poorly developed. Another important morphological character to separate the Mexican species is the ornamentation of the corion of the egg: in *C. galinsogae*, *C. evansi*, and *C. rosae* the corion has distinct punctations, and in *C. torreyanae*, and *C. salina* it is smooth. The size of the cyst of *C. rosae* on average is 650 µm; between 523 to 575 µm are *C. galinsogae* and *C. torreyanae*, and the smallest cyst is *C. evansi* with 459 µm in length. Other morphological characters are the cuticle ornamentation of the cyst between the vulva and anus and the distance between these characters. All of these morphological characters are important to separate the currently known Mexican species.

RECORDS OF FIFTEEN RING NEMATODES (NEMATODA: CRICONEMATIDAE) REPORTED FOR THE FIRST TIME IN COSTA RICA [REGISTRO DE QUINCE NEMATODOS DE ANILLOS (NEMATODA: CRICONEMATIDAE) REPORTADOS POR PRIMERA VEZ EN COSTA RICA]

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This study focuses on the family Criconematidae, a taxon commonly known as the ring nematodes due to the accentuated transverse annulation of the cuticle. Criconematidae nematodes have been found worldwide except on the Antarctic continent. All species are plant parasites and they have a cosmopolitan distribution. It has been documented that many species attack vegetable crops and fruit trees. The occurrence of criconematidae nematodes was studied in several crops, vegetation and regenerated forest. This study presents the results of a survey primarily focused on cultivated soils of Costa Rica, including an area of regeneration. The host plant, pictures and morphological characters of the nematode species were documented. A total of fifteen species were recovered and described from 33 local soil samples collected between 2010 and 2013 in 30 cities in Costa Rica. These nematodes belong to 5 genera in 15 families, including *Criconema mutabile*, *C. neopacificum*, *C. graminicola*, *Criconemoides silvicola*, *C. informis*, *Crossonema civellae*, *Mesocriconema onoense*, *M. sphaerocephalum*, *M. rusticum*, *M. ornatum*, *M. anastomosis*, *M. denoudenii*, *Ogma decalineatus*, *O. octangularis*, and *Ogma* sp. All species except *Mesocriconema sphaerocephalum* are new records in Costa Rica. It was not possible to identify an *Ogma* species and according to all measurements it is a new species, but not reported for the world.

DIVERSITY AND DISTRIBUTION OF NEMATODE ASSEMBLAGES IN PUNTA FRANCÉS CORAL REEF, CUBA [DIVERSIDAD Y DISTRIBUCIÓN DE LAS COMUNIDADES DE NEMATÓDOS MARINOS EN EL ARRECIFE CORALINO DE PUNTA FRANCÉS, CUBA]

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We studied the diversity and distribution of free-living nematodes in Punta Frances coral reef (Cuba). The selected habitats were seagrass bed, sand flat, dead coral, and hard bottom in two sites within the reef (Punta Francés y Cabezo de Moya) in 2010. A total of 7,903 nematodes were identified, measured and assigned to different biological traits. Nematodes were more abundant in soft bottoms (seagrass beds and sand flats) because possibly exploit advantageously the interstitial environment of these bottoms. Nematodes in hard bottoms mostly were characterized by morphological adaptations to the epibenthic life style particularly to reduce the reworking by currents. The species richness was 156 ± 9 species indicating a high diversity of the group in the coral reef. The body size and biomass was influenced by the type of habitat and possibly by the quality and availability of the food. Larger nematodes dominated in the seagrass beds probably because a larger gut enhances the digestion of the refractory detritus produced by seagrasses. Epigrowth feeders of comparatively smaller size dominated in the sand flats possibly because they use more efficiently the interstitial space and biofilm supported by the sand grains.

ROOT KNOT NEMATODES: NEW INSIGHTS INTO PARASITISM SUCCESS [NEMATODOS AGALLEROS: NUEVAS PERSPECTIVAS RESPECTO AL ÉXITO DEL PARASITISMO]

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The root knot nematode (RKN) *Meloidogyne incognita* is a widespread and polyphagous obligate asexual endoparasite of plants that causes serious and growing problems to agriculture. This feeding habits causes dramatic changes of plant cells into specialized feeding sites, which are induced by secreted proteins by the nematode, so-called parasitism effectors. An integrated approach of molecular techniques has been used to functionally characterize nematode parasitism proteins. The complete genome sequence of *M. incognita* revealed

that the assembled sequence consists of homologous but divergent segment pairs that might represent former alleles in this species. Based on comparative genomics, we identified in RKN genome a set of genes preserved during the evolution and of plant-parasitic nematodes only shared with organisms having a plant parasitic lifestyle. Using a series of bioinformatic screens, we selected a set of genes that were further analyzed with design of siRNAs and infestation test experiments after silencing. In total about 10 inactivated genes showed a significant reduction in the number of egg masses or gall numbers compared to the control. These genes represent new targets that may allow development of new methods to control plant-parasitic nematodes but harmless for the environment and the consumer health. Thus, RKNs constitute a unique model system to study the links between variation in genome structure, mode of reproduction, and adaptation to environment and hosts, in relation with parasitic success.

CAMBIOS CUANTITATIVOS DE COMPUESTOS FENILPROPANOIDES EN PLANTAS DE CHILE (*CAPSICUM ANNUUM* L.) CM334 INDUCIDOS POR *NACOBBUS ABERRANS* THORNE AND ALLEN, 1944 [QUANTITATIVE CHANGES OF PHENYLPROPANOID COMPOUNDS IN CM334 CHILLI PEPPER PLANTS (*CAPSICUM ANNUUM* L.) INDUCED BY *NACOBBUS ABERRANS* THORNE AND ALLEN, 1944]

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El genotipo de chile CM334 es resistente a las principales especies del genero *Meloidogyne*; sin embargo, susceptible a *Nacobbus aberrans*. Su resistencia ha sido asociada con la ruta de biosíntesis de los fenilpropanoides. *N. aberrans* podría inducir cambios en esa ruta metabólica y lograr su establecimiento exitoso en CM334. En este trabajo se determinó el contenido de fenoles solubles totales (FST), identificaron y cuantificaron los ácidos fenólicos y flavonoides en diferentes tiempos de muestreo (7 y 21 días después de la inoculación con *N. aberrans*). El contenido de lignina también fue determinado. En ambos tiempos de muestreo, las plantas inoculadas tuvieron menor contenido de FST respecto a plantas sin inocular ($P < 0.05$). En el sistema radical de las plantas de chile CM334 se encontraron los ácidos: p-hidroxibenzoico (p-HBA),

gálico, cafeico, siríngico, ferúlico, vanílico, y clorogénico. En algunos tiempos de muestreo, las plantas inoculadas tuvieron el menor contenido de ácidos fenólicos respecto a las plantas sin inocular, particularmente a los 7 días posteriores a la inoculación con el nemátodo ($P < 0.05$). El ácido clorogénico fue el compuesto más abundante. Los niveles de éste ácido variaron de 209 a 543 $\mu\text{g}\cdot\text{g}^{-1}$ de materia seca en plantas inoculadas y sin inocular, respectivamente. En todos los tiempos de muestreo, las plantas inoculadas con *N. aberrans* presentaron menor contenido de los ácidos p-HBA, ferúlico y clorogénico en comparación con las plantas sin inocular ($P < 0.05$). Por otro lado, el único flavonoide identificado en el sistema radical fue rutina (quercetin-3-rutinósido). La inoculación con *N. aberrans* indujo cambios en el nivel de éste flavonoide únicamente a los 7 (ddi) en comparación con las plantas control, no así en el contenido de lignina ($P < 0.05$). Éstos resultados sugieren que *N. aberrans* induce cambios en el metabolismo fenilpropanoide que podrían asociarse con su establecimiento en sistema radical del genotipo CM334.

2D-COS CHARACTERIZATION OF TOMATO PLANTS INFECTED BY *MELOIDOGYNE ENTEROLOBII* [CARACTERIZACIÓN 2D-COS DE PLANTAS DE TOMATE INFECTADAS CON *MELOIDOGYNE ENTEROLOBII*]

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The evolution over time of the infrared spectra of tomato plants infected by *Meloidogyne enterolobii* vs. uninfected plants by infrared spectroscopy was studied. After infection, young leaves were removed from the plants once a week and analysed in a Fourier Transformed Infrared spectrometer with an attached Attenuated Total Reflectance accessory. The data was evaluated by two dimensional correlation spectroscopy (2DCOS IR spectroscopy). This method allows studying the response of a process in a spatial-temporal perturbation, and it is accounted as a correlation

spectrum and its Noda-Hilbert transform. A statistical comparison of the correlation spectra showed significant differences between the control and the infected plants. An analysis of the synchronous spectra of the band has shown that the bands in the carbonyl region, specifically ester groups or amide I, are important infection indicators, and the modification of these bands affects the rest of bands. On the other hand, the asynchronous spectra reveal that these modifications occur before the change of the rest of bands, except the bands around 1200 cm⁻¹, possibly C-O single bonds. These results suggest an increase of oxidative processes during the infection, i.e., formation of phenolic compounds, decreasing chlorophyll, carotenoids and protein. All of these processes are governed by an increment of the phenoloxidase and/or peroxidase enzymes as reported in the literature. In the near future this technique could be used for early diagnosis of nematode infections or even other pathogen in plants before symptoms appearance and without soil and root sampling.

RENIFORM NEMATODE MANIPULATION OF HOST ROOT GENE EXPRESSION DURING SYNCYTIUM FORMATION IN UPLAND COTTON [MANIPULACIÓN DE LA EXPRESIÓN DE GENES DE LA RAÍZ DE ALGODÓN DURANTE LA FORMACIÓN DEL SINCICIO DEL NEMATODO RENIFORME]

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Reniform nematode (*Rotylenchulus reniformis*) is a major yield-limiting pest of multiple crops in the tropics and sub-tropics, including upland cotton (*Gossypium hirsutum*). Parasitism by reniform nematode involves significant developmental changes in plant roots, leading to the formation of multicellular feeding structures called syncytia. Here, we present transcriptome data from syncytial and non-syncytial cotton roots sampled across a 12-day time course. Total mRNA samples extracted from infected and uninfected roots were sequenced on the Illumina HiSeq 2000 platform, generating over 593 million paired-end reads. A *de novo* root transcriptome for *G. hirsutum* was assembled with the Trinity pipeline, and reads from 24 individual RNA samples were mapped back to the transcriptome to quantify gene expression at 3, 6, 9, and 12 days after inoculation (DAI). Overall, 162, 117, 197, and 40 genes were significantly up-regulated and 16, 4, 161, and 49 genes were down-regulated in infected vs. uninfected roots at 3, 6, 9 and 12 DAI, respectively (FDR = 0.05). Thirteen genes were consistently up-regulated, including sulfite reductase, expansin b1,

an abc transporter, and a zinc finger transcription factor. Two genes were consistently down-regulated: anextensin and a non-specific lipid transfer protein. A cytochrome p450 gene with reported functions in gibberellin deactivation and cell wall biosynthesis was depressed at 3 DAI but up-regulated on subsequent days. Gaining new insights into the mechanisms of plant response to reniform nematode has practical significance for nematode control through the development of resistant crop varieties.

QUITINASE AND β -1,3 GLUCANASE ACTIVITY IN SOYBEAN CULTIVARS INOCULATED WITH *PRATYLENCHUS BRACHYURUS* AND TREATED WITH ACIBENZOLAR-S-METIL [ACTIVIDAD QUITINASA Y β -1,3 GLUCANASA EN CULTIVARES DE SOYA INOCULADOS CON *PRATYLENCHUS BRACHYURUS* Y TRATADOS CON ACIBENZOLAR-S-METIL]

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This study aimed to evaluate the enzymatic activity (quitinase and β -1,3-glucanase) in soybean cultivars inoculated with *Pratylenchus brachyurus* and treated or not treated with acibenzolar S-metil (ASM). The experiment was conducted under greenhouse conditions in a completely randomized design, in a 5 x 2 x 2 factorial scheme, with 8 replications. Factor A was cultivars (BRSGO Caiaponia, Emgopa 313 RR, TMG 132 RR, BRSGO 8560 RR and M-Soy 8360 RR), factor B was two levels of ASM (0 and 10 g a.i./100 L) and factor C, two levels of inoculum (0 and 300 *P. brachyurus*/plant). Each plot consisted of two plants grown in a plastic bag containing autoclaved soil and sand (1:1). Evaluations of quitinase and β -1,3-glucanase were performed at 14, 21, and 28 days after inoculation (DAI) (24 hours after ASM spray). Nematode reproduction factor (RF) was evaluated at 60 DAI. Results showed higher quitinase and β -1,3-glucanase activity in plants inoculated with *P. brachyurus* and not treated with ASM. Higher activity was found in cultivars BRSGO Caiaponia and BRSGO 8560 RR during the three evaluation periods. Cultivars Emgopa 313 RR and M-Soy 8360 RR had an increase in the enzyme activity at 21 DAI, while in the other cultivars, this activity decreased along the period of evaluation. Among the cultivars, BRSGO Caiaponia had the lowest RF. Plants treated with ASM presented lower RF compared with not treated plants.

TROPHIC INTERACTIONS LEADING TO THE LOSS OF CITRUS ROOT HEALTH [INTERACCIONES TRÓFICAS QUE CONDUCEN A LA PÉRDIDA DE LA SALUD DE LAS RAÍCES DE LOS CÍTRICOS]

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Huanglongbing (HLB) is a devastating disease of citrus, caused by phloem-limited, gram-negative bacterium *Candidatus Liberibacter asiaticus* (Las). Early symptoms of HLB include fibrous root loss, followed by yield decline and canopy dieback. Early root loss is quantified as a 30-50% reduction in root density, is dependent on local bacterial infection, and does not cause phloem plugging in the roots. Root loss occurs before above ground foliar symptoms and precedes phloem plugging of the canopy that eventually leads to carbohydrate (CHO) starvation in roots. Root cages were buried under trees of increasing HLB canopy decline and replaced every two months to measure new root growth. Sampling of trees in different stages of HLB decline revealed that root loss occurs in two stages. The second phase of root loss (70-80%) begins at the early stages of canopy thinning. During this phase, CHO supply to the roots is limited by canopy phloem-plugging. Surprisingly, during both phases of canopy decline root growth was stimulated compared to presumed healthy trees. Root growth in the cages was positively correlated with the presence of Las in these new roots suggesting that bacterial infection stimulates CHO allocation to roots. Root loss is exacerbated by biotic and abiotic stresses in the rhizosphere. The prevalent root pathogen in citrus roots is *P. nicotianae* (*Pn*). To understand the possible interaction between the two root pathogens, seedlings of two citrus rootstocks, one resistant and one susceptible to *Pn*, were inoculated with Las, *Pn*, both or neither. Infection by Las increased *Pn* infection on both rootstocks indicating that Las reduced tolerance to *Pn*. Both pathogens caused significant root loss, but Las in combination with *Pn* did not cause additional root loss compared to Las alone.

OCCURRENCE AND PERSISTENCE OF *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA* IN QUARANTINED FIELDS WITHOUT HOST PLANTS UNDER NORDIC CONDITIONS [OCURRENCIA Y PERSISTENCIA DE *GLOBODERA ROSTOCHIENSIS* Y *G. PALLIDA* EN CAMPOS BAJO CUARENTENASIN PLANTAS HUÉSPED EN CONDICIONES NÓRDICAS]

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In 1955, potato cyst nematode (PCN) was recorded in Norway. After the detection, regulations were implemented and extensive surveys were carried out. Fields with PCN were placed under strict quarantine. To prevent introduction of new PCN populations import and movement of seed-potato was prohibited. In addition to this, crop rotation involving resistant cultivars was enhanced. Surveying production areas with certified seed potatoes started in 1956. Seed potato areas have been under monitoring for 59 years and are so far free of PCN. Separation of *Globodera rostochiensis* and *G. pallida*, and evidence of resistant-breaking populations changed the use of resistant cultivars. Today, non-virulent *G. rostochiensis* is managed by crop rotation, while infestations by *G. pallida* or virulent *G. rostochiensis* results in 40-years ban on growing potato. Reduction of quarantine period would have positive effects for Norwegian farmers and enterprises. In a recent project, the identity of PCN populations from the main potato districts was studied. PCR amplification of ITS regions identified the majority of populations as *G. rostochiensis*, with the exception of one, which belonged to *G. pallida*. PCR amplification and sequencing of the non-coding scmt mitochondrial region confirmed the species identification, and demonstrated a close relationship to European populations. Studies on vap-1 patterns demonstrated several variants of the vap-1 gene to be present in each population, and that differences in allele frequencies between populations are minor. To investigate the decline in PCN infectivity in absence of host plants, quarantined fields for 32, 18, and 12 years were selected. These fields were infested by *G. rostochiensis* (Ro3), *G. rostochiensis* (Ro1) and *G. pallida* (Pa 2/3) respectively. In each soil sample cysts were extracted and baited on a susceptible potato in pots. After 4 months, the soil was analysed for new cysts. Viable PCN were found in all soil

samples. This study, which is the first of its kind on the Nordic area, demonstrated that in the absence of host-plants *G. rostochiensis* can survive for 32 years, and *G. pallida* has survived for 12 years so far.

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF NEEDLE NEMATODES (*LONGIDORUS* SPP.) ON DATE PALM IN ARIZONA, CALIFORNIA, AND FLORIDA [CARACTERIZACIÓN MORFOLÓGICA Y MOLECULAR DE NEMATODES PICADORES DE LA RAÍZ (*LONGIDORUS* SPP.) SOBRE PALMERA DATILERA EN ARIZONA, CALIFORNIA Y FLORIDA]

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For decades, ornamental growers in Florida have imported date palms (*Phoenix dactylifera*) from Arizona and California. Needle nematodes (*Longidorus* spp.) with conoid and hemispherical tails have been detected consistently in these imported palms in Florida. The populations with conoid tails have been identified routinely as *Longidorus africanus* and those with hemispherical tails as *L. belloi*, *L. belondiroides*, and *L. orientalis*. Recent molecular and morphological analyses have confirmed the identification of *L. africanus* and also that of *L. orientalis*, reported by R. Esser, in Florida, in 1995 on date palms from California. These findings disprove the reports of detection of *L. belloi* and *L. belondiroides*, not found again, and validate that of *L. orientalis* as a continental record in the United States and the Americas. *Longidorus africanus* is a known parasite of vegetables in Southern California, whereas *L. orientalis* is a parasite of date palm in the Middle East. The populations of *L. orientalis* contained a few males, not reported in the original description. *Longidorus orientalis* was commonly found in association with *L. africanus* and was able

to survive for at least four years, at low densities in warm and humid environments of Florida, on transplanted date palms imported from California and Arizona. Phylogenetic relationships of *L. orientalis* with closely related *Longidorus* species were reconstructed using D2-D3 of 28S rRNA, ITS1 rRNA, and partial coxI gene sequences. The PCR-D2-D3 of 28S rDNA-RFLP diagnostic profile was obtained and proposed for the identification of this species. *Longidorus orientalis* shows high intraspecific variation (up to 15.5%) in coxI mtDNA sequences. Incongruence between ITS1 rRNA and coxI mtDNA gene trees was noticed from the analysis of phylogenetic relationships between *L. orientalis* populations, indicating selective introgression of mtDNA through gene flow as a consequence of hybridization of populations from different origin.

THE GIANT AFRICAN LAND SNAIL (*LISSACHATINA FULICA*), AN INTERMEDIATE HOST OF THE RAT LUNGWORM (*ANGIOSTRONGYLUS CANTONENSIS*) - A PARASITIC RELATIONSHIP POSING A THREAT TO AGRICULTURE AND POTENTIALLY TO HUMANS IN FLORIDA [EL CARACOL GIGANTE AFRICANO (*LISSACHATINA FULICA*) COMO HOSEPEDADOR INTERMEDIARIO DEL NEMÁTODO DEL PULMÓN (*ANGIOSTRONGYLUS CANTONENSIS*) - UNA ASOCIACIÓN PARASITARIA QUE AMENAZA LA AGRICULTURA Y POTENCIALMENTE AL HOMBRE EN FLORIDA]

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In September of 2011, the giant African land snail, *Lissachatina fulica* was observed in Florida for the first time since its complete eradication following an introduction in 1966. Upon this recent find, a joint eradication program sponsored by FDACS and USDA was established. Currently, the snail has only been found in Miami-Dade County where there have been 21 cores established. Cores consist of an initial detection site with a 1 mile diameter circular buffer. In October of 2012, scientists from FDACS confirmed the presence of the rat lungworm (*Angiostrongylus cantonensis*) in samples of *L. fulica* collected during the ongoing eradication program. This nematode has the ability to infect humans and cause eosinophilic meningitis. So far, over 125,808

snails have been collected and destroyed. Sub-samples for a total of 175 snails have been separated from the collected snails and monitored for presence of the rat lungworm. These snails were mechanically processed in order to extract *A. cantonensis*. Nematode juveniles were identified on the basis of their morphological characteristics using light microscopy. Only 15 snails, (approximately 9%) have been found positive. The plant damage caused by the snail in the Miami area is so far limited to suburban residential properties where the vectored nematode has the potential to become a human pathogen.

THE INCREASING IMPORTANCE OF ROOT-KNOT NEMATODE SPECIES (*MELOIDOGYNE* SPP.) IN INTERNATIONAL TRADE, WITH PARTICULAR EMPHASIS ON *M. ENTEROLOBII* AND *M. MALI* IN EUROPE [LA IMPORTANCIA CADA VEZ MAYOR DE NEMATODOS DE LA RAÍZ (*MELOIDOGYNE* SPP.) EN EL COMERCIO INTERNACIONAL, CON ESPECIAL ATENCIÓN A *M. ENTEROLOBII* Y *M. MALI* EN EUROPA]

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Root-knot nematodes (RKN) have regularly been intercepted in the international trade of plants, but in the last 20 years a significant increase in consignments has resulted in an increase in the number and diversity of interceptions. Most records refer to '*Meloidogyne* spp.' because RKN pose many challenges for full identification; not all life stages may be present, or perhaps very few, poor specimens are found. However, a rise in the observed pathogenicity and hence phytosanitary importance of some species has led to an increased effort to utilise biochemical tools. Very recently, DNA technology, combined with established identification techniques, has allowed phytosanitary services in many countries to distinguish economically important species from less pathogenic or native species. In Europe this has resulted in species such as *M. enterolobii* and *M. mali* being added to 'alert' lists and 'risk' registers which has raised their profile and allowed targeted control strategies.

PROTECCIÓN CONTRA FITONEMATODOS CUARENTENARIOS EN CUBA. ALCANCE Y RESULTADOS [PROTECTION AGAINST QUARANTINED NEMATODES IN CUBA, SCOPE AND RESULTS]

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En la República de Cuba está establecido en las estrategias de cuarentena exterior e interior, un sistema de vigilancia fitosanitaria para enfrentar los riesgos de introducción de plagas cuarentenarias, donde se incluyen acciones encaminadas a la prevención y detección de fitonematodos y su control oficial. En la lista de organismos cuarentenarios de Cuba están registradas 17 especies del grupo A1, una A2 y 7 no cuarentenarias reglamentadas, reconocidas en el ámbito internacional por su impacto en cultivos de importancia económica. El análisis para el diagnóstico en las importaciones de productos vegetales abarca un promedio general anual de 2282 muestras con 64 intercepciones; 11 cuarentenarias, entre ellas *Ditylenchus dipsaci* en ajo, cebolla, habas y tubérculos de papa; *Meloidogyne chitwoodi* en bulbos de gladiolo; *Globodera rostochiensis*, y *G. pallida* en papa; *Heterodera schachtii* como contaminante de semillas de acelga y suelos de papa. Por otra parte en el territorio nacional está implementado un Programa de Defensa que abarca la ejecución, por cuadrantes cartográficos, de encuestas de detección en los cultivos de ajo, cebolla, papa, arroz, caña de azúcar, cítricos, tabaco, café, soya y coníferas para los cuales se ha rastreado un promedio anual de 421 440 ha, que generaron 1461 muestras. Fueron detectadas mayormente *Aphelenchoides subtenuis*, *Aphelenchoides* spp., *Aphelenchus* sp., *Bursaphelenchus* sp., *Cactodera amaranthi*, *Helicotylenchus* spp., *Heterodera fici*, *Meloidogyne enterolobii* (A2), *M. incognita*, *Pratylenchus coffeae*, *P. zaeae*, *Rotylenchulus reniformis*, *Tylenchorhynchus annulatus*, *Trophurus* sp., y *Xiphinema* sp., especies comunes en sus ecosistemas. Los resultados indican que no ha ocurrido la introducción de ninguna especie cuarentenaria en Cuba.

CHARACTERIZATION OF THE PINWOOD NEMATODE, *BURSAPHELENCHUS XYLOPHILUS*-PINUS SYSTEM IN PORTUGAL: PHYTOCHEMICAL, HISTOPATHOLOGICAL, MOLECULAR, AND BIOTECHNOLOGICAL APPROACHES [CARACTERIZACIÓN DEL NEMATODE DEL PINO, *BURSAPHELENCHUS XYLOPHILUS*-PINUS SISTEMA EN PORTUGAL: FITOQUÍMICO, HISTOPATOLÓGICO, MOLECULAR, Y BIOTECNOLÓGICO]

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The pinewood nematode, *Bursaphelenchus xylophilus*, is one of the main threats affecting Portuguese maritime pine (*Pinus pinaster*). Several research teams have joined efforts to better understand the plant-nematode system. Over 150 essential oils (EO), as well as several EO fractions and decoction waters have been evaluated, *Ruta graveolens*, *Satureja montana*, *Thymbra capitata*, *Thymus pulegioides*, and *T. vulgaris* EOs being the most nematotoxic. Two-year-old *P. pinaster*, *P. pinea*, *P. sylvestris*, and *P. halepensis* were inoculated with a virulent PWN Portuguese isolate, and comparatively evaluated with non-inoculated and wounded plants to understand the plant-nematode interaction and the role of plant volatiles. Histological studies showed that the number of nematodes increased in *P. pinaster* and *P. sylvestris* with disease progression, and 7 weeks after inoculation all pine tissues were severely damaged. PWN distribution in *P. pinea* and *P. halepensis* was nearly restricted to the inoculated area; no clear change was observed in the stem tissues. Pine species volatiles showed the existence of chemotypes in some cases. Key volatile organic compounds, such as 4-hexen-1-ol, involved in *P. pinaster* response against the nematode have also been identified using non-destructive methods, with the potential to be used as biomarkers for early detection of infected trees. *In vitro* co-cultures of the host with parasite have also been established as a biotechnological tool to evaluate the effect of nematotoxic addition and assess their phytotoxicity to the host. Molecular approaches have addressed

the changes in α -pinene synthase gene expression in susceptible *P. pinaster* and non-susceptible *P. pinea*, following nematode invasion. Preliminary results showed an increased expression of this gene in *P. pinea*, contrary to *P. pinaster* which revealed the same expression level in infected and non-infected controls. Acknowledgments: Studies partially funded by FCT, under SFRH/BD/43738/2008, PEst-OE/EQB/LA0023/2011, PTDC/AGR CFL/117026/2010 and PTDC/AGR-CFL/120184/2010.

***CACTODERA TORREYANAE* NEW SPECIES OF CYST NEMATODE (NEMATA: HETERODERIDAE) ITS MORPHOLOGY AND SOME BIOLOGICAL ASPECTS [CACTODERA TORREYANAE UNA ESPECIE NUEVA DE NEMATODO FORMADOR DE QUISTE (NEMATA: HETERODERIDAE) SU MORFOLOGÍA Y ALGUNOS ASPECTOS BIOLÓGICOS]**

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During the spring of 2012, we detected the second stage juveniles and males of a cyst nematode from soil around of *Suaeda torreyana* (Chenopodiaceae) plants, growing in saline soil and known by the Mexican people as romerito, a typical plant consumed during Christmas. After more sampling, we found cysts and at the beginning of the rainy season we observed and collected abundant white females attached to the roots. Female bodies were oval-shaped with conspicuous vulva cones and only the neck was introduced into the root. Some are attached to very thin roots and others to thicker roots. Most of the white females are pearly white and produced a transparent and conspicuous gelatin, but no eggs were observed inside; the body becoming light brown with clearly observed eggs inside. In some mature females the posterior end had irregular annulations. Cysts were lemon-shaped, light brown to dark brown with vulva cone and vulva not in a deep depression. Cyst surface with zigzag pattern at the middle of body, not pronounced on the surface of the vulva cone. Cone without bullae and denticles. Anus in a small depression with smooth surface and a minute pore. Eggs with smooth corion, lacking

punctuations. Males with stylet knobs rounded. Spicules with slightly bifid. Gubernaculum small. Cloacal tubus present. Second-stage juvenile with stylet dorsal knob rounded and subventrals sloping lightly posteriorly. Genital primordium to anterior end 215 - 307 (260 ± 4.5) μm . Hyaline part tail 15 - 25 (18 ± 0.41) μm . Phasmid a minute pore at level of the beginning the hyaline part of the tail. The most striking distinction of *Cactodera torreyanae* n.sp. from all the species of *Cactodera* is the biology. Advanced second-stage juveniles (J2A) are found in the soil having developed from recently hatched j2, also the third and four stage bodies are attached outside the roots. The adult females are pearly white color, with only part of the neck attached in the cortical tissue of the roots; the development of this species is as a sessile ectoparasite.

DORYLAIMOIDEA FROM ANTARCTIC REGION OF ARGENTINA [DORYLAIMOIDEA DE LA REGIÓN ANTÁRTICA ARGENTINA]

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Samples taken by E. Mondino in February 2000 around *Sanionia uncinata* and *Deschampsia antarctica* from Base Brown, Costa Danco, Antarctic Peninsula ($64^{\circ} 53' 43''$ S $62^{\circ} 52' 15''$ O) yielded two Dorylaimidae and one Qudsianematidae species reported for the first time in this region. *Mesodorylaimus signatus* agrees with the description given by Loof (1975): vulva longitudinal, advulvar cuticle not wrinkled, female prerectum 74-102 μm . Nevertheless, lateral chordone-third of body diameter (against one-fifth) and the stylet length is 13 a 15 μm (against 16-18 μm), although the length of the spear agrees with the original description. The *M. imperator* population presents the diagnostic characters of this species: advulvar cuticle irregularly wrinkled, lateral chordone-third of body diameter, female prerectum 69-70 μm length μm . The genus *Amblydorylaimus* was erected by Andrassy (1998) to place *Eudorylaimus isokaryon* Loof, 1975 which presents some characters that differ from the other species of the genus *Eudorylaimus*, as the precloacal pair of genital papillae that lies at a greater distance from cloaca than in general and stylet weakly sclerotized, consisting of an anterior conus and posterior gradually widened shaft, also guiding ring fold like, dorsal oesophageal nucleus fairly posterior in position and equally sized mid-oesophageal nuclei. The specimens present the characters given

by Andrassy for the genus, also the inverted position in the replacement stylet. Nevertheless, the juveniles and adults of our *A. isokaryon* population present stylet tubular as described by Loof, without conus and shaft differentiated. Andrassy disregarded the taxonomical importance in the shape of stylet, but these characters are in *Amblydorylaimus* description. Therefore, we make the question about what of both stylet shapes must take into account to the genus diagnosis.

NEMATODO FOLIAR APHELENCHOIDES SP., ASOCIADO A LA MANCHA ANGULAR DE LA HORTENSIA (HYDRANGEA) EN EL ORIENTE DE ANTIOQUIA- COLOMBIA [NEMATODE LEAF APHELENCHOIDES SP., ASSOCIATE WITH THE SPOT ANGLE OF HYDRANGEA IN THE EAST OF ANTIOQUIA-COLOMBIA]

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La Hortensia (*Hydrangea macrophylla* Tumb), es una de las especies de flor de corte atractiva en los mercados de Estados Unidos, India y Japón principiante, lo que convierte a este cultivo en un producto de exportación para estos mercados, estimulando de esta forma el incremento del área de siembra en Colombia. En la actualidad se cultivan aproximadamente 700 hectáreas en la región productora de Antioquia; de este cultivo dependen económicamente cerca de 1.500 familias. La Hortensia se ve afectada por diversos patógenos, entre ellos, el Mildeo polvoso (*Oidium* sp.), *Alternaria* sp., manchas bacteriales (*Pseudomonas* sp.), virus y nematodos del género *Pratylenchus* sp. En Colombia son pocos los estudios asociados a la nematofuna de cultivos de Hortensia; sin embargo, en Europa y Estados Unidos se relaciona la presencia de *Pratylenchus* sp., *Meloidogyne* sp., *Aphelenchoides* sp., y *Ditylenchus dipsaci* (Filip), este último no ha sido registrado en Colombia para esta planta. En años recientes, para algunas variedades comerciales de Hortensia, se han presentado afecciones foliares necróticas delimitadas por las nervaduras de las hojas y ocasionalmente lesiones necróticas en los tallos florales. Con el objetivo de determinar la causa asociada a esta sintomatología, en tejidos foliares (hojas y tallos), procedentes de diferentes localidades de la zona productora de Hortensia, en Antioquia; en el Laboratorio de Sanidad Vegetal

de la U.C.O, mediante análisis de los tejidos afectados y por métodos de extracción tradicional Baermann modificado con bandejas, se identificó el fitonematodo *Aphelenchoides* sp., como causa de esta sintomatología; plantas afectadas por este nematodo, disminuyeron la calidad de la flor para exportación. Es de anotar, que los agricultores y técnicos han confundido estos síntomas con los ocasionados por hongos o bacterias.

GENOME AND BIOLOGY OF *P. CHLAMYDOSPORIA*: A FUNGAL ENDOPHYTE WITH BIOFERTILIZER AND NEMATODE BIOCONTROL CAPABILITIES [GENOMA Y BIOLOGÍA DE *P. CHLAMYDOSPORIA*: UN ENDÓFITO FÚNGICO CON CAPACIDADES DE BIOFERTILIZANTE Y AGENTE BIOCONTROL DE NEMATODOS]

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I will provide an overview of our work on the biology and applications of the nematophagous fungus *Pochonia chlamydosporia* (Pc). We have used Pc isolates from soils suppressive to nematodes (and other sources) in Spain and worldwide. We have developed techniques for enhancing the fungus in the rhizosphere. A GFP stable Pc transformant has proven useful to analyse egg-parasitism and root endophytism by the fungus. Our initial cell and biochemical studies have been followed by *omics* approaches. We consider Pc an endophyte with biofertilizer and nematode biocontrol capabilities. Pc is able to colonize the rhizosphere of crops (barley and tomato) and the model plant *Arabidopsis*. Using histochemistry (papillae and systemic defences detected) and transcriptomics (up-regulation of heat shock protein and defence genes) we have proven that Pc induces a moderate plant response to stress. In bioassays (up to greenhouse scale) with tomato susceptible to root-knot nematodes (RKN), some Pc isolates are able to promote growth (especially in roots) reducing flowering time and increasing yield. Nutrient solubilisation (e.g., P) or enhancing plant hormones by Pc could be involved in these effects. Metabolomics has identified root exudate signals putatively involved in tomato-RKN-Pc interactions. We aim to develop these to reduce crop damage by interfering with nematode-plant communication. Pc genome has shown large similarities with

entomopathogenic fungi and endophytes. Hydrolytic enzymes, transcription factors and signal transduction components are expanded in the Pc genome. This explains the nematophagous and endophytic habits of the fungus. Concluding, our studies support the multitrophic lifestyle of Pc. Rhizomodulation is our current paradigm to manage Pc-crop interactions for improving crop yield and reducing damage by plant parasitic nematodes.

EXPRESSION PROFILE OF NON-CODING SMALL RNAs IN TOMATO ROOTS DURING *POCHONIA CHLAMYDOSPORIA* ENDOPHYTISM [PERFIL DE EXPRESIÓN DE PEQUEÑOS RNAs NO CODIFICANTES EN RAÍCES DE TOMATE EN EL ENDOFITISMO DE *POCHONIA CHLAMYDOSPORIA*]

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Small RNAs play a key role in the plant-parasite interaction, regulating critical effector genes needed for infection. However, little is known about the effects of endophytes on non coding (nc)-RNAs expression in plant. To elucidate micro (mi) RNAs and other ncRNAs regulatory participation in plant-endophyte interactions, we used Illumina's NGS technology to sequence small RNAs (sRNAs) in tomato roots inoculated and not inoculated with the fungus *Pochonia chlamydosporia*. In both treatments, Sly-miR166a/b was the most abundant tomato miRNA, followed by sly-miR166c-3p. The two miRNAs together accounted for 81% and 74.1% of the annotated tomato miRNAs in *P. chlamydosporia* not inoculated or inoculated roots. Such highly expressed miRNAs are likely to have important roles in roots, considering that in epigeal parts of tomato and other plants the most abundant miRNA reported is miRNA-156. Endophytism by *P. chlamydosporia* affected miRNAs and other nc-sRNAs expression, with 26 miRNAs differentially expressed between the two treatments (up regulated with fold changes 2 to 9). Their 154 potential target genes involve apoptosis, primary metabolism and binding functions, i.e., Squamosa promoter binding-like protein. Comparative analysis showed that 48 out of 5055 *P. chlamydosporia* down-regulated tomato genes, from a previous RNAseq experiment, are miRNA targets (with fold changes 2 to 16). Furthermore, five miRNAs (sly-miR9473-5p, sly-miR169c, sly-miR169a, sly-miR9476-5p and sly-

miR1918) were found only in presence of the fungus. We also identified many other classes of sRNAs, including transfer RNA (tRNA)-derived sRNAs, some of which were also differentially expressed between the two treatments. Data provide valuable clues to understand the properties of sRNAs with a new insight on the role of miRNAs and other sRNAs in the host-endophyte interaction. A better understanding of ncRNA-mediated plant-endophyte interaction may sustain management of pests and diseases, and promote growth. MiRNA-based manipulations as gene suppressors, i.e. artificial miRNAs, may emerge as a new alternative approach for the improvement of crops and control of nematode pests.

POCHONIA CHLAMYDOSPORIA VAR. MEXICANA: UN AGENTE DE CONTROL BIOLÓGICO PARA MELOIDOGYNE ARENARIA EN ZANAHORIA [POCHONIA CHLAMYDOSPORIA VAR. MEXICANA: A BIOLOGICAL CONTROL AGENT FOR MELOIDOGYNE ARENARIA ON CARROT]

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Tres nuevos aislados de *Pochonia chlamydosporia* para el control de *Meloidogyne* spp. fueron obtenidos de la región hortícola del Valle de Tepeaca, Puebla, México. Los aislados presentaron morfología colonial y microscópica similar a *P. chlamydosporia*, pero con variación en la forma y tamaño de los conidios y la presencia de otras estructuras similares a conidios de forma redonda y pequeña. Los aislados se caracterizaron molecularmente usando la región ITS. Los valores de identidad nucleotídica mostraron un 83.6% de similitud con las secuencias de referencia. Los iniciadores específicos para dos de las variedades conocidas del hongo, RFLP's, gen *vcp1* de *P. chlamydosporia* y la inferencia filogenética en conjunto mostraron que los aislados pertenecen a una nueva variedad: *P. chlamydosporia* var. *mexicana*. En pruebas de laboratorio los aislados colonizaron > 80% de la rizósfera y parasitaron > 60% de los huevos de *M. incognita* y *M. arenaria*. En el invernadero se llevó a cabo un experimento utilizando como tratamientos la incorporación de hojas de brócoli, Furadan® y el hongo (5000 clamidosporas por g de suelo del aislado nativo Pcp 21), solos y en combinación, con la finalidad de reducir los daños en zanahoria por *M. arenaria*. El experimento se mantuvo en invernadero

durante 12 semanas, repitiéndose un año después. El aislado Pcp21 disminuyó significativamente el daño provocado por *M. arenaria* en zanahoria. Además la combinación del hongo con brócoli o con Furadan resultaron efectivos para disminuir el número de agallas.

INTEGRACION DE KLAMIC® EN UNA ESTRATEGIA DE BIOMANEJO DE MELOIDOGYNE SPP. EN CULTIVOS PROTEGIDOS [INTEGRATION OF KLAMIC® IN A BIOMANAGEMENT STRATEGIC OF MELOIDOGYNE SPP. IN PROTECTED CROPS]

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En los últimos 18 años, un equipo multidisciplinario del CENSA ejecutó la investigación-desarrollo e innovación del bionemático biológico KlamiC®, a base de una cepa seleccionada del hongonematófago *Pochonia chlamydosporia* var. *catenulata* IMI SD 187. El producto es obtenido mediante unatecnología de fermentación en estado sólido en bolsas siguiendo una Guía de Buenas Prácticas de Fabricación acorde a las Normas ISO 9001. KlamiC cuenta con Registro Comercial en Cuba, Nicaragua y en fase de presentación en República Dominicana y Panamá. Una estrategia de manejo exitosa en sistemas de producción protegida de hortalizas, requiere de un detallado conocimiento del establecimiento del hongo en las condiciones particulares de estos sistemas de cultivo: abundante fertirriego, excesivo uso de plaguicidas y rotación solo con cultivos susceptibles a *Meloidogyne* spp. Se presentan los resultados obtenidos con la aplicación de KlamiC® en condiciones de cultivos protegidos en diferentes escenarios de Cuba y sus nuevos usos, a partir de la demostración de su actividad endófito facultativa en plantas cultivadas en estos sistemas, como estimulador del crecimiento e inductor de tolerancia al efecto adverso que confieren las sales sobre las mismas. Se propone una estrategia de integración con otros bioproductos desarrollados en Cuba sobre la base de sus diversos mecanismos de acción.

AISLADOS NATIVOS DE *TRICHODERMA* SPP. EN LA SUPRESIÓN DE *MELOIDOGYNE INCOGNITA* [NATIVES ISOLATES OF *TRICHODERMA* SPP. IN THE SUPPRESSION OF *MELOIDOGYNE INCOGNITA*]

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En un experimento de control de *M. incognita* en plantas de *Capsicum chinense* Jacq. bajo condiciones protegidas; aplicaciones de 1×10^6 esporas por mL de cepas nativas de *T. harzianum* (Th43-13 y Th43-14), *Trichoderma* sp. (Th07-05), *T. atroviride* (Th09-06), *T. harzianum* (Th02-01), y el testigocomercial (Fithán®). Redujeron significativamente ($P \leq 0.01$) la formación de agallas del 27.71 al 79.09%, el número de huevos por gramo de raíz licuada del 83.53 al 98.48% y el número de hembras por gramos de raíz teñida del 43.51 al 70.88%; comparadas con el testigo sin inoculantes de *Trichoderma* spp. También se estimaron efectos significativos ($P \leq 0.01$) en las variables de crecimiento y desarrollo del cultivo; en altura se incrementó del 11.93 al 27.25%, en el diámetro del tallo del 15 al 31.14%, en la biomasa seca aérea del 27.27%, en el volumen de raíz del 15.56 al 41.48% y en el peso del fruto fresco de 9.81 al 38.12%, en relación con el testigo sin inoculantes microbianos. Las cepas nativas de *T. harzianum* registradas como Th43-14 y Th02-01 se consideraron las mejores para suprimir las poblaciones de *M. incognita* y favorecer el crecimiento y desarrollo del cultivo.

BIOLOGICAL CONTROL OF PLANT PARASITIC NEMATODES IN BANANAS [CONTROL BIOLÓGICO DE NEMATODOS PARÁSITOS DE BANANAS]

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Results of research conducted over 15 years demonstrated that endophytic fungi had an effective control of plant-parasitic nematodes not only in green house conditions but also in commercial banana plantations. Two strains of *Trichoderma atroviride*, were evaluated in 4 countries: Panama, Venezuela, Dominican Republic, and Costa Rica and reduction of the population of plant-parasitic nematodes were found. In addition, a better healthy root system was found as result of the plant growth promotion of the fungi as well as the less infection of plant parasitic

nematodes. Currently tissue cultured plants are used to establish commercial banana plantations and the protection of these plants with endophytes in greenhouse conditions is a good alternative to manage plant-parasitic nematode in bananas. It is important to mention that some additional good agricultural practices enhance the biocontrol activity of the endophytes such as improving the drainage, adding organic matter and inoculation of fungi in the fields. It is also important to stress that combining all these technologies improves the effectiveness of endophytes and all these technologies can be applied at the moment to make renovation of banana plantations, which can be also accompanied with a fallow period. Using all these technologies we can keep banana plantations with very low population of nematodes for 3 to 4 years without any application of nematicides.

RESPONSE TO RATIONAL AND TECHNICAL NEMATICIDE APPLICATION ON BANANA ROOT NEMATODE CONTROL AND YIELD [RESPUESTA A LA APLICACIÓN RACIONAL Y TÉCNICA DE NEMATICIDA EN EL CONTROL DE NEMATODOS EN RAÍCES DE BANANO Y EL RENDIMIENTO]

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The field experiment was conducted in a 15-year-old commercial banana (*Musa* AAA cv. Grand Naine) plantation from November 2011 to February 2013. To evaluate the response of rational and technical nematicide application, the nematode population density, the root weight and yield were recorded. Additionally, the relationship between costs and benefits of the nematicide application was estimated. Four treatments were evaluated: 1. Three rational and technical nematicide (Nemacur®, Mocap®, Vydate®) cycles per year with a 4-month interval, 2. Two rational and technical nematicide (Nemacur®, Mocap®) cycles per year with a 6-month interval, 3. Nematicide applied based on nematode threshold (8.000 nematodes per 100 g of roots) which resulted in two applications Nemacur® and Mocap® with a 7-month interval, and 4. Untreated control. Averaging the 12 root nematode samplings, the lowest *R. similis* ($P = 0.0008$), *Helicotylenchus* spp. ($P < 0.0001$), and total nematode ($P < 0.0001$) population were observed in the plots treated with three nematicide cycles per year. Compared with the untreated control,

the three nematicide cycles reduced *R. similis* in 53%, *Helicotylenchus* spp. in 48% and the total nematode population in 53%. Even though three nematicide cycles per year resulted in higher *R. similis* control efficacy with 42.4%, no difference ($P = 0.6372$) was detected with two 32% at 6-month interval and two 33% cycle per year, based on nematode population threshold. For *Helicotylenchus* spp. ($P = 0.0047$) and total nematodes ($P = 0.0018$), three cycles were better than two at 6-month interval or two cycles per year based on nematode population threshold, with 65.3 and 58.5% of efficacy on nematode control, respectively. No difference in total root weight ($P = 0.9812$) and functional root weight ($P = 0.7742$) was observed among treatments, varying from 88 to 90 and 73 to 79 g per plant, respectively. At the beginning of the experiment, no difference was observed in the number of hands per bunch ($P = 0.8680$) and bunch weight ($P = 0.5621$), which varied from 5.2 to 5.3 hands and 13.8 to 14.8 kg per bunch, respectively. At the harvest done 12 months after the nematicide application, bunch weight was increased ($P = 0.0013$) in 7.2 (41%), 4.8 (27%) and 4.7 (27%) kg per bunch resulting in an extra gain of \$2468, \$1660, and \$1427 ha⁻¹ with three, two at 6-month interval, and two cycles per year based on nematode population density, respectively.

MANEJO DE FITONEMATODOS EN EL CULTIVO DE LA VID [PHYTONEMATODES MANAGEMENT IN THE CULTURE OF THE VINE]

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El realizar aplicaciones de productos nematicida en vides, no se justifica sólo debido a la presencia de nemátodos fitoparásitos en las raíces, sino que debe evaluarse el equilibrio entre las raíces y las partes aéreas de las plantas, cuanto de las raíces se deberá proteger y, los nemátodos fitoparásitos, y si luego de analizar este conjunto de variables se decide utilizar un nematicida, deberá ser únicamente con el objetivo de proteger las raíces y debemos posicionar en un condición de menor importancia atacar a los nemátodos presentes en esa rizósfera. Los análisis de nemátodos que se hagan de la rizósfera son necesarios, por dar cuenta de los fitoparásitos presentes, y sus poblaciones cuantificadas, determinando el volumen de raíces que se encuentran más afectadas por los nemátodos. Dependerá de esos volúmenes de raíces la necesidad o no de actuar con

aplicaciones de nematicida, y una vez decidida la aplicación, se deberá ser cuidadosos con las dosis de producto que se va a utilizar, debido a que todos los nematicidas que han existido y existirán, tienen o tendrán una concentración de ingrediente activo, o de unidades formadores de colonias, óptima, aunque esta información no es explícita, excepto para pocos de ellos, debido a que los antecedentes disponibles en los envases, definen dosis, pero pocos explicitan una concentración de aplicación. Cuando las recomendaciones de aplicación se dan en tiempo (minutos), se genera un problema porque los volúmenes de agua que aportan los sistemas de riego, son muy variables obteniéndose resultados erráticos. Si en los productos “antiguos” había desinformación en etiquetas, que se transfirieron por años a su utilización, la proyección hacia los productos de las nuevas generaciones se hace más delicada, debido a la mayor sensibilidad de estos productos a las concentraciones de aplicación, y la información debiera ser técnicamente clara. En paralelo, los resultados de uso de productos biológicos y extractos actuales, son sensibles a la fecha de aplicación o estado fenológico del cultivo, y a las condiciones de suelo.

MANEJO DE NEMATODOS AGALLADORES EN HORTALIZAS DE MÉXICO [ROOT-KNOT NEMATODE MANAGEMENT IN VEGETABLE CROPS OF MEXICO]

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En la última década, la mayoría de las regiones productoras de hortalizas de México fueron afectadas en menor o mayor grado por *Meloidogyne* spp. y *Nacobbus* spp. causando pérdidas económicas importantes, incremento el nivel de daño en asociación con otros patógenos. *Nacobbus* spp. inició su dispersión en campos de Chapingo, Edo. de México en 1967 y actualmente se encuentra reportado en 12 estados hortaliceros de México. En esta contribución, reseñaremos el histórico de dispersión de ambos géneros, especies disponibles y las controversias generadas entre especialistas. También describiremos las tácticas de combate que se utilizan en los diferentes sistemas de producción (cielo abierto, tecnificado y no tecnificado, bajo cubierta o protegido y sus modificaciones dependientes de los mercados de hortalizas). Daremos un inventario

de tácticas químicas y no químicas que se utilizan comercialmente en los diferentes sistemas de producción, sus ventajas y desventajas en función a la realidad socioeconómica de los productores. Nos permitiremos en sugerir para el futuro lo que se debe hacer para mejorar el combate de nematodos agalladores en los sistemas de producción de hortalizas en México.

VARIABILITY WITHIN A SILT LOAM SOIL ON THE RESPONSE OF A FUMIGANT TO *ROTYLENCHULUS RENIFORMIS* [VARIABILIDAD DE UN SUELO FRANCO LIMOSO A LA RESPUESTA DE *ROTYLENCHULUS RENIFORMIS* A UN FUMIGANTE]

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Many of the alluvial soils in the southern U.S.A. have considerable variation in soil texture within individual fields. Soil texture changes within fields can currently be mapped using apparent electrical conductivity (ECa) to provide a shallow (ECa-sh) and deep (ECa-dp) reading of 0.3 and 0.9 m, respectively. A 2 year study was conducted to evaluate the impact of soil texture changes within a Commerce silt loam soil on population development of *Rotylenchulus reniformis* and response of cotton to the application of a fumigant. The test field was divided into six soil zones based on ECa-sh and ECa-dp values ranging from 21.9 to 47.4 and 26.9 to 58.5 mS/m, respectively. Half of each of these zones was treated with 1,3-dichloropropene at 28.1 l/ha each year and the other half left untreated prior to planting. The overwintering populations of *R. reniformis* were significantly lower in the two zones with the lowest values for ECa. A similar pattern was observed for populations of *R. reniformis* found within zones after harvest where the highest levels were found as ECa values increased. Populations did begin to significantly decline in the zone with the highest ECa value. Zones significantly impacted yield of cotton with the lowest yields occurring in the lowest ECa values and highest yields with the highest ECa values. The fumigant resulted in a significant increase in yield in the four zones with the lowest ECa values. Although the fumigant did give a numerical increase in yield in the two highest ECa zones, the difference would not have been economical. This study indicated that fields with sufficient variability in soil texture can be successfully developed into management zones for *R. reniformis*.

USING SOIL SAMPLES TO PREDICT YIELD LOSS DUE TO *PRATYLENCHUS PENETRANS* [USO DE MUESTRAS DE SUELO PARA PREDECIR LAS PÉRDIDAS EN LOS RENDIMIENTOS PROVOCADAS POR *PRATYLENCHUS PENETRANS*]

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The Root Lesion nematode, *Pratylenchus penetrans*, is damaging to a wide range of crops. All vermiform life stages are capable of feeding on roots as ectoparasites or endoparasites. The majority of chemical options to mitigate yield loss must be applied before or at the time of planting, so the damage potential of *P. penetrans* must be estimated in advance of the crop. The utility of soil samples for estimating population densities of Root Lesion nematodes is controversial and some advisory services only assay living roots to determine the damage potential of *P. penetrans*. The goal of our project was to develop critical point models for yield loss based on population densities of nematodes in soil samples without live roots. Our data set represented more than ten experiments conducted over multiple years. We used a two-step system for recovering nematodes by passing the soil sample, in water, through nested sieves. Fragments of dead roots retained on the top (250 mm) sieve were incubated on Baermann funnels. Soil and nematodes retained on the bottom (38 mm) sieve were cleaned using sugar flotation and centrifugation. Data from the two assays were collected separately. Counts of nematodes from the bulk soil fraction and the root fragment fraction were used alone or in combination in regression models to predict yield loss for corn, soybean, and potato. Estimates based on the combined assays were superior to soil only -or root fragment only- estimates for predicting yield and significant relationships between Pi and yield were demonstrated for all three crops. This work is useful for managing *P. penetrans* and demonstrates the significance of this pest to crops important worldwide.

BIOLOGICAL CONTROL OF GALL NEMATODES: ITS INCLUSION IN AN INTEGRATED PEST AND DISEASES MANAGEMENT APPROACH [CONTROL BIOLÓGICO DE NEMATODOS AGALLADORES: SU INCLUSIÓN EN UN MANEJO INTEGRADO DE PLAGAS Y ENFERMEDADES]

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Diverse biological control agents, including the nematophagous fungus *Pochonia chlamydosporia*, have been screened against root-knot (*Meloidogyne* spp.) and false root-knot (*Nacobbus aberrans* sensu lato) nematodes also known as ‘gall nematodes’. *Pochonia chlamydosporia* has been developed as a commercial bionematicide in some countries. The fungus is usually recommended for use in combination with other chemical and non-chemical nematode control methods, and as part of an integrated pest management program. However, information that may affect the fungus effectiveness as a biological control agent, e.g., soil analysis (including organic matter content), initial infestation level of the target nematode, presence of other native strains of the fungus, interactions with other microorganisms, etc., are rarely reported or taken into account before fungus application is recommended. Nowadays, the inundative and conservative approaches to biological control are moving towards an integrated biological management, potentially capable of delivering a sustained crop yield while preserving soil health. This approach increasingly requires in depth background information about soil quality and health and deals with the soil as a living, rather than an inert, system. The importance of a better understanding of the effect of agricultural practices on soil quality, soil biodiversity and multitrophic interactions, will be discussed as part of an integrated pest management and/or an integrated biological management approach using gall nematodes as an example.

PRESENCIA DE NEMATODOS DE IMPORTANCIA EN LOS CULTIVOS DE ÑAME (*DIOSCOREA* SP. L.) Y YUCA (*MANIHOT ESCULENTA* CRANTZ) EN CUBA [PRESENCE OF IMPORTANT NEMATODES IN YAM (*DIOSCOREA* SPL.) AND CASSAVA (*MANIHOT ESCULENTA* CRANTZ) IN CUBA]

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Los cultivos de ñame y yuca constituyen las principales fuentes de carbohidratos en países en vías de desarrollo. La producción mundial de ñame y yuca se estima en 54.05 y 230 millones de toneladas respectivamente. En Cuba ambos cultivos tienen gran participación en la producción de raíces y tubérculos. De ambos cultivos el ñame presenta la mayor susceptibilidad a nematodos, donde las especies *Scutellonema bradys* y *Pratylenchus coffeae*, ocasionan la llamada “pudrición seca” y considerables pérdidas, mientras la yuca puede ser ocasionalmente afectada por *P. brachyurus* en suelos arenosos. Se tuvo como objetivos evaluar cultivares comerciales de varias especies de ñame, frente a *P. coffeae* en condiciones semicontroladas y valorar la presencia de daños ocasionados por *P. brachyurus*, en cultivares de yuca sembrados en varias zonas de la provincia de Pinar del Río. En ambos estudios para la extracción de los nematodos, se utilizó el método de tamizado y embudos Baermann. Los cultivares de ñame con mayor daño fueron “Blanco de Guinea” y “Belep”, pertenecientes a *D. rotundata* y *D. alata*, respectivamente. Para el cultivo del ñame se recomienda realizar las siembras con tubérculos certificados libres de nematodos en todas las especies y utilizar bulbillos aéreos en los cultivares de *D. alata*. En las áreas de yuca de la provincia de Pinar del Río, *P. brachyurus*, ocasionó graves daños al sistema radicular y poca producción de raíces de almacenamiento. Estos campos habían sido rotados con tabaco en suelos arenosos, que estaban infestados con dicha especie y que tenían niveles de humedad adecuados. Se ha recomendado específicamente en zonas tabacaleras de Pinar del Río, que no se rote el tabaco con el cultivo de yuca en áreas con antecedentes de la especie *P. brachyurus*.

IDENTIFICACIÓN DEL NEMATODO NODULADOR *MELOIDOGYNE* SP. ASOCIADO AL CULTIVO DEL CAFÉ EN NICARAGUA [IDENTIFICATION OF ROOT-KNOT NEMATODE *MELOIDOGYNE* SP. ASSOCIATED WITH COFFEE PLANTATION IN NICARAGUA]

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Los nematodos noduladores del género *Meloidogyne* son de amplia distribución en zonas cafetaleras de la región centroamericana y constituyen uno de los principales problemas que afectan al cultivo de café; sin embargo, son pocos los estudios realizados que indiquen las especies de nematodos noduladores que afectan este cultivo en Nicaragua, por tal razón este estudio se condujo con el fin de identificar las especies de *Meloidogyne* asociadas al cultivo. Se colectaron 18 aislados de *Meloidogyne* en diferentes zonas cafetaleras de Nicaragua. Para la identificación se usaron patrones perineales de hembras, marcadores SCAR y la secuencia parcial de la región 18S y 28S del ADN. Los resultados indicaron la presencia de *M. exigua* y *M. incognita*. *M. exigua* fue la de mayor distribución, encontrándose en todas las muestras, en cambio *M. incognita* fue identificada solamente en uno de los aislados. La secuencia combinada de la región 18S y 28S del rADN mostró que ambas especies son haplotipos únicos y cuando se comparó la región 18S y 28S rADN de varias especies de *Meloidogyne* incluyendo a aquellas que comparten mecanismos similares de reproducción, en la secuencia de *M. exigua* de este estudio se detectaron sitios variables únicos dentro de la región 18S y 28S rADN, este hallazgo es de gran importancia para el diseño de SCAR específicos para esta especie. Los resultados de este estudio pueden considerarse de gran ayuda para el diseño de estrategias de manejo de los nematodos noduladores en el cultivo de café.

SPATIAL-TEMPORAL BANANA (*MUSA* AAA) ROOT NEMATODE DISTRIBUTION IN ECUADORIAN PLANTATIONS FROM 2008 TO 2014 [DISTRIBUCIÓN ESPACIAL Y TEMPORAL DE LOS NEMATODOS DE LAS RAÍCES DEL BANANO EN PLANTACIONES DE ECUADOR DEL 2008 AL 2014]

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Banana root samples taken from 2008 to 2014 in the banana (*Musa* AAA) plantations of Ecuador were analyzed in NEMALAB and used for this study in order to provide quantitative information about their population densities. Nematodes were extracted from 25 g of fresh roots that were macerated in a kitchen blender and then recovered in a 0.025 mm pore size (No 500 mesh). Data were subjected to ANOVA and frequency analysis, and the absolute frequency was calculated for each genus. Four plant parasitic nematodes were detected, and based on their frequencies and population densities their relative importance was established as follows: *Radopholus similis* > *Helicotylenchus* spp. > *Meloidogyne* spp. > *Pratylenchus* spp. *Radopholus similis* was most abundant accounting for 46 to 61% of the overall root population throughout the sampling years, followed by *Helicotylenchus* spp. which varied from 29 to 47%. From a total of 11,596 root samples, 7,201 (60%) had more than 2500 individuals per 100 g of roots, which is the economic threshold suggested by INIAP to decide chemical treatment. When all nematodes present were pooled (total nematodes) 10,465 (90%) of the samples were over the economic threshold. Considering all plant-parasitic nematode present, 11,587 root samples contained at least one nematode. High populations of total nematodes were found in all the years, months and provinces. The four nematode genera found are parasites of the banana roots, living within the roots, weakening plant anchorage, restricting water and nutrients uptake, retarding leaf emission and reducing photosynthesis, bunch weight, ratio, ratooning, and plant longevity. When interpreting nematode analysis, it is indispensable to consider the total phytonematodes population.

**POPULATION DYNAMICS OF
MELOIDOGYNE SPP. IN A COMMERCIAL
GRAPEVINE FIELD IN THE VALLEY OF
CIENEGUILLO, PIURA, PERU [DINÁMICA
POBLACIONAL DE MELOIDOGYNE SPP.
EN UN CAMPO COMERCIAL DE UVA EN EL
VALLE DE CIENEGUILLO, PIURA, PERÚ]**

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Meloidogyne spp. is the main pathogen of grapevine roots; nematode populations are highly virulent and aggressive, early affecting the growth and vigor. For three consecutive years (2012, 2013, and 2014) the population dynamics of *Meloidogyne* spp. were evaluated in a commercial field of table grapes 2-year-old Red Globe variety grafted on Harmony rootstocks, located in the valley of Cieneguillo Center, Piura region. Monthly samples of soil and roots with an auger (18 cm x 6.5 cm) to a depth of 40 cm of the soil and a horizontal distance of 40 cm from the trunk were performed. In 2012 the population density of J2 + eggs in soil did not differ significantly between summer, autumn, and early winter; during late winter and spring populations had declined considerably. Between 2013 and 2014 the population increased progressively between summer and early winter, decreasing significantly in the spring. The density of absorbing roots (mg/ m³ soil) decreased progressively between 2012 and 2014, an estimated decrease of more than 50%. No direct relationship between the population dynamics of the root nematode density was observed. Pruning of plants appear to have a significant effect on root density.

**OVERVIEW OF PHYTOCHEMICALS
AVAILABLE FOR THE MANAGEMENT OF
NEMATOLOGICAL PROBLEMS [VISIÓN
GENERAL DE LOS FITOQUÍMICOS
DISPONIBLES PARA EL MANEJO DE
PROBLEMAS NEMATOLÓGICOS]**

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Plants produce compounds for their protection against pests and adversity. These phytochemicals can be directly toxic to the pests or can generate environments suppressive to the pests. The nature and biological activities of the chemicals are as varied as the plant species. Plants in the order

Brassicales produce isothiocyanates with broad spectra of activities against bacteria, fungi, insects, and nematodes, and with considerable herbicidal properties. Their high vapor pressure endows them with fumigant action in soil. Examples of the chemicals and plants that generate them are: allyl isothiocyanate [horse radish, *Armoracia rusticana*]; benzyl isothiocyanate [papaya, *Carica papaya*; pepper weed, *Lepidium virginicum*]; n-butyl isothiocyanate [turnip, *Brassica rapa*]; phenylethyl isothiocyanate [watercress, *Nasturtium officinale*]. These compounds do not generally occur free in the plants but as glucosinolates that are released in soil by myrosinase type enzymes. Some aromatic and condiment plants base their defense mechanisms on other types of compounds. Oregano [*Origanum vulgare*] and sweet marjoram [*O. majorana*] produce carvacrol [2-Methyl-5-(1-methylethyl)-phenol] in significant quantities, and thyme [*Thymus vulgaris*] its isomer thymol [1-isopropyl-4-methyl-2-phenol] compounds with pronounced antiseptic and pesticidal properties. Benzaldehyde derived from enzymatic hydrolysis of amygdalin is produced by almond [*Prunus dulcis*] and other Rosaceae possesses pronounced pesticidal properties. The legume [*Crotalaria spectabilis*] and other *Crotalaria* spp., rely on cyanogenic glycosides for their defense against pests. A different type of nematicidal activity is based on the production of saponin, a soap, present in the bark of *Quillaja saponaria*. These examples indicate that there are ample sources of nematicidal activities in common plants. Compounds can be extracted, synthesized, or simply delivered to the soil with the plants that generate them. The method chosen for delivering these natural pesticides will depend on the properties of the specific compounds and very much on economic considerations.

CUBAN PLANTS AS SOURCE OF BIOACTIVE METABOLITES FOR NEMATODE MANAGEMENT [PLANTAS CUBANAS COMO FUENTES DE METABOLITOS BIOACTIVOS PARA EL MANEJO DE NEMATODOS]

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Plants are an important source of bioactive metabolites, which may be used for managing nematodes effectively. These compounds may be naturally released from plants or residues into the environment and/or extracted using different techniques. Interactions among plant metabolites, target pests and other nontarget organisms also need to be considered in the context of soil chemical ecology. The potential of Cuban plants, belonging to families Asteraceae, Piperaceae, Rubiaceae, and Poaceae, for *Meloidogyne incognita* management was evaluated in laboratory and semicontrolled conditions. Aqueous extracts from species from Asteraceae and Rubiaceae produced the highest percentage of mortality of second-stage juveniles of *M. incognita*. Soil treatments with water extracts and plant material from the two most promising plants were effective in decreasing the population of the nematode and the best effect was obtained for Asteraceae's species. These results suggest that the species of Asteraceae may be used as green manures to reduce *M. incognita* populations and plant extracts of the active principles could be used as nematicides.

EFFECTOS NEMATICIDAS DE LOS RESIDUOS ACUOSOS DE LA PRODUCCIÓN SEMI-INDUSTRIAL DEL ACEITE ESENCIAL DE ARTEMISIA ABSINTHIUM [NEMATICIDAL EFFECTS OF WASTE WATER FROM SEMI-INDUSTRIAL PRODUCTION OF ARTEMISIA ABSINTHIUM ESSENTIAL OIL]

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Artemisia absinthium L., llamada comúnmente ajenojo, es una planta herbácea medicinal nativa de las regiones templadas de Europa, Asia y África de gran interés etnofarmacológico. La composición de su aceite esencial, así como sus efectos biológicos (antimicrobianos, acaricidas, insecticidas, antifúngicos), han sido ampliamente estudiados. En este estudio se evalúa la actividad nematicida frente al nematodo formador de nódulos *Meloidogyne javanica* de los residuos acuosos (AQ) de la producción semi-industrial de aceite esencial de *A. absinthium*, var. Candial, domesticada y en fase de cultivo experimental, así como la caracterización de los compuestos activos. Los ensayos *in vitro* demuestran una alta efectividad de los AQ que inducen el 100% de mortalidad en juveniles infectivos (J2) de *M. javanica* a 24, 48, y 72 h de tratamiento. Los fuertes efectos nematicidas se mantienen en diluciones de hasta el 50%. Así mismo, los AQ estudiados inducen la inhibición significativa de la eclosión de huevos y de la capacidad infectiva de los J2 en plántulas de tomate. En los ensayos en maceta sobre plantas de tomate la aplicación de los AQ reduce significativamente la tasa de reproducción del nematodo. Se ha realizado el fraccionamiento biodirigido por técnicas cromatográficas (VLC, flash y HPLC-semipreparativo) con objeto de identificar, mediante RMN y espectrometría de masas, las moléculas bioactivas. Los resultados obtenidos indican el gran potencial de estos residuos acuosos generados en la producción del aceite esencial del *A. absinthium* como fuente de compuestos nematicidas.

ASPECTOS FÍSICOS, QUÍMICOS, Y BIOLÓGICOS, INHIBIDORES, Y PROMOTORES DE CRECIMIENTO DE RAÍCES, QUE POTENCIAN O DEBILITAN LOS RESULTADOS DE APLICACIONES DE PRODUCTOS NEMATICIDAS [PHYSICAL, CHEMICAL, AND BIOLOGICAL ASPECTS OF GROWTH INHIBITORS AND PROMOTERS OF ESTATE THAT BOOST OR WEAKEN THE RESULTS OF APPLICATIONS OF GOODS NEMATICIDES]

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Si en lo conceptual del manejo y control de los nemátodos fitoparásitos desplazamos el foco desde los nemátodos hacia las raíces de las plantas, la planificación de las estrategias permite visualizar herramientas aplicables en la defensa de raíces que antes de este cambio de foco no eran visibles, generándose una transición virtuosa desde el uso exclusivo de productos fumigantes y sustancias que normalmente trabajan actuando directamente en contra de la actividad de los nemátodos, hacia una visión integral de factores de los ámbitos de la física, la química y la biología, que permiten mayores logros en los procesos de recuperación de las plantas y sus raíces, y acortar los tiempos para recuperar el equilibrio de convivencia necesario para una adecuada expresión de las raíces. Bajo este esquema de manejo, los productos que tradicionalmente se utilizan en presencia de las plantas, adquieren vida nueva, que les permite expresarse con mayor plenitud, aunque se hace necesario conocer con detalle las fortalezas y debilidades de estos productos, como por ejemplo la perduración de su actividad en diferentes tipos de suelo desde el momento de su aplicación, la concentración de ingrediente activo, o de producto comercial, o unidades formadores de colonias, óptimos, entre otras. Este nuevo enfoque centrado en las raíces, permite mejores resultados usando productos modernos, biológicos como hongos, y bacterias, y extractos vegetales, que de forma muy significativa se están integrando constantemente a agricultura en el mundo para el manejo de nemátodos fitoparásitos. Particularmente los productos biológicos requieren de ciertas condiciones mínimas de materia orgánica, humedad o aireación, que de no estar presentes anulan por completo los beneficios de su aplicación.

DELADENUS SIRICIDICOLA INTERACTIONS WITH FUNGI: WHO EATS WHOM? [INTERACCIONES DEL NEMATODO DELADENUS SIRICIDICOLA CON HONGOS; ¿QUIÉN COME A QUIÉN?]

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Deladenus siricidicola is a parasitic nematode used to control invasive pine-killing *Sirex noctilio* woodwasps in the Southern Hemisphere. The nematode is inundatively released in several countries, and it is one of the most successful examples of classical biological control. Both nematode and woodwasp rely on the white rot fungus, *Amylostereum areolatum*, for continued survival. The fungus is carried in specialized internal organs of female *Sirex* woodwasps and is deposited into pine trees during oviposition. It is critical for woodwasp survival in that it serves to digest wood for the developing larvae. The nematode *D. siricidicola* has a dual life strategy where part of the time it is a parasite of *S. noctilio*, and part of the time it is free-living and feeds on *A. areolatum*. The free-living stage is exploited for commercial mass-production of the nematode. After observing what appeared to be fungus overgrowing nematode eggs, we tested the hypothesis that a role reversal can occur wherein fungal hyphae invade and kill nematode eggs. *D. siricidicola* eggs were exposed to *A. areolatum* to quantify the number of eggs lost to fungal invasion. Additionally, *A. areolatum* and *A. chailletii* were observed via cryogenic scanning electron microscopy and fluorescence microscopy in order to document their ability to parasitize eggs and adults of *D. siricidicola*, *Deladenus proximus*, and an undescribed *Deladenus* species. This study reports evidence of a basidiomycete destroying nematode eggs, as well as a novel trapping mechanism used to capture and parasitize three species of adult female *Deladenus* nematodes.

NEW INSIGHTS IN THE ENTOMOPATHOGENIC NEMATODES ATTRACTION TO THE RED PALM WEEVIL IN DATE PALMS [NUEVAS PERSPECTIVAS SOBRE LA ATRACCIÓN DE NEMATODOS ENTOMOPATÓGENOS PARA EL PICUDO ROJO EN LAS PALMERAS DATILERAS]

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The red palm weevil (RPW) *Rhynchophorus ferrugineus* is the most dangerous pest of date palms. Its larvae bore deep into the trunk, disrupt the vascular tissues, and kill the infested trees. Delivering control means, chemicals or biological, to the infested trunk is a big challenge. The RPW is known to be susceptible to infection by entomopathogenic nematodes (EPNs). Behavioral features of EPNs reflected by attraction and distribution patterns, is a fundamental aspect in determining their parasitic ability and potential management of RPW. We studied the attraction behavior of the EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to the RPW under simulated natural conditions in columns to evaluate their infective potential. In all experiments a major proportion (38 to 48%) was attracted to the host. Both *H. bacteriophora* and *S. carpocapsae* were efficient crawlers, climbing up and descending when locating their insect host. They were efficiently attracted to the various larval sizes and stages of the RPW life cycle. Host localization by ascending movement was more prominent in *S. carpocapsae* than in *H. bacteriophora*. Using the same system we determined the effect of the vibrations caused by the insect on the behavior of both nematode species. Furthermore, these studies provide the foundation of developing an efficient application system for control the RPW in palm trees by EPNs.

HERE BUT NOT THERE: THE PHYSIOLOGICAL BASIS OF HABITAT ADAPTATION IN ENTOMOPATHOGENIC NEMATODES [AQUÍ, PERO NO ALLÍ: BASES FISIOLÓGICAS DE ADAPTACIÓN AL HÁBITAT EN LOS NEMATODOS ENTOMOPATÓGENOS]

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The geospatial patterns of entomopathogenic nematode (EPN) species in citrus orchards across Florida are congruent with ecoregions that are characterized primarily by the depth to groundwater. Two closely related *Steinernema glaseri*-group species occupy different habitats. *Steinernema diaprepesi* occurs primarily in orchards on the deep (drier) sandy soil of the central ridge ecoregion, whereas *Steinernema* sp. has only been detected in orchards on the shallow (wetter) sandy soils of the several flatwoods ecoregions. The two species respond to soil water potential differently in controlled studies. In soil columns with moisture gradients, *Steinernema diaprepesi* migrated toward drier soil and *Steinernema* sp. toward wetter soil. Both species survived longest at water potentials to which they were attracted. Total proteins were extracted from each species and separated by 2D-PAGE Gel electrophoresis after 48 h in wet (18% moisture) or dry (6%) sand. Proteins that were expressed differentially were identified using LC-MS-MS. We hypothesize that a membrane protein(s) senses and initiates responses to moisture levels differentially in each species. In *S. diaprepesi*, the expression of muscle proteins including paramyosin, actin, and LET-99 required for the proper orientation of spindles after the establishment of polarity varied between wet and dry soil. Some energy enzymes, possibly helping the nematode migrate to preferred soil moisture, also varied between the two conditions. Interestingly, we found that some of these proteins in *Steinernema* sp. varied oppositely in the two conditions, suggesting that it uses some of the same mechanisms to respond differently than *S. diaprepesi* to soil moisture.

FACULTATIVE SCAVENGING BEHAVIOUR IN ENTOMOPATHOGENIC NEMATODES: A SURVIVAL STRATEGY [CARROÑERISMO FACULTATIVO DE NEMATODOS ENTOMOPATÓGENOS: UNA ESTRATEGIA DE SUPERVIVENCIA]

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Entomopathogenic nematodes (EPNs) are very specialized bacteria-feeding organisms. Normally they penetrate an insect releasing a symbiotic pathogenic bacteria and feed on them once the host is dead when the bacteria have transformed the cadaver tissues in a nutritive broth. However, these nematodes can also colonise dead insects and complete their life cycle in the same manner acting as scavengers. We consider scavenging as the ability of entomopathogenic nematodes to penetrate, develop and produce offspring in insects which have been killed by causes other than the nematode-bacteria complex. This behaviour is mediated by nematode species (*Steinernema glaseri* can scavenge dead *Galleria mellonella* larvae which have been dead for 13 days previous nematode colonization, whereas *H. indica* colonize *G. mellonella* killed only 3 days before), species-cadaver found (some species are better for being scavenged than others; including non-insect cadavers), competition for other scavengers (depending on the time and number of individuals, other rhabditid nematodes can use the cadavers as food resource before EPNs). There is no measured evidence of scavenging in nature, EPNs prefer to colonize dead *G. mellonella* larvae rather than to infect live ones, probably trying to overcome the insect immune system (30% more dead larvae were penetrated than live ones). When the nematode species were combined, the results varied among the combinations, but the dead larvae were always used as a host. The significance of scavenging in nature remains under study but the increase of their host range, numbers of colonizing nematodes and preference for dead hosts have been proven.

UNRAVELING THE IMPACT OF THE FREE-LIVING NEMATODES *OSCHEIUS* SPP. IN THE ENTOMOPATHOGENIC NEMATODE REPRODUCTION [DESCIFRANDO EL IMPACTO DE NEMATODO DE VIDA LIBRE *OSCHEIUS* SPP. EN LA REPRODUCCIÓN DE LOS NEMATODOS ENTOMOPATÓGENOS]

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In augmentation biological control, good persistence and natural recycling is the most profitable scenario. Little is known about the post-application biology and ecology of the entomopathogenic nematodes (EPNs). Besides harsh abiotic factors and natural enemies of the infective juveniles, free-living bacterivorous nematodes (FLBN) might compete for the cadaver, displacing EPN reproduction, and hence, decreasing their recycling in the field. Recently, a mixture of the FLBN *Oscheius* spp. in the nematode progeny emerging from insect used as bait revealed an intense competition for the cadaver. We hypothesized that i) *Oscheius* spp. show a scavenging behavior rather than entomopathogenic, ii) *Oscheius* spp. can reproduce in dead larvae, even those killed by EPN, and iii) low EPN quantities and mixed EPN species in combination favors *Oscheius* spp. reproduction. To unravel this relationship, we isolated and characterized new FLBN isolates, tested the outcome of their interactions with *Steinernema kraussei* and/or *Heterorhabditis megidis*, developed species-specific primers/probe for quantitative real time PCR, and evaluated their occurrence in the field in the context of the EPN soil food web. Molecular analysis (ITS and D2D3) designed MG67 to *Oscheius* sp.-2 and MG68 as *O. tipulae* (*Dolichura*-group). None caused mortality to the insects, but they were able to reproduce in >60% of cadavers. Ongoing experiments have shown that larval mortality might not be affected by mixed infection of FLBNs with low (3 IJs) or high (20 IJs) EPN when compared with EPN applied alone; however, both nematode guilds reproduce simultaneously, with possible EPN displacement if low numbers are present. Species-specific primers/probe showed high proportion of *Oscheius* spp. in the nematode progeny from soil-bait cadavers combined with EPN, which was in agreement with their persistence in the field. Learning about this cadaver-competitor is particularly important in those systems where native EPNs populations are low and host availability are limited, such as annual crops.

DISCOVERY OF MULTIPLE PHASMARHABDITIS SPP. IN NORTH AMERICA AND THEIR POTENTIAL FOR BIOCONTROL OF INVASIVE GASTROPODS [DESCUBRIMIENTO DE MÚLTIPLES PHASMARHABDITIS SPP. EN NORTE AMÉRICA Y SU POTENCIAL PARA EL BIOCONTROL DE GASTERÓPODOS INVASORES]

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Invasive snails and slugs are among the most important pests of agriculture, horticulture and other high-value crops. They cause direct losses in crop yield and quality (fecal and mucus contamination) and increase management costs. They are vectors of plant and human pathogens, e.g., *Alternaria brassicicola*, pathogen of brassica dark leaf spot and *Angiostrongylus cantonensis* that causes eosinophilous meningo-encephalitis. In the US, gastropods are managed almost exclusively with molluscicides, metaldehyde being the most widely used. Although generally effective and efficient, it is very toxic to some mammals including dogs and its activity decreases under high relative humidity. Iron phosphate and sodium ferric EDTA are often used as alternatives but both are toxic to non-target soil invertebrates like earthworms. Methiocarb is a restricted use material and is toxic to birds, bees, aquatic taxa and mammals including humans. Other non-chemical control approaches (e.g., use of copper barriers or hand removal) are impractical for large areas. Hence, there is an urgent need for effective and highly targeted strategies such as biocontrol. *Phasmarhabditis hermaphrodita* is commercially available for slug biocontrol in Europe but the nematode has not been found in the US. We recently discovered this species for the first time in CA, along with two *Phasmarhabditis* that are new to science. These species were isolated from three invasive slugs: *Arion hortensis*, *Deroceras reticulatum*, and *Lehmannia valentiana*. Nematodes were identified using combined morphology, morphometrics, and molecular sequence data for internal transcribed spacer (ITS-1, 5.8S, ITS-2) region, D2-D3 expansion segments of the large subunit (LSU or 28S) and nearly complete small subunit (SSU or 18S) ribosomal

DNA. Bacterial associates were identified using 16S rDNA sequences. *Phasmarhabditis* discovery in the US opens possibilities for a more sustainable and safe biocontrol strategy, either alone or as a component of an integrated pest management program for invasive gastropods.

HEALTHY VEGETABLE SEEDLING SYSTEMS: DO THEY WORK UNDER HIGH PEST AND DISEASE FIELD PRESSURE? [SISTEMAS DE PLÁNTULAS DE VEGETALES SALUDABLES: ¿PUEDEN FUNCIONAR BAJO ALTAS PRESIONES DE PLAGAS Y ENFERMEDADES?]

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We are all aware that healthy planting material is a key component to good agricultural practice towards reducing production losses, especially those incurred by pests and disease. Across smallholder cropping systems in sub-Saharan Africa, the health of seed and planting material remains a key challenge, including vegetable seedlings. Smallholder vegetable farmers consistently sow their seed into nurseries or directly into fields, which can result in the immediate infection of germinating seedlings with soil-borne pests and diseases. This includes plant-parasitic nematodes, especially root-knot nematodes. Infested seedlings consequently rarely attain their full potential production, but become further challenged as the season progresses. The promotion of healthy seedlings, produced through sustainable seedling systems would appear a positive step. Such seedlings would however incur higher outlay by the farmer, and so would need to be economically justifiable through improved production. Under the high pest and disease challenges prevailing in smallholder systems, studies were undertaken to establish the benefit of using more costly healthy seedlings compared with traditional farmer produced seedlings. This was further evaluated using seedlings enhanced with the microbial antagonists *Trichoderma asperellum* and *Bacillus subtilis*. The results are discussed.

TREATING BULBS PRIOR TO PLANTING FOR MANAGING LESION NEMATODE ON EASTER LILIES [TRATAMIENTO DE BULBOS ANTES DE LA PLANTACIÓN PARA EL MANEJO DEL NEMATODO LESIONADOR EN LIRIOS DE PASCUA]

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Easter lily bulbs for greenhouse forcing are produced in Del Norte County, California, and Curry County, Oregon, USA. Lesion nematode, *Pratylenchus penetrans*, infestation of roots seriously affects growth of field-grown bulbs. During 2 consecutive years of field trials, commercially prepared formulations of essential oils were compared to an untreated control, and a chemical standard combination of 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN), plus metam sodium (Vapam, Amvac, Los Angeles, CA) [FU] was applied pre-plant followed by Thimet (Phorate, Amvac, Los Angeles, CA) at-planting, to determine their value in improving plant health in the presence of lesion nematode. Three essential oil products from USAgritech (Paso Robles, CA): 1) Duogard, 2) EF400 All Purpose Fungicide, 3) EF300 All Purpose Insecticide; and Cinnamite (Mycotech, Butte, MT) were tested as pre-plant dips to bulblet planting stock. The essential oils were tested either alone, in combination with Thimet at-planting, at planting following FU, or in combination with Thimet at planting following FU. The organophosphates Ethoprop (Mocap, Amvac, Los Angeles, CA), and Fosthiazate (Nemathorin, Syngenta International AG, Basel, Switzerland) were also tested either alone, at a reduced rate combined with a reduced rate of Thimet, or in combination with Thimet and FU. Each trial consisted of the same 22 treatments. Logistics did not permit testing all products in all combinations. Trials were statistically evaluated at $P = 0.05$. In both trials, 10 treatments consistently had a greater bulb circumference at harvest than the control, and four treatments had a greater foliage weight at harvest than the control. In consecutive years, four treatments had healthier-appearing roots than the control. Nine treatments consistently had lower levels of lesion nematode within roots at harvest than the control. The new products tested show promise for use in integrated pest management (IPM) programs for management of lesion nematode.

FOUR YEAR SOUTHERN CALIFORNIA FIELDASSESSMENT OF NEW NEMATICIDES AGAINST ROOT-KNOT NEMATODES IN PROCESSING TOMATO [ENSAYO DE CAMPO DE CUATRO AÑOS EN EL SUR DE CALIFORNIA DE NUEVOS NEMATICIDAS CONTRA NEMATODOS AGALLEROS EN TOMATE PROCESADO]

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California grows approximately 90% of the US processing tomatoes. Crop damage caused by root-knot nematodes has been estimated to be about 10% despite the widespread use of Mi-resistant tomato cultivars or nematicides. Increasing occurrence of resistance-breaking root-knot nematode strains in California's production fields and concerns about air quality issues related to soil fumigants have rekindled interest in novel nematicides. For the past 4 years, we have tested more than a dozen development products for their efficacy in mitigating tomato crop damage in *Meloidogyne incognita*-infested fields. Each summer trials were conducted at the University of California South Coast Research and Extension Center, Irvine, CA (SCREC). The soil was a sandy loam with a relatively uniform *M. incognita* infestation (average Pi2011-2014: 62, 46, 43, 67 J2/100 cm³, respectively). All trials were set up as a randomized complete block with 8-10 treatments and 5 replications. Test materials were predominantly sprinkled onto the beds and rototilled into the top 10 cm or applied with a low volume irrigation system. Root gall ratings were conducted 6-7 weeks after transplanting and at harvest. Fruits were picked once for yield determination. Most products tested failed to show any efficacy at the early evaluation and were not different in harvest disease ratings or yield to the non-treated control. In contrast, Nimitz treatments (a.i., fluensulfone) reduced root galling significantly each year and increased tomato yield on average by 31% over the non-treated control. Although several products in development showed similar efficacy, Nimitz leads the competition having already attained federal US EPA registration in the fall of 2014.

EFFECTIVIDAD DE DIFERENTES ALTERNATIVAS PARA EL CONTROL DE NEMATODOS NODULADORES EN PEPINO BAJO CONDICIONES DE CULTIVOS PROTEGIDOS [EFFECTIVENESS OF DIFFERENT ALTERNATIVES FOR THE ROOT-KNOT NEMATODE CONTROL IN CUCUMBER CROP GROWING UNDER PROTECTED CONDITIONS]

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En la actualidad el desarrollo de métodos de control contra nematodos en cultivos protegidos ha tenido auge como medidas alternativas al uso de pesticidas. En este estudio se determinó la efectividad biológica de diferentes alternativas para el control de nematodos noduladores en el cultivo del pepino (*Cucumis sativus* L.) en la unidad Casa de Cultivos Protegidos de la Empresa Agropecuaria Horquita municipio Abreus, provincia Cienfuegos. Los productos biológicos utilizados fueron *Tsukamurella paurometabola* cepa C-924 (HeberNem[®]), *Micorrizas arbuscular* (EcoMic[®]) y los químicos spirotetramat (Movento[®] OD 150) y dazomet (Dazomet P 98), se utilizó un diseño experimental de bloque al azar, con cuatro réplicas, empleándose variantes con dazomet a dosis 40 g PC/m², el spirotetramat a dosis 0.7 y 0.6 l PC ha⁻¹, en dos y tres aplicaciones foliares a partir de los siete días después del trasplante, *Hebernem* cuatro aplicaciones a dosis de 1 ml/m² cada siete días, aplicación de EcoMic[®] al suelo a razón de 5g/planta y combinaciones de estos, spirotetramat a dosis 0.7 y 0.6 l PC ha⁻¹, en dos y tres aplicaciones foliares más EcoMic[®] y cuatro tratamientos de Hebernem[®] + un tratamiento de EcoMic[®] a dosis 1ml/m² + 5 g/planta respectivamente. El grado medio de infestación por *Meloidogyne incognita* (Kofoid & White) Chitwood se determinó mediante la escala de Zeck modificada en cinco grados. La infestación inicial en el área experimental fue de 2.4, logrando con todas las variantes disminución del grado medio a 1.4, excepto las variantes con spirotetramat a 0.6 L PC ha⁻¹. Los tratamientos con dazomet, EcoMic[®], Hebernem[®], y spirotetramat a 0.7 L PC ha⁻¹ en todas las combinaciones fueron efectivas no permitiendo el desarrollo de los estados juveniles del nematodo,

mientras que spirotetramat a 0.6 L PC ha⁻¹ solamente redujo las poblaciones en combinación con EcoMic[®], resultando la efectividad técnica más promisoría la combinación con spirotetramat a dosis 0,7 L ha⁻¹/ha (2 tratamientos foliares) con un 74.3 % de efectividad, seguida por Hebernem[®] que alcanzó un 55.6% de efectividad técnica. El spirotetramat aplicado a dosis de 0.7 L ha⁻¹ ejerce un efecto nemastático sobre *Meloidogyne incognita* (Kofoid & White) Chitwood bajo sistemas de cultivos protegidos.

ALTERATIONS IN THE PHENYLPROPANOID METABOLISM AND PEROXIDASE ACTIVITY INDUCED BY *NACOBBUS ABERRANS* IN CHILLI (*CAPSICUM ANNUM* L.) CM334 RESISTANT TO *PHYTOPHTHORA CAPSICI* LEO [ALTERACIONES EN EL METABOLISMO FENILPROPANOIDE Y ACTIVIDAD DE PEROXIDASAS INDUCIDAS POR *NACOBBUS ABERRANS* EN CHILE (*CAPSICUM ANNUM* L.) CM334 RESISTENTE A *PHYTOPHTHORA CAPSICI* LEO]

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The genotype of chilli CM334 is resistant to *M. arenaria*, *M. incognita*, *M. javanica*, and *Phytophthora capsici* (Pc), but susceptible to *Nacobbus aberrans* (Na). Apparently, in this genotype, the resistance in root is independent of foliar resistance. In this study, the content of total soluble phenols (TSP), peroxidase (POD) and L-phenylalanine ammonia-lyase (PAL) activity in foliage of CM334 plants inoculated with Na, Pc, or both pathogens (Na - Pc) were determined. Furthermore, the profiling and characterization of soluble phenolic acids and flavonoids were analyzed. Usually, plants inoculated with Pc alone had higher contents of TSP ($P \leq 0.05$) (7.4 mg tannic acid g⁻¹ dry matter) and plants inoculated with Na or Na - Pc had lower levels (4.1 and 3.8 mg) than those non-inoculated (4.8 mg). The highest POD activity (6.6 µM tetraguaiacol mg⁻¹ protein min⁻¹) was registered in plants inoculated only with Pc, while those inoculated only with Na - Pc showed the lowest (4.4 µM) ($P \leq 0.05$). PAL activity was 28.2 nM trans-cinnamic acid µg⁻¹ protein min⁻¹ in plants inoculated

only with Pc, and it was lower (11.7 nM) and similar in non-inoculated plants or those with Na and with Na - Pc ($P \leq 0.05$). The p-HBA, gallic, caffeic, syringic, sinapic, ferulic, vanillic, p-coumaric, and chlorogenic acids were found in foliage of CM334 plants. Chlorogenic acid was the phenolic acid in the highest quantity in foliage (from 277 to 458 $\mu\text{g}\cdot\text{g}^{-1}$ of dry matter). In all sampling points, plants inoculated with Na showed lower content of p-hidroxy benzoic and chlorogenic acid than control ($P < 0.05$). Rutin, apigenin and luteolin were the flavonoids found in shoot. Significant differences ($P < 0.05$) in flavonoids content between inoculated and non-inoculated plants were found. Taking all together, the results obtained in the present study suggest that *N. aberrans* reduced the defence responses in foliage and could be enough for the establishment of foliar pathogens in CM334 chilli pepper plants.

THE EFFECT OF METHYL JASMONATE FOLIAR SPRAY IN TOMATO ON THE LIFE CYCLE OF THE ROOT-KNOT NEMATODE *M. INCOGNITA* [EFECTO DEL SPRAY FOLIAR DE METIL JASMONATO SOBRE EL CICLO DE VIDA DEL NEMATODO *M. INCOGNITA* EN TOMATE]

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Plant defense elicitors are synthetic or natural compounds which induce systemic resistance in plants. This plant based resistance is multigenetic and could offer a sustainable alternative for the use of resistant cultivars which carry a single resistance gene. The durability of these resistance genes is questionable as infection of resistant Mi tomatoes by virulent populations of *Meloidogyne* spp. have been reported several times. The root-knot nematode *Meloidogyne*, is a soil-borne parasite of the plant root system, and its host range includes economically important crops, both monocots and dicots, including rice and many common vegetables. As such, they cause damage worth of billions of dollars worldwide. One of such elicitors is methyl jasmonate, a plant hormone involved in the plant defense system. In the present study we have examined whether repeated spraying of this hormone could influence the life cycle of the nematodes. We will discuss an experiment carried out with the tomato cv. Marmande. The plants were sprayed with the elicitor and 2 days later, inoculated with freshly hatched second-stage juveniles (J2) of

M. incognita. Thirty days after inoculation the plants were harvested, several plant variables assessed and the root galling index determined. The roots were collected and stained with acid fuchsin to visualize the nematodes microscopically and the egg masses were visualized by phloxine B and counted. Surprisingly, the results show a growth-promoting effect of methyl jasmonate on nematode development but not on the amount of egg masses. Furthermore, a qRT-PCR has been carried out to determine the induction of the resistance at several time points after spraying, of both inoculated and uninoculated plants in both leaves and roots. We will further discuss the results and implications of the use of defense elicitors.

BIODESINFECCIÓN DE SUELOS: ALTERNATIVA SUSTENTABLE PARA EL MANEJO DEL SUELO. EXPERIENCIA DE CUBA [SOILS BIODESINFECTION: SUSTENTABLE ALTERNATIVE FOR SOIL NEMATODES MANAGEMENT. CUBA EXPERIENCE]

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En Cuba, se desarrollan proyectos multidisciplinarios que estudian y proponen alternativas para una gestión agroecológica de los sistemas agrarios, donde la aplicación de agroquímicos tuvo impactos negativos sobre los suelos y los agroecosistemas. Para el manejo de nematodos, así como la mejora en los parámetros físicos, químicos y biológicos de los suelos, se utilizan residuos del sector agrícola e industrial entre los que se destacan la materia orgánica y vinaza de caña de azúcar, estudiando su eficacia tanto solos como combinados, así como se evalúa su efecto sobre organismos benéficos, analizando su acción en el crecimiento, nutrición, índice de nodulación y en particular sobre las propiedades del suelo, centrándose los estudios en el cultivo de las hortalizas, reduciendo el consumo de agua y fertilizantes, aumentando la microfauna edáfica y el rendimiento de los cultivos. Los aportes obtenidos han demostrado que se disminuye más de un 80% los fitoparásitos y se aumentan los organismos benéficos, además que se aumenta en un 95% el

rendimiento cuando se combina los dos residuos, demostrando que se les confieren un valor añadido cuando se utiliza de manera eficiente, destacando a su vez el impacto que desde el punto de vista social ofrecen. Se propone esta alternativa por la acción desinfectante de las sustancias que se originan durante la biodescomposición de la materia orgánica para el manejo de los patógenos de las plantas.

EFFECTO DEL NEMACUR® 10 G Y THIMET® 10 G EN EL CONTROL DE *GLOBODERA PALLIDA* Y LA PRODUCCION DE PAPA (*SOLANUM TUBEROSUM* L) VARIEDAD FLORESTA [EFFECT OF NEMACUR® 10 G AND THIMET® 10 G ON POTATO (*SOLANUM TUBEROSUM* L) VAR. FLORESTA *GLOBODERA PALLIDA* CONTROL AND CROP YIELD]

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En un terreno infestado de nematodos (*Globodera pallida*) y cultivado con papa (*Solanum tuberosum*) variedad Floresta se evaluó el efecto de Nematicur® y Thimet® en el control del nematodo y la producción del cultivo. Los nematicidas se aplicaron a la siembra o a la siembra y aporca. A los 45 días de la siembra, los nematicidas aplicados a la siembra redujeron ($P = 0,0023$) el número de quistes y en las plantas de las parcelas tratadas con la mezcla Nematicur® 10 G - Thimet® 10 G se observó la menor población de juveniles con 189 nematodos por 100 g de raíces. Con la aplicación de Nematicur® 10 G a la siembra y a la aporca se encontró un 10% de reducción en la población de quistes de los 45 a los 155 días después de la siembra resultando en el menor crecimiento de la población. La densidad inicial de 0,6 quistes por gramo de suelo en el testigo sin control, resultó en una reducción del 48% en producción. En promedio, los tratamientos nematicidas superaron ($P = 0,0537$) al testigo en 5,6 toneladas ha⁻¹ y el Nematicur® 10 G a la siembra y la aporca, y la mezcla de Nematicur® - Thimet® 10 G a la siembra, lo superaron en 10,7 ($P = 0,0131$) y 6,2 toneladas ha⁻¹ ($P = 0,0455$), lo que representó una ganancia neta de \$2743, \$6383 y \$3625 por hectárea, respectivamente. Los aumentos en producción encontrados variaron de un 19-106%, siendo mayores cuando la aplicación se hizo tanto a la siembra como a la aporca.

VIRULENCIA DE POBLACIONES DEL GÉNERO *MELOIDOGYNE* EN PORTAINJERTOS DE FRUTALES DE CAROZO UTILIZADOS EN CHILE [VIRULENCE OF THE GENUS *MELOIDOGYNE* IN STONE FRUIT ROOTSTOCKS USED IN CHILE]

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Los nematodos del género *Meloidogyne* son considerados unos de los más dañinos en el mundo. En frutales de carozo pueden ocasionar pérdidas cercanas al 15%. Con el objetivo de determinar la virulencia del género *Meloidogyne* en portainjertos de *Prunus* sp. se colectó 20 poblaciones en el Valle Central de Chile. A partir de ellas se inició la crianza de líneas puras en plantas de tomates bajo condiciones de invernadero. Estas líneas están siendo caracterizadas con marcadores morfológicos, moleculares e isoenzimáticos. Posteriormente, de cada línea se obtuvieron 10.000 huevos para inocular cada maceta. Se utilizó tres portainjertos, incluyendo dos descritos como resistentes, Nemaguard (*Prunus persica* x *P. davidiana*) y Marianna 2624 (*P. cerasifera* x *P. munsoniana*), y un susceptible, Pomona (*P. persica*), los que fueron sometidos al test. Luego de 5 meses se evaluó el agallamiento del sistema radical y el índice reproductivo R (pf/pi). El análisis estadístico utilizó un diseño completamente aleatorio con 5 repeticiones. Los resultados muestran un fuerte agallamiento y reproducción sobre el portainjerto Pomona, mientras que en Nemaguard se observó una virulencia diferencial siendo algunas poblaciones de *M. ethiopica* y *M. javanica* las más virulentas. Por su parte el patrón Marianna 2624 fue inmune a todas las poblaciones. Por lo tanto, este portainjerto es un material parental promisorio para un programa de mejoramiento genético destinado a la búsqueda de resistencia a *Meloidogyne* spp.

NEMATODE PARASITES OF POTATO (*SOLANUM TUBEROSUM* L.) AND GLOBALIZATION: NEW CHALLENGES AND OPPORTUNITIES [NEMATODOS PARÁSITOS DE LA PAPA (*SOLANUM TUBEROSUM* L.) Y LA GLOBALIZACIÓN: NUEVOS RETOS Y OPORTUNIDADES]

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When potato was brought from South America to Europe centuries ago, the potato cyst nematode (*Globodera* sp.) came along. This event is probably one of the earliest consequences of globalization, a term used for international integration of (agri) cultural and economic activities, as well as environmental and social issues. Quarantine measures for important nematode species like *G. rostochiensis* and *G. pallida*, the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*, and the false root-knot nematode *Nacobus aberrans*, ensure that these species remain restricted to certain regions of the world. However, their spread continues within these regions, indicating there are still gaps in the way we deal with them. More nematode-resistant potato cultivars, improved handling of contaminated waste products, better detection methods and paying more attention to processes occurring in the rhizosphere, can help in solving these gaps. Global awareness of the negative impacts of chemicals led to restriction of nematicides in the 90s, at least in Europe. At the same time, global warming is influencing nematode life cycles and impacting their survival. These events probably contributed to the observed “revival” of potato damage provoked by *Pratylenchus* spp. and Trichodorids. Stimulating potato cropping in (sub) tropical regions to keep up with global food demand should take into account that certain local nematode species can impact yields, e.g., *Ditylenchus* spp., *Meloidogyne* sp., and even *Scutellonema bradys*, the yam nematode. Modern technology, increasingly accessible, can be used to educate farmers and traders worldwide, ranging from software applications showing damage symptoms, to decision support systems for choosing appropriate management tools. Global exchange has increased tremendously in the last decades: people, machineries, plants, but also nematodes move intensively between continents. As education, technology and research efforts are exchanged too, globalization offers as many opportunities as challenges and could contribute to lessen the impact of nematodes on potato.

ROOT AND TUBER CROPS AND THEIR ASSOCIATED NEMATODE PROBLEMS IN AFRICA [CULTIVOS DE RAÍCES Y TUBÉRCULOS Y SUS PROBLEMAS DE NEMATODOS EN ÁFRICA]

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Root and tuber crops are key components of human diets and essential sources of nutrition across the globe. In less developed countries, small holders may rely on such crops for survival. However, plant parasitic nematodes remain a serious challenge to roots and tubers, arguably proportionally more so in less developed countries than elsewhere. With a focus on Africa, we will discuss the nematode problems of key root and tuber crops to the region. Cassava, sweet potato, yam and potato are four principal root and tuber crops grown across tropical and sub-tropical regions in Africa that are relied on by millions of people as starch staple crops. Cassava is commonly respected for its ability to withstand various constraints and be able to produce under taxing climatic and physical conditions. It is also generally viewed as having no nematode problem. Sweet potato is similarly not recognised as having any significant nematode problem, but surveys and basic studies demonstrate the potential of nematodes to seriously reduce productivity. Yam is traditionally important in West Africa, but is extending its importance across sub-Saharan Africa. A key quality characteristic of yam is its ability for long storage, enabling food security during periods of poor productivity. The yam nematode, however, is perhaps most devastating during storage, significantly undermining yam productivity and quality. Potato is nematologically best recognised for the impact of potato cyst nematode (PCN) on this crop. In sub-Saharan Africa PCN has traditionally not been a cause for concern however, while damage by root knot nematode has largely been overlooked. In general only limited attention has been paid to nematode problems of root and tuber crops in general, in part due to ignorance of the potential for nematode damage and in part due to limited expertise to explore this area.

PLANT PARASITIC NEMATODES AS IMPORTANT CONSTRAINTS FOR YAM (*DIOSCOREA* SPP.) PRODUCTION IN WEST AFRICA: ASSESSMENT OF DAMAGE ON TUBERS COLLECTED FROM FIELDS, MARKETS AND STORES [NEMÁTODOS PARÁSITOS DE PLANTAS COMO IMPORTANTES LIMITACIONES PARA LA PRODUCCIÓN DE ÑAME (*DIOSCOREA* SPP.) EN ÁFRICA OCCIDENTAL: EVALUACIÓN DE DAÑOS EN TUBÉRCULOS RECOGIDOS EN LOS CAMPOS, MERCADOS Y TIENDAS]

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Surveys were conducted to assess the incidence of plant parasitic nematodes and associated damage to yam (*Dioscorea* spp.) tubers in fields, markets and stores in Benin and Nigeria. In Benin, 695 yam local accessions including 2500 tuber samples from 202 farmers' fields were visually assessed for typical nematode (*Scutellonema bradys* and *Meloidogyne* spp.) damage symptoms during harvest period. In Nigeria, visual assessment of nematodes was carried out on 1141 yam heaps from 218 vendors in different markets and 26 farmers' stores located in the vicinity of the surveyed markets. Damage assessment of fields' tubers showed significant variability in severity and incidence of galls, cracking, and dry rot symptom between accessions and between agroecological zones. Severity of tuber galling, cracking, and dry rot symptom was scored by up to 5 over 5 for some accessions such as "Môrôkô", "Wodjoa", and "Wôrgôninti", respectively. Incidence was also high for many accessions such as "Tchaboulangapa" and Kabletona (100% for galling, cracking, and dry rot symptom). The markets' survey indicated an incidence of galling ranging from 52% (Derived Savanna) to 59% (Humid Forest), dry rot symptom from 17% (Southern Guinea) to 44% (Humid Forest), and tuber cracking between 4% (Southern Guinea) and 7% (Derived Savanna). In the farmers'

stores, the gall incidence ranged from 22% (Derived Savanna) to 27% (Southern Guinea) while the dry rot incidence was up to 9% (Derived Savanna). The crack incidence varied from 1% to 3% with the highest incidence recorded in the Derived Savanna. In addition, mixed nematode damage symptoms were observed on yam tubers in fields, markets, and farmers' stores. This study further established the evidence that nematodes are important constraints for yam production, suggesting that effective yam nematode management strategy is an urgent need for sustainable yam production in West Africa.

NEMATODOS DE IMPORTANCIA ECONOMICA EN BANANO, PLATANO, TUBERCULOS Y RAICES COMESTIBLES EN CUBA [NEMATODES OF ECONOMICAL IMPORTANCE IN BANANA, PLANTAIN AND ROOT AND TUBER CROPS IN CUBA]

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Cuba es un país altamente consumidor de bananos y viandas en su dieta diaria, entre las más demandadas están las musáceas (*Musa* spp), malanga (*Xanthomonas violaceum* Schoott y *Colocasia esculenta* S.), boniato (*Ipomoea batatas* L.) y la papa (*Solanum tuberosum* L.), donde los nematodos parásitos constituyen una plaga de consideración. El trabajo recoge los resultados en más de 15 años, con las principales especies de fitonematodos asociadas, sus daños, comportamiento varietal y algunos componentes del manejo. En las musáceas se registran como principales especies a *Radopholus similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus* y *Meloidogyne* spp. La introducción de híbridos FHIA produjo cambios importantes como la manifestación de resistencia parcial a *R. similis*, particularmente en los híbridos FHIA 01, 02, 18, 21 y la variedad SH-3436. El cultivo de malanga presenta principalmente problemas con *M. incognita*, *M. javanica*, y *M. arenaria*; todas las variedades sembradas en el país han mostrado ser susceptibles a estas especies. En el cultivo del boniato se manifiesta *M. incognita* y *Rotylenchulus reniformis*, la primera provoca rajaduras en los tubérculos, estos daños en ocasiones son subestimados o menospreciados; la

mayoría de los clones de boniato son susceptibles a estos patógenos, aunque el clon CEMSA 78-354 tiene alta resistencia y es un excelente cultivo de rotación en áreas con altos niveles de *M. incognita*. En Cuba, la papa se siembra a nivel del mar, bajo estas condiciones los nematodos formadores de agallas del género *Meloidogyne* se han informado entre las plagas más importantes; *M. incognita* y *M. arenaria* son las más comunes en diferentes tipos de suelos. Todas las variedades comerciales y precomerciales de papa evaluadas han manifestado una alta susceptibilidad a estas especies.

NEMATODOS ASOCIADOS A LAS HORTALIZAS BAJO CULTIVO PROTEGIDO EN TRES LOCALIDADES DE CUBA. ESTUDIOS DE CASO [NEMATODES ASSOCIATED WITH VEGETABLES UNDER PROTECTED CULTIVATION IN THREE LOCALITIES OF CUBA. STUDY CASES]

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Las hortalizas bajo cultivo protegido presentan problemas de plagas, entre ellas los nematodos del género *Meloidogyne*, que afectan la producción y calidad. El presente trabajo brinda los resultados obtenidos en tres localidades de La Habana (Wajay, Atabey y Las Guásimas), respecto a las especies de nematodos parásitos asociadas y sus daños, así como características de los sistemas de cultivo y su influencia sobre los problemas nematológicos y la capacitación. Se analizaron muestras de suelo, agua de riego, raíces y las materias orgánicas empleadas. El género *Meloidogyne* se presentó en todas las localidades (78,8% de las casas de cultivos muestreadas), con predominancia de las especies *M. incognita* (78,8%) y *M. arenaria* (12,1%); no obstante otros como *Rotylenchulus reniformis*, *Xiphinema basiri*, *Helicotylenchus dihystra*, y *Tylenchorhynchus* sp., aparecieron en la localidad de Las Guásimas. Se observaron daños apreciables en las raíces solamente con *Meloidogyne* y *Xiphinema*. Fueron detectadas contaminaciones en algunos sistemas de colecta de agua para regar, que contribuían a la diseminación de los parásitos y aumento de los problemas en los campos. Igualmente se encontraron infestaciones por *Meloidogyne* en zonas no sembradas con anterioridad, producto de la presencia de malezas hospedantes y contaminaciones

por arrastre. La Biofumigación con restos de col, produjo disminuciones significativas (más de 75%) de los niveles de infestación por *M. incognita*, mientras que la aplicación de *Trichoderma viride* (cepa TS-3) y *Bacillus thuringiensis* (cepa LBT-25) permitió una reducción del 50% de plantas con altos grados de infestación. Se realizaron en el periodo de 14 meses cuatro talleres participativos sobre el tema y se editaron tres plegables divulgativos.

NEMATODOS ASOCIADOS CON UCHUVA (*PHYSALIS PERUVIANA* L.) EN 24 MUNICIPIOS DE COLOMBIA [NEMATODES ASSOCIATED TO (*PHYSALIS PERUVIANA* L.) PLANTS IN 24 COLOMBIAN MUNICIPALITIES]

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La producción de uchuva (*Physalis peruviana* L.) en Colombia, actualmente, está limitada por un complejo patológico que involucra al hongo *Fusarium oxysporum* y nematodos, entre otros factores. Esta interacción, ocasiona devastadoras epidemias que acortan el ciclo productivo de 3 años a 12-18 meses y convierten el cultivo en itinerante, debido a la contaminación de suelos. En un levantamiento de poblaciones de nematodos realizado en cuatro estados y 24 municipios, se encontraron 8 géneros de nematodos fitoparásitos. En raíces se presentó *Meloidogyne*, *Pratylenchus*, y *Helicotylenchus* en poblaciones promedio de 464, 23, y 225 nematodos por 1 g de raíces; en suelo de la rizosfera se encontró *Meloidogyne*, *Pratylenchus*, *Xiphinema*, *Hemicycliophora*, *Helicotylenchus*, *Heterodera*, trichodoridos y criconematidos, en poblaciones promedio de 4687, 356, 121, 415, 6660, 66, 162, y 56 nematodos por 100 cc de suelo, respectivamente. El nematodo espiral y el nematodo del nudo fueron los taxones predominantes en raíces y suelo, seguidos por el nematodo de la lesión, en raíces; y por trichodoridos, en suelos. En pruebas preliminares de parasitismo con plantas de *Physalis peruviana*, se reprodujeron todos los géneros, excepto criconematidos y *Heterodera*, taxones para los cuales aún no se tiene información.

EVALUACIÓN DE LA CAPACIDAD HOSPEDERA DE CULTIVARES Y PORTAINJERTOS DE VID A *PRATYLENCHUS THORNEI*, *P. VULNUS*, Y *P. PENETRANS* EN PLANTAS CULTIVADAS EN MACETAS [ASSESSMENT OF THE HOST CAPACITY OF GRAPEVINE CULTIVARS AND ROOTSTOCKS TO *PRATYLENCHUS THORNEI*, *P. VULNUS*, AND *P. PENETRANS* IN A STUDY WITH POTTED PLANTS]

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Los nematodos del género *Pratylenchus* son muy comunes en suelos cultivados con vides, sin embargo poco se sabe acerca de la incidencia de las especies más frecuentes en el vigor de las plantas y la capacidad hospedera de estas. Un estudio para determinar la capacidad hospedante de plantas de vid francas y portainjertos a *Pratylenchus thornei*, *P. vulnus*, y *P. penetrans* fue realizado en macetas inoculadas en forma artificial. Las especies puras fueron cultivadas en discos de zanahoria y una vez obtenidos los ejemplares necesarios, se procedió a inocular plantas de 8 meses establecidas en macetas de 10 L de capacidad con un sustrato en base a tierra agrícola, arena y tierra vegetal en proporción 1:1:1, con una población de 1000 ejemplares de nematodos por maceta. Se utilizó plantas francas cv Chardonnay y los portainjertos 101-14 y Harmony, de amplio uso de vides en Chile, dispuestos en un diseño completamente al azar con 8 repeticiones por combinación. Para la evaluación de los resultados se utilizó el Índice Reproductivo Pf/Pi, el cual indica la tasa reproductiva de las especies, evaluando los ejemplares en suelo y raíces 1 año después de la inoculación. Los resultados indican que los 2 portainjertos y el cultivar franco no son buenos hospederos para ninguna especie de *Pratylenchus*, ya que los valores de R fluctuaron entre 0 y 0.25 como máximo.

PINE WILT DISEASE AND THE PINWOOD NEMATODE, *BURSAPHELENCHUS XYLOPHILUS*: RECENT PROGRESS AND UPDATED INFORMATION FROM THE EU PROJECT “REPHRAME” [MARCHITEZ DEL PINO Y EL NEMATODE *BURSAPHELENCHUS XYLOPHILUS*: PROGRESO RECIENTE E INFORMACIÓN ACTUALIZADA SOBRE EL PROYECTO EUROPEO “REPHRAME”]

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Bursaphelenchus xylophilus, the pinewood nematode (PWN), the causal agent of pine wilt disease (PWD), was detected for the first time in 1999, in Europe, in Portugal. The PWN has been detected in new forest areas in the center of the country, in 2008, despite efforts developed by the national forestry and quarantine authorities to control the nematode and its insect vector (*Monochamus galloprovincialis*). The nematode has also recently been reported to be present in Madeira Island and Spain. Circulation of non-treated wood and wood products may explain the spread of the nematode. Control strategies have been focused on the vector by using chemical traps, by cutting down symptomatic trees, heat-treatment of lumber, and monitoring of main roads and ports through which lumber and wood products are transported, by the Portuguese authorities. The nematode constitutes a threat to the rest of Europe if proper measures are not taken by European governments. The issue constitutes a major challenge to nematology in regards to nematode (and insect) bioecology, pathogenicity, use of molecular biology in diagnostics and detection, histopathology, etc. Many gaps in the knowledge of this complex biological system persist. The involvement of bacteria, associated with the PWN in causing pine wilt, has been claimed. New quick detection methods and understanding of the nematode population dynamics are being developed. Nematode genomics may provide some insight to better understand the pathogenic effects caused inside the plant. Pathogenicity testing of susceptible pine species is imperative. A EU 7th Framework project (REPHRAME) has recently ended, and several major findings and recommendations have been produced as a result of this 3-yr, 10-partner consortium. A review of recent progress and major conclusions of this project are hereby presented.

HIGH GENETIC DIVERSITY AND GEOGRAPHIC SUBDIVISION OF *HOPLOLAIMUS STEPHANUS* IN THE UNITED STATES [ALTA DIVERSIDAD GENÉTICA Y SUBDIVISIÓN GEOGRÁFICA DE *HOPLOLAIMUS STEPHANUS* EN ESTADOS UNIDOS]

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Lance nematodes (*Hoplolaimus* spp.) feed on the roots of a wide range of plants, some of which are agronomic crops. Morphometric values of amphimictic lance nematode species overlap considerably, and useful morphological characters for their discrimination require high magnification and significant diagnostic time. Given their morphological similarity, these *Hoplolaimus* species provide an interesting model to investigate hidden diversity in crop agroecosystems. In this scenario, *H. galeatus* may have been over-reported and the related species that are morphologically similar could be more widespread in the United States than has been recognized thus far. The main objectives of this study were to delimit *H. stephanus* and morphologically similar species by using morphology, phylogeny, and a barcoding approach, and to estimate the genetic diversity and population structure of the species found. Molecular analyses were performed using sequences of the cytochrome c oxidase subunit 1 (Cox1) and the internal transcribed spacer (ITS1) on 23 populations. *Hoplolaimus galeatus* exhibited low genetic diversity and the shortest genetic distances among populations. In contrast, *H. stephanus*, the species with the fewest reports from agricultural soils, was the most common and diverse species found. Results of this project may lead to better delimitation of lance nematode species in the United States by contributing to the understanding the diversity within this group.

INFLUENCE OF *SOLANUM SISYMBRIIFOLIUM* AND THE BIOLOGICAL CONTROL FUNGI *TRICHODERMA HARZIANUM* AND *PLECTOSPHAERELLA CUCUMERINA* ON CONTROL OF *GLOBODERA PALLIDA* [INFLUENCIA DE *SOLANUM SISYMBRIIFOLIUM* Y LOS HONGOS CONTROLES BIOLÓGICOS *TRICHODERMA HARZIANUM* Y *PLECTOSPHAERELLA CUCUMERINA* EN EL CONTROL DE *GLOBODERA PALLIDA*]

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The effect of the trap crop *Solanum sisymbriifolium* (litchi tomato; LT) combined with the biocontrol agents *Trichoderma harzianum* or *Plectosphaerella cucumerina*, on population decline of *Globodera pallida* (pale cyst nematode; PCN) was assessed. Effects were determined under three simulated cropping systems (potato, *S. sisymbriifolium*, or soil only-fallow), amended with either *P. cucumerina*, or *T. harzianum* or non-amended. Soil was infested with PCN at a rate of 5 eggs/g soil and planted with either potato or *S. sisymbriifolium*. The soil-only treatment was amended with the biocontrol agent but not planted. Treatments were as follows: fallow-no agent added, fallow-*P. cucumerina*, fallow-*T. harzianum*, LT-no agent added, LT-*P. cucumerina*, LT-*T. harzianum*, potato-no agent added, potato-*P. cucumerina*, potato-*T. harzianum*. After 16 wk in the greenhouse, plants were removed; soil containing cysts was chilled for 8 wk, and then planted into a potato bioassay. Cyst counts were determined after an additional 16 wk. PCN populations were significantly reduced in a potato after litchi tomato cropping system compared to potato after potato. Not only was a reduction in cyst numbers observed, but a decrease in nematode fecundity (eggs per cyst) was also observed. *P. cucumerina* decreased the final populations of PCN (eggs/g soil) in both the potato-after-LT and the potato-after-potato treatments. *T. harzianum* reduced PCN populations only in the potato-after-LT. Our results suggest that litchi tomato has potential to significantly reduce PCN populations, and also that the cropping system may play a significant role in the efficacy of biological control agents.

EXPERIENCIAS EN LA PRODUCCIÓN DEL BIONEMATICIDA KLAMIC® EN CUBA [MASS PRODUCTION EXPERIENCES OF BIONEMATICIDE KLAMIC® IN CUBA]

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KlamiC® es un bionemático que se produce y comercializa en Cuba, cuyo ingrediente activo son las clamidosporas producidas por el hongo nematófago *Pochonia chlamydosporia* var. *catenulata* (IMI SD 187). Para su producción se desarrolló una tecnología de Fermentación en Estado Sólido en Bolsas con filtro (FESB) bajo normas de calidad, que permitió elevar la producción de clamidosporas. Actualmente se evalúa la ampliación de las capacidades productivas y el mejoramiento de la viabilidad del producto. En los últimos 7 años en el CENSA se produjeron 231 lotes, con un incremento en el escalado productivo a nivel de Planta Piloto y la obtención de más de 2366 kg de KlamiC®, que se han empleado para el manejo de nematodos formadores de agallas en diferentes sistemas intensivos de producción agrícola en el territorio nacional y para la investigación. La evaluación de los lotes mostró una producción media de $1,5 \times 10^7$ clamidosporas.g⁻¹ de sustrato con una viabilidad superior a 88%, parasitismo de huevos de *M. incognita* de 76%, contenido de agua 5,9% y contaminación microbiana de $6,99 \times 10^4$ UFC.g⁻¹, cumpliendo con los indicadores de calidad del producto, lo cual constituye uno de los mayores retos del escalado de las producciones. Estos resultados demuestran la reproducibilidad y consistencia de los lotes mediante esta tecnología bajo un sistema que asegura la calidad y que recientemente se transfirió con éxito en Nicaragua al Laboratorio de producción de hongos biocontroladores Biotor Labs S.A. La metodología de superficie respuesta permitió proponer un diseño para la evaluación de la estabilidad en almacén de formulaciones de KlamiC® en forma de polvo humedecible, conteniendo zeolita como relleno, en diferentes condiciones de almacenamiento y se localizó un punto de máxima viabilidad de clamidosporas con el empleo de 30 a 40% de relleno almacenando el producto a 15°C, lo cual sirve de base para la evaluación de nuevas formulaciones en base a otros excipientes disponibles compatibles con el hongo.

EXOENZYMES AND METABOLITES RELATED TO THE NEMATICIDAL EFFECT OF RHIZOBACTERIA ON *XIPHINEMA INDEX* THORNE & ALLEN [EXOENZIMAS Y METABOLITOS ASOCIADOS A RIZOBACTERIAS CON CARACTERÍSTICAS NEMATICIDAS SOBRE *XIPHINEMA INDEX* THORNE & ALLEN]

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Plant-growth promoting rhizobacteria (PGPR) are known to produce antibiotics, antimicrobial metabolites, hydrolytic enzymes and other components, some of which have been reported in the control of plant-parasitic nematodes. A series of bacterial strains were isolated from soils in central Chile, and previous investigations promoted four of them as potential *X. index* controllers and promoted plant growth. For a reliable identification and also to evaluate the presence of metabolites with nematicide potential, these isolates were considered for *in vitro*, biochemical and molecular studies. The direct effect of the bacterial filtrates was *in vitro* evaluated on *X. index* larvae and adults. Hydrogen sulfide, hydrogen cyanide liberation, and also protease, chitinase, collagenase, and lipase presence were verified for the four isolates. To identify them, up to five housekeeping genes and one ITS 16-23S RNAr were sequenced and analysed. Results show that all strains belong to *Bacillus* genera, among them there are three pure species: *B. megaterium* FB133M, and two isolates of *B. thuringiensis* (FB833T, FS213P), while the fourth isolate FR203A, proved to be in fact a consortium of two different *Bacillus* species, *B. amyloliquefaciens*, and *B. subtilis*. All bacterial filtrates presented 54 to 100% mortality evaluated at 72 h of nematodes exposure. Rhizobacterial strains presented proteases activity, two of them (strain FB833T and FR203A) showed reliable collagenase and chitinase activities, and three of them showed a strong lipolytic activity (FB833T, FR203A, and FS213P). Strain FB133M had no lipases activity and also presented the lowest nematicidal effect on *X. index*. The rhizobacterial strains tested possess nematicidal compounds, which may be an interesting alternative and a potential tool in control on *X. index*.

UTILIZACIÓN DE *TRICHODERMA* SPP. PARA EL MANEJO DE *MELOIDOGYNE* EN TOMATE (*LYCOPERSICON ESCULENTUM* MILL.) EN LA PROVINCIA DE MATANZAS, CUBA [USE OF *TRICHODERMA* SPP. FOR *MELOIDOGYNE* MANAGEMENT IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) IN MATANZAS PROVINCE, CUBA]

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En Cuba uno de los principales problemas fitosanitarios que se presentan en la producción de hortalizas bajo diferentes sistemas de cultivo, lo constituye la incidencia de nematodos pertenecientes al género *Meloidogyne*. Como alternativa biológica de manejo de *M. incognita* en la provincia de Matanzas, se valoró en condiciones de producción (sistema de organopónicos), la efectividad técnica del biopreparado sólido de las tres cepas de *Trichoderma* que se reproducen en los Centros de Reproducción de Entomofagos y Entomopatogénos (*T. harzianum* A-34 y A-53; *T. viride* TS-3) y además se valoró el efecto de la aplicación de *T. viride* cepa TS-3, en forma líquida, en el Sistema de Cultivos Protegidos; en todos los casos se realizó la primera aplicación un día antes de la siembra. La aplicación de los biopreparados sólidos de todas las cepas en condiciones de organopónicos, produjo resultados alentadores en la reducción de la infestación por los nematodos, con valores superiores al 50% en la dosis de aplicación de 30 kg/Ha; no obstante con la cepa A-53 a dosis de 20 kg/Ha se lograron reducciones similares. Estos resultados se acompañaron de un incremento significativo de los rendimientos respecto a las zonas no tratadas. Bajo las condiciones de cultivo protegido, la utilización del biopreparado líquido de TS-3 con 4 aplicaciones a dosis de 30 l/Ha, redujo la infestación en valores significativos, que incidieron en los resultados de rendimiento y rentabilidad de las instalaciones que utilizaron el procedimiento. Se recomendó la generalización de los resultados en las zonas contaminadas por este nematodo en la provincia.

USO COMBINADO DE *METARHIZIUM ANISOPLIAE* Y *BEAUVERIA BASSIANA* CON *HETERORHABDITIS BACTERIOPHORA* PARA EL MANEJO DE LA PALOMILLA DORSO DE DIAMANTE BAJO CONDICIONES DE INVERNADERO [COMBINED USE OF *METARHIZIUM ANISOPLIAE* AND *BEAUVERIA BASSIANA* WITH *HETERORHABDITIS BACTERIOPHORA* FOR CONTROL OF DIAMONDBACK MOTH IN GREENHOUSE CONDITIONS]

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La palomilla dorso de diamante (*Plutella xylostella*) es una de las principales plagas del cultivo de brócoli en el mundo. Ocasiona defoliación, minas superficiales y genera pérdidas anuales superiores al 80%. Una de las alternativas de manejo es la aplicación de hongos y nematodos entomopatogénos; sin embargo, no se ha estudiado la aplicación combinada. En este estudio se evaluó la interacción de *B. bassiana* Bb9205 y *M. anisopliae* Ma9236 con *H. bacteriophora* HNI0100 para su control en condiciones de invernadero. Para determinar la susceptibilidad de la plaga frente a los entomopatogénos los experimentos se hicieron en plantas de brócoli, cada una con 4 larvas (300 plantas). Los juveniles infectivos se suspendieron en agua destilada con Tween 80 y se inocularon sobre las hojas de brócoli en las dosis 0, 3×10^2 , 6×10^2 y $1,2 \times 10^3$ JIs/cm². La mortalidad de las larvas y daño en la planta se evaluó por una semana. Para los hongos entomopatogénos se realizó la misma metodología con las dosis de 0, 1×10^5 , 1×10^6 y 1×10^7 conidios/cm², la mortalidad se evaluó durante dos semanas. La aplicación de *H. bacteriophora* HNI0100 generó una mortalidad superior al 87% en las tres dosis, escogiendo 1×10^2 JIs/cm² por causar mayor mortalidad en menor tiempo. Así mismo *P. xylostella* fue susceptible a los hongos encontrando que a dosis de 1×10^5 con/cm² de *B. bassiana* Bb9205 y *M. anisopliae* Ma9236 la mortalidad es superior al 85%. En los ensayos de interacción entre hongos y nematodos se inocularon inicialmente los hongos seguido de los nematodos en diferentes días (0, 2, 4, y 6). Se estableció al día 0, 2, y 4 una interacción antagonica a diferencia del día 6 donde se presentó sinergismo. Estos resultados indican que la interacción entre nematodos y hongos entomopatogénos es una alternativa innovadora para el control de *P. xylostella*.

**CONTROL DE *STENOMA CECROPIA*
Y *STRATEGUS ALOEUS* PLAGAS DE
PALMA DE ACEITE, POR NEMATODOS
ENTOMOPATÓGENOS [CONTROL OF
STENOMA CECROPIA AND *STRATEGUS*
ALOEUS PEST PALM OIL FOR
ENTOMOPATHOGENIC NEMATODES**

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El gusano cuernito menor (*Stenoma cecropia*) y el barrenador (*Strategus aloeus*), son plagas principales del cultivo de la palma de aceite en Colombia. Los daños atribuidos son principalmente a la defoliación y perforación del bulbo de la palma. El objetivo de éste estudio fue evaluar la susceptibilidad de larvas de tercer instar de *Stenoma cecropia* y *Strategus aloeus* a *Steinernema columbiensis* SNI0198, *Steinernema websteri* JCL006, *Steinernema feltiae* SCIENTI, *Steinernema* sp3 JCL027, *Heterorhabditis bacteriophora* HNI0100 y *Heterorhabditis* sp. SL0708, aislados de la región andina colombiana. Se evaluaron las dosis de 160 JIs/cm² (*Strategus aloeus*) y 200 JIs/cm² (*Stenoma cecropia*). Para *Stenoma cecropia*, *Heterorhabditis bacteriophora* HNI0100 generó una mortalidad del 75% entre 24 a 96 horas y *Steinernema feltiae* SCIENTI generó mortalidad de 93,3% entre 24 y 48 horas, las demás especies presentaron un tiempo de mortalidad superior a las 96 horas. En cuanto a *Strategus aloeus*, fue susceptible a todas las especies de nemátodos entomopatógenos, siendo *Steinernema* sp3 JCL027, la que mostró mayor porcentaje de mortalidad (19 ± 8%), seguida de *H. bacteriophora* HNI0100 (5 ± 9%). De acuerdo a los resultados para las dos especies se evaluaron las dosis de 0, 100, 150, 200, 250 JIs/ cm² (*Stenoma cecropia*) y 160, 290, 420, 550, 680, 810 JIs/cm² (*Strategus aloeus*), además de la producción de JIs/larva de *Stenoma cecropia*. En cuanto a las mejores dosis, estas correspondieron a 195 y 680 JIs/cm² respectivamente. En la producción de juveniles infectivos de *Stenoma cecropia*, *S. feltiae* SCIENTI presentó su mayor producción con 41981 JIs al cuarto día y *H. bacteriophora* HNI0100 4523 JIs al tercer día. El uso de las especies de nematodos, es una alternativa eficiente e innovadora para el control de estas dos plagas del cultivo de la palma y podría ser replicado en condiciones de campo como método de control.

**USE OF ENTOMOPATHOGENIC
NEMATODES AND FARMERS'S
PERCEPTION ABOUT THEIR EFFICACY
FOR PEST MANAGEMENT IN CUBA [USO
DE NEMATODOS ENTOMOPATÓGENOS
Y PERCEPCIÓN DE LOS AGRICULTORES
ACERCA DE SU EFICIENCIA EN EL MANEJO
DE PLAGAS EN CUBA]**

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The use of biological control of agrarian pest in Cuba is a practice that has accompanied the farmers for more than seven decades. Since the 70s, the entomopathogenic nematodes (NEP) have been becoming, step by step, popular biological control agents in our country. Farmers from different municipalities have been informed in several workshops, television spots and leaflets about the use of EPN. These biological control agents are reproduced in 33 cottage laboratories belonging to the sugarcane industry enterprise group, with yields of some 700 million of infective juveniles per month. In laboratories or semi-controlled conditions, the EPN were evaluated against more than 20 insect species, but in field have been used in pest management with efficacy in yam or cocoyam (*Xanthosoma sagittifolium*), sweet potato, rice, cabbage, watercress (*Nasturtium officinale*), banana - plantain (*Musa* spp.), sugar cane, common bean (*Phaseolus vulgaris*), cucumber, guava (*Psidium guajava*), grape vine (*Vitis vinifera*), pineapple (*Ananas comosus*), and grasses, among other crops. A survey about the use of EPN was recently performed and farmers from 11 provinces answered it. The EPN have been used for management of two recurrent pests in our country, the diamondback moth and the sweet potato weevil. In spite of several field trials with EPN performed in Cuba, details about concentration and application frequency are scarce. Nevertheless, the farmers, in their innovative experimentation, a common activity in our country, have determined to be necessary two or three applications each cycle in temporary crops like sweet potato, cabbage, squash, and tomato, whereas in permanent crops (coffee, pineapple), the frequency must be according to the key pest life cycle, among other factors.

IMPORTANCE OF AGRICULTURAL NEMATOLOGY EXTENSION [IMPORTANCIA DE LA EXTENSIÓN EN NEMATOLOGÍA AGRÍCOLA]

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In several countries there are many problems due to soil nematodes despite the research done on the subject. The results are published in scientific journals that are not read by farmers or their technical advisors. Time passes, interesting data are accumulated in the field of research, but crops are still affected by several species of phytophagous nematodes. In any investigation, even the most abstract, you can collect some original information to make it known by those who are not scientists. Such information should be presented clearly and accurately in the appropriate field. Farmers' associations and related organizations are the places where the information must be submitted. It is necessary to broaden the knowledge of farmers and technicians to make them aware of the potential danger posed by these parasitic organisms. Thus, research will result in benefits for scientists, farmers, technicians and society as a whole.

PRINCIPLES FOR ROOT-KNOT NEMATODE MANAGEMENT IN PROTECTED CULTIVATION [FUNDAMENTOS PARA EL MANEJO DE NEMATODOS FORMADORES DE AGALLAS EN CULTIVOS PROTEGIDOS]

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Protected cultivation in greenhouses allows out-of-season cultivation of high value crops and continuous year-round supply to consumers. Growers specialized usually in a reduced number of crops that are cultivated in rotation with little time between successive crops. Plant-parasitic nematodes, particularly those of the genus *Meloidogyne*, are major constraints for vegetable production under protected cultivation worldwide. Nematode management in these conducive systems is a major challenge due to the wide host range of *Meloidogyne*, crop intensity, short fallowing, and environmental conditions that favor the development of the disease. A complete growing season approach, rather than a single crop, will be required as management actions in a given crop would affect the subsequent crop. It is

necessary to understand the host-parasite relationship in the rotational crops of the production system for estimating nematode damage thresholds, predicting yield losses and modelling population dynamics. For most susceptible annual crops, the critical population density is that at planting (P_i), and this is negatively related with the reproduction rate (P_f/P_i), P_f being the soil population density at harvest. Plant growth and yield are also related to P_i and there is a direct positive relationship between P_i and yield losses. The magnitude of the yield losses depend on the size of the P_i , the nematode species, the crop susceptibility and temperature during the cropping cycle. Root galling provides information on the severity of damage, and root gall indexes and yield losses have a linear relationship. Management strategies include chemical, physical, biological, and cultural methods as the use of non-host or resistant plants. The strategies can be used simultaneously or sequentially to reduce population densities and increase yield. The method selected will depend on factors such as the climatic conditions and level of technological advancement in the region, and the expected economic return.

MANAGEMENT OF *MELOIDOGYNE* SPP., IN PROTECTED CROPS IN TROPICAL CONDITIONS: STUDIED CASE CUBA [MANEJO DE *MELOIDOGYNE* SPP., EN LA PRODUCCIÓN PROTEGIDA DE HORTALIZAS EN CONDICIONES TROPICALES: CASO DE ESTUDIO CUBA]

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In Cuba, the vegetable production in protected crops systems has been developing in the last 15 years. In tropical conditions, the root-knot nematodes represent a threat for vegetables in this intensive system. Tomato, cucumber, melon, and pepper growing all seasons in nearly 123 hectares of protected crops in Cuba and *Meloidogyne* spp., represent the key pests in tomato and cucumber crops, with high populations in all provinces. Their management and the presence of emerging species as *Rotylenchulus reniformis* have been identified as a main challenge in Cuba, for nematologists and stakeholders relative to these cultivation systems. The national survey conducted in 9 provinces showed that *Meloidogyne incognita* was the most distributed specie with relative abundance of 81.5%, but *Meloidogyne*

arenaria and *Meloidogyne enterolobii* were found in some areas, making their management complex. All commercial tomato genotypes evaluated were susceptible to *M. incognita*. Seven genotypes from Solanaceae were evaluated for herbaceous grafting and *Solanum lycopersicum* cv. Rossol and Motelle and *Solanum torvum* were resistant, selecting Rossol for tomato grafting. Tactics, such as traps in crops, soil biofumigation or biodesinfección and biological control (with commercial products as KlamiC® and HeberNem®, or bioproducts as different *Trichoderma* spp. strains) have been used by farmers with different levels of efficacy. This variability in efficiency with the same tactics between different places was due to factors like: differences in soils type and organic material content, among amendment types and rates available in each site, lack of proper understanding about interactions within a soil system (among techniques and/or microorganisms), insufficient knowledge, or preparation of farmers and others stakeholders and lacking an integrative and multidisciplinary experiments. The results of different studies must be presented for discussion.

FIELD AND MICRO-PLOT TRIALS ON USE OF MUSTARD-TYPE CROPS TO MANAGE ROOT-KNOT NEMATODES IN CARROT AND TOMATO IN CALIFORNIA [ENSAYOS DE MICROPARCELAS Y CAMPO EN EL USO DE CULTIVOS DEL TIPO MOSTAZA PARA EL MANEJO DE NEMATODOS AGALLEROS EN ZANAHORIA Y TOMATE EN CALIFORNIA]

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Carrots are one of the 10 most important fresh-market vegetables in the USA, with a value of approximately \$600 million annually. The majority (> 80%) of US carrots are grown in California. Tomato, particularly those used for processing, is another important vegetable crop that, within the US, is mainly grown in California (90%). In both these major vegetable crops, root-knot nematodes (*M. incognita*, *M. javanica*) cause serious problems. In tomato, observations that nematode-resistant varieties are being infested and developing obvious root symptoms (*i.e.*, galling), have become more frequent over the last several years. In carrot, resistance to root-knot nematodes is not (yet) available in commercial varieties, and as a consequence, growers are relying on fumigant nematicides such as 1,3-D and metam-sodium where nematodes are a problem. Because of the environmental and human health problems associated with use of soil fumigants, potential alternatives that are less toxic and more sustainable

are being evaluated. One potential alternative is the use of mustard-type cruciferous crops as “bio-fumigants”. In micro-plot (tomato) and field (carrot) trials on root-knot nematode infested sites, we evaluated the impact of cultivating and then soil-incorporating different varieties of cruciferous crops on root-knot nematode population levels, nematode symptoms and crop yield over a 2-yr-period. The cruciferous cover crops generally did not sufficiently reduce root-knot nematode population levels to avoid severe root damage in the following carrots. In tomato however, some of the cruciferous crop varieties resulted in significant yield increases, in spite of the root systems of these tomato plants still showing obvious root galling. We conclude that some nematode multiplication occurred on the cruciferous cover crops, which was not offset entirely by the subsequent bio-fumigation period. In contrast, using mustard meal as a pre-plant soil amendment did avoid nematode multiplication, while it still provided the bio-fumigant effect.

INTERNET-BASED RESOURCES FOR TEACHING NEMATOLOGY [RECURSOS BASADOS EN INTERNET PARA LA ENSEÑANZA DE LA NEMATOLOGÍA]

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Teaching in general, and in the plant protection disciplines in particular, is a challenging endeavor in the 21st century. A large blackboard and a package of multi-colored chalk are increasingly being displaced by a range of multi-media and distance-education formats. Expensive and non-editable textbooks are being supplemented with software bound instructional modules. In order to successfully “engage” students, instructors must come to class armed with seasoned experience in the subject, a keen awareness of the students’ foundation in science and a genuine enjoyment for the teaching activity. Introduction to Nematodes, a multimedia, multi-platform, multi-layered slide and video production by E. C. McGawley, C. Overstreet, M. J. Pontif and A. M. Skantar brings this new technology to the teaching of nematology. The presentation, 2 yr in production, is available for free (for educational purposes) download from the website of The Society of Nematologists, <nematologists.org>, with links from The Organization of Nematologists of Tropical America, <ontaweb.org>, The European Society of Nematologists, <Esn-online.org>, The Russian Society of Nematology <russiannematologists.com>, The Japanese Nematological Society <senchung.

ac.affrc.go.jp> and <nematode.net>. The “Teaching Resources” section of the website of the Society of Nematologists also has a wealth of other teaching resources available for free download in addition to those mentioned above. The nematology and parasitology communities worldwide are encouraged to contribute to these ongoing education resources designed to enhance the quality of instruction and produce an increased awareness of the importance of nematodes in agriculture and human health.

THE DEVELOPMENT AND IMPLEMENTATION OF SITE-SPECIFIC TECHNOLOGY FOR MANAGING COTTON NEMATODES IN THE UNITED STATES [EL DESARROLLO Y LA IMPLEMENTACIÓN DE LA TECNOLOGÍA DE SITIO-LOCALIZADO PARA EL MANEJO DE NEMATODOS DEL ALGODÓN EN LOS ESTADOS UNIDOS]

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Geospatial technologies have greatly impacted production agriculture in the U.S. during the past decade. The application of the Global Positioning System (GPS) provides unprecedented precision within a field for yield monitors, auto-steering, pesticide application, soil texture mapping (apparent electrical conductivity), or data collection such as nematode samples. A number of scientists in the southern U.S. were involved in evaluating the potential of these technologies for site-specific application of nematicides to specific areas within fields of cotton for management of *Meloidogyne incognita*, *Rotylenchulus reniformis*, or *Hoplolaimus columbus*. A major component of this research was to use soil texture as a tool to define treatment areas within a field. The Veris 3100 Soil EC Mapping System was used to measure apparent electrical conductivity (ECa) and serves as a surrogate for soil texture in the wide range of soils found in the southern U.S. The ECa data combined with some nematode information could then be used to divide fields into different management zones. Trials were conducted in producers fields in Arkansas, Georgia, Louisiana, and South Carolina to evaluate this concept for nematode management. Management zones could successfully be developed and accurately designated as to the level of management required. These trials clearly showed the value of this technology to apply nematicides in a very specific manner only where needed. Information from these studies has been promoted at numerous field days, producer meetings, publications, news releases, and workshops. Additionally, many other trials have been conducted in producers' fields to demonstrate the effectiveness

of this method of nematode management. This site-specific technology has been adopted by hundreds of producers in the southeast U.S. and to a lesser extent in the mid-south U.S.

TEACHING NEMATOLOGY IN COSTA RICA [LA ENSEÑANZA DE LA NEMATOLOGÍA EN COSTA RICA]

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Costa Rica is a Neotropical country of agricultural vocation, with a planted area of 450,000 hectares where products are grown for local consumption and exportation. The tropical conditions favor the occurrence of plant health problems and nematodes are not an exception. The country imports annually an average of 1.5 tons of nematicides. This reflects the importance of nematodes in the agricultural economy and may suggest the need for the country of professionals in this field. However, nematology training from the early 70s to the present day has been linked primarily to introductory courses in the curriculum of agricultural engineering at the University of Costa Rica (UCR) and the Universidad Nacional (UNA). Occasionally specialized courses in nematology have been offered in the Master's programs of CATIE and UCR. Changes in curriculum programs over time has meant that the course of nematology changed from obligatory to an elective status. The Escuela de Ciencias Agrarias de la Universidad Nacional has offered the course of nematology since its foundation and now has a properly equipped Nematology Laboratory for research and teaching, instructional leaflets on identification of plant parasitic and free living nematodes and a reference collection with more than 22,000 permanent slides that include free-living and plant parasitic nematodes over 60 crops. The laboratory also provides diagnostic services to independent growers and agribusinesses and offers training to professionals interested in this field.

COMBINING TRAINING AND RESEARCH ON THE EDUCATION AND MANAGEMENT OF PLANT-PARASITIC NEMATODES [COMBINAR LA ENSEÑANZA E INVESTIGACIÓN EN LA EDUCACIÓN EN MANEJO DE NEMATODOS PARÁSITOS DE PLANTAS]

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Although plant-parasitic nematodes still are responsible for tremendous reduction on the production of staple foods worldwide, the universities are closing professorial and scientific staff positions in nematology results in fewer nematology courses being offered and fewer students who are interested in plant-parasitic nematodes. A functional and practical strategy to continue to have activities in nematology is combining short training activities and conducting research on plant parasitic nematodes. Specific short workshops and field days are effective to demonstrate the importance of the subject, due to the students and farmers can see very clearly the damage as well as identify the causal agent of the problem. In the case of research, often less money is allocated to plant-parasitic nematodes. However, the trends is to find biological products which can control nematodes, so that this is an important window to work in close collaboration with plant pathologists, bacteriologists, and soil ecologists in order to conduct bio prospection of native strain of microorganism with potential antagonistic activity against plant parasitic nematode. In Central America, we had the opportunity to work in regional and international institutions like CATIE and EARTH, where we received students from more than 50 countries worldwide and this strategy of combining training and research allowed us to continue to work on nematology. Later, the alumni and farmers who have been trained come back to their countries and can teach and train more students and farmers on plant-parasitic nematodes in their homeland.

PROVIDING SIMPLE NEMATOLOGY TRAINING TO CREATE AWARENESS AND INTEREST IN AFRICA [PROPORCIONANDO ENTRENAMIENTO SIMPLE EN NEMATOLOGÍA PARA CREAR CONCIENCIA E INTERÉS EN ÁFRICA]

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Creating awareness of nematology within the agricultural research and development environment is an essential component to a holistic and wholesome approach to pest and disease management. Demystifying the science and discipline of nematology to the uninitiated, however, can be quite a daunting task. Maintaining a simplistic approach, combined with accurate information and generating genuine interest are key ingredients to a successful recipe. A fine balance is required though to ensure these ingredients are delivered effectively and moreover, that the techniques are adopted and taken aboard. In particular, encouraging our next generation of nematologists is of paramount importance in maintaining capacity and managing these pests in the future. In many African countries there may be limited or no expertise in nematology. The need to create awareness and provide support in nematology is acute. At the International Institute of Tropical Agriculture in close partnership with colleagues from Universities, National Programmes and Commercial Enterprises, a basic training course has been devised, based on a 'Practical Nematology Guide' to provide the basic essentials needed for nematology assessment. We believe this serves a useful basis for building further, without overwhelming participants – but encourages interest and intrigue to take things further. The course manual and additional techniques will be discussed.

TEACHING AND MENTORING FUTURE GENERATIONS OF NEMATOLOGISTS [ENSEÑANZA Y MENTORES PARA LAS FUTURAS GENERACIONES DE NEMATÓLOGOS]

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Training and education of human resources in crop protection requires integration of various knowledge areas, one of which is nematology. Depending on the curricula offered by higher education institutions, training and education programs generally require students to attend courses and complete an original research project (*i.e.*, thesis, or dissertation) in order to qualify. In both training and education, an environment of continuous stimuli and encouragement provided to students by staff members and the teacher can make the students more productive and innovative. Didactic and interactive materials available on the internet and their free access has benefitted the teaching-learning process, teachers, and students in nematology courses. The internet has also facilitated virtual teaching, continuing and distance education courses (online). Nevertheless, research and training of highly qualified scientists is still done under the supervision of an expert authority who can also play the role of a mentor: an expert to whom the student or new graduate can refer to share and interchange experiences about planning and developing (progressing) a career. Nowadays the role of a mentor has greater importance in a changing, uncertain working environment that demands greater flexibility from the individual than formerly. As a result, the contents of higher education curricula for future generations necessitate a more holistic approach that not only meets the high academic and scientific standards required, but also adds creativity, media communication, and entrepreneurial skills – new skills that may increase the graduate's opportunity for employment within nematology or other related areas.

CONSIDERATIONS ON GLOBALIZATION, FOOD PRODUCTION AND NEMATOLOGY – WHERE WE ARE AND WHAT WE NEED TO DO [CONSIDERACIONES SOBRE LA GLOBALIZACION, LA PRODUCCION DE ALIMENTOS Y LA NEMATOLOGIA – DONDE ESTAMOS Y QUE TENEMOS QUE HACER]

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Contemporary crop production in the industrialized world provides for adequate food for an increasing population, supporting the view that it is possible to maintain our food production rate ahead of population development. Food production today is the result of processes begun in the late XVIth century involving global exchanges in crop species, the introduction of organic fertilizer [*Peruvian guano*], followed by natural inorganic N sources [chilean nitrate] and cheap synthetic N. Concomitant with these changes there has been a trend in progressive simplification of cropping systems from production based poly-crop and diverse management practices to one presently based on very few crops or even monoculture. These changes required increased energy inputs dependent on fossil fuels and mining of irreplaceable elements [P, K]. Simplification and the stylizing of food production systems has resulted *inter alia* in impoverishment in soil biological components, geographical expansion and diversification of pest problems and reduction in the “biological buffer capacity” of cropped areas against a multiplicity of pest problems. In nematology there are documented: the expansion *Rotylenchulus reniformis*, a typical tropical species, into temperate and even cool areas of the world; the presence of *Heterodera glycines*, and other *Heterodera* spp., in tropical environments; the ubiquitous distribution of *Globodera rostochiensis*, and *G. pallida* in temperate and cool areas; *Radopholus similis* and *Meloidogyne javanica* in greenhouse and covered agriculture in cool climates. There is need to intensify research on diversification of cropping systems to understand the interactions of nematodes with the various components of the soil biota. Key to the research is the accurate and rapid identification of the nematofauna, the effects of diverse crop management systems on nematode activities, and the economic feasibility of the systems. There is great need for world-wide interdisciplinary cooperative research.

POSTER ABSTRACTS/RESÚMENES DE CARTELES

3D MODELLING OF THE AMPHIDIAL APERTURE IN THE GENUS *MALENCHUS* [MODELADO 3D DE LA APERTURA ANFIDIAL DEL GENERO *MALENCHUS*]

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Three-dimensional (3D) modelling has shown an increasing number of applications in different fields as it eases the understanding of complex 3D structures. Traditionally, a set of transmission electron microscopy (TEM) images from serial sections is needed for a single reconstruction, which is a time-consuming and laborious task. In this study, the shape of the typical amphidial aperture of the genus *Malenchus* was modeled with the program Autodesk Maya™; light microscopy pictures of *M. nanellus* were used as a reference to understand the general morphology of the head and scanning electron microscopy (SEM) images provided information for a more detailed reconstruction. The outline of the head shape was drawn following the profile of a SEM picture; this basic 2D line was then swept in a circular direction around the global axis to create a 3D polygon. The en face view was also based on SEM images, and polygons (representing morphological structures) were created directly on the image with the program tools. Details of these polygons were improved according to SEM pictures and the modelling of inner structures was based on light microscopy images. Compared to traditional 3D reconstruction, this method is less accurate but the procedure is much simpler and faster. The proposed tool is a versatile method to represent morphological structures, complementary to pictures and drawings, and has a wide spectrum of uses in nematological teaching and research.

CHARACTERISATION OF THE PHARYNGEAL GLAND CELLS OF *BURSAPHELENCHUS XYLOPHILUS* – FROM MORPHOMETRICS TO MOLECULAR BIOLOGY [CARACTERIZACIÓN DE LAS CÉLULAS GLANDULARES FARÍNGEAS DE *BURSAPHELENCHUS XYLOPHILUS* – DE LA MORFOMETRÍA A LA BIOLOGÍA MOLECULAR]

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Bursaphelenchus xylophilus, the pinewood nematode (PWN), is the causal agent of Pine Wilt Disease, one of the major economic and ecological threats to pine forests all over the world. The life cycle of this nematode is characterised by two phases, a phytophagous and a mycetophagous. While feeding on the cytoplasm of the plant cells and invading host tissues, it is thought that, like other plant-parasitic nematodes, PWN delivers a set of secreted proteins from its pharyngeal gland cells. In aphelenchids, including *B. xylophilus*, these glands are composed of two subventral gland cells and one dorsal gland cell. In this study the *B. xylophilus* (Portuguese isolate BxPt750H) pharyngeal gland cells were characterized in adults and juveniles based on measurements and laser scanning microscopy photographs obtained by Differential Interference Contrast. Our results showed that the dorsal gland, which was less well developed in the juvenile stage, is more developed in the adult stage and, by contrast that the subventral glands do not grow between juvenile and adult stages. The present study gives not only a new insight in the characterization of the *B. xylophilus* gland cells but also a new perception on the localisation of these structures which might be helpful in future molecular studies with the aim of localising proteins and mRNAs in nematode tissues.

IN VIVO VOLATILE ORGANIC COMPOUNDS ANALYSIS IN *PINUS PINASTER* AFTER INFECTION WITH *BURSAPHELENCHUS XYLOPHILUS* [ANÁLISIS IN VIVO DE COMPUESTOS ORGÁNICOS VOLÁTILES EN *PINUS PINASTER* TRAS LA INFECCIÓN CON *BURSAPHELENCHUS XYLOPHILUS*]

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The pine wilt disease (PWD), caused by the nematode *Bursaphelenchus xylophilus*, is devastating for *Pinus pinaster* plantations, leading to the loss of thousands of trees every year worldwide. Despite the important research efforts of the past decades, no effective strategies against the PWN have been developed and the physiological aspects related to disease resistance remain largely unknown. In this work, 1-yr-old *P. pinaster* plants were inoculated with ca. 1000 *B. xylophilus* nematodes or with water (controls). Three and 21 days post inoculation (dpi), volatile organic compounds (VOCs) were analysed through a non-invasive methodology: VOCs were concentrated in a personalized acrylic chamber for 60 min and subsequently collected with a DVB/CAR/PDMS fibre for 30 min, after which they were identified by gas chromatography/mass spectrometry (GC/MS). An additional set of inoculated plants was sacrificed three and 21 dpi for total stem nematode counting. Results showed that nematodes successfully reproduced throughout the experimental period, reaching ca. 3000 nematodes per plant just 21 dpi. Compared with control plants, total VOCs, especially monoterpenes, significantly increased three dpi in inoculated plants, probably as part of *P. pinaster* chemical defence mechanisms against the pathogen. VOCs dramatically decreased 21 dpi, most likely due to tissue damage induced by *B. xylophilus*, demonstrating the susceptible character of *P. pinaster*. At 3 dpi, there was increased biosynthesis of almost all VOCs, specially α -pinene and β -myrcene (ca. 4-fold), known to accumulate in leaf tissues to repel herbivores and pathogens. Sabinene and 3-carene also increased significantly in infected plants (4.1- and 5-fold, respectively), 21 dpi, which suggests that the biosynthesis of different defence-related VOCs is triggered at different stages of the disease. 4-hexen-1-ol, a known allelopathic agent, was only found in inoculated plants, perhaps as a strategy to attract *B. xylophilus* predators. These findings could be of

utmost importance for the precocious diagnostic of the PWD.

DELIMITACIÓN DE ESPECIES DE NEMATODOS AGALLADORES FOLIARES AFECTANDO *AGERATUM CONYZOIDES* MEDIANTE TAXONOMÍA INTEGRATIVA EN LOS VALLES ALTOS DE TLAXCALA, MÉXICO [SPECIES DELIMITATION OF FOLIAR-GALL NEMATODES AFFECTING *AGERATUM CONYZOIDES* USING INTEGRATIVE TAXONOMY IN HIGH VALLEYS OF TLAXCALA, MEXICO]

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Algunos miembros de la familia Anguinidae producen síntomas típicos de agallas en raíces y partes aéreas de cultivos y malezas en México. Con el objetivo de delimitar la especie del nematodo agallador en *Ageratum conyzoides* con un enfoque de taxonomía integrativa, se realizaron estudios morfológicos, fisiológicos y moleculares. Las hembras, machos y J2 fueron extraídos mediante disección de agallas de hojas y tallos, montados en agua-agar 2% para su análisis morfométrico. Las características y valores obtenidos se encuentran reportados para el género *Subanguina*; sin embargo, los valores no entran en los rangos de las especies reportadas a la fecha. La prueba fisiológica preliminar en 42 ornamentales de Asteraceae, el nematodo infectó e indujo síntomas solo en *Ageratum conyzoides*. Se realizó la amplificación por PCR y secuenciación de las regiones ITS1-5.8S-ITS2 (700 pb) y los segmentos de expansión D2-D3 del gen 28S (686 pb) del rDNA a partir de especímenes individuales. La búsqueda de homología por BLAST con secuencias del NCBI no mostró resolución significativa en cobertura e identidad. Los estudios filogenéticos preliminares de las secuencias de ambos marcadores moleculares con secuencias de géneros y especies de anguinidos ya reportados indican que no existe una clara afinidad de agrupamiento de los nematodos encontrados en los valles altos de Tlaxcala. Es necesaria la exploración de otras regiones moleculares que

permitan la discriminación clara de los especímenes detectados en Ageratum.

ESTUDIO DE LA DIVERSIDAD DE NEMATODOS ASOCIADOS AL SUSTRATO COMO INDICADORES DE LA CALIDAD DEL SUELO EN CULTIVOS HORTÍCOLAS DE LA PLATA, BUENOS AIRES, ARGENTINA [STUDY OF THE DIVERSITY OF NEMATODES ASSOCIATED WITH SUBSTRATE AS INDICATORS OF SOIL GRADE IN HORTICULTURAL CROPS IN LA PLATA, BUENOS AIRES, ARGENTINA]

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La estimación de la biodiversidad de los nematodos edáficos, identificación y abundancia de los diferentes grupos tróficos, permite estimar el grado de perturbación de los suelos. La rápida respuesta a alteraciones ambientales, como el laboreo o aplicación de insumos químicos, los convierte en un indicador clave en la evaluación del efecto del manejo agrario sobre los ecosistemas. En la Provincia de Buenos Aires, la horticultura es una de las actividades agrícolas más importantes, presentando diferentes estilos (convencionales, intensivos a orgánicos). En el presente trabajo se realizó un monitoreo ambiental empleando nematodos edáficos, como herramienta para determinar el grado de perturbación de suelos sometidos a diferentes sistemas de manejo en zonas del Gran La Plata, Buenos Aires, Argentina. Se analizaron muestras de suelo provenientes de áreas dedicadas a la horticultura intensiva (cultivos de tomate) entre Septiembre-Noviembre del 2014. Los nematodos fueron obtenidos por centrifugación (método de flotación en azúcar). Se determinaron los distintos grupos tróficos según morfología del aparato bucal y esófago. Los resultados mostraron un elevado porcentaje de bacteriófagos en suelos tratados con plaguicidas, respecto a los otros grupos. Este porcentaje fue considerablemente inferior en cultivos orgánicos con escaso o nulo uso de agroquímicos, lo que reflejaría la estrategia adaptativa de estos nematodos (colonizadores). Fitoparásitos se encontraron casi exclusivamente en cultivos orgánicos, con una escasa presencia en suelos con plaguicidas. Se observaron representantes de los géneros *Helicotylenchus* sp., *Aglenchus* sp., *Tylenchus* sp., *Hemicalosia* sp., *Hemicyclophora* sp., y *Nacobbus aberrans* (fitoparásitos), *Mesorhabditis* sp., *Cruzema* sp., *Paradoxorhabditis* sp., *Distolabrellus* sp., y

Rhabditis sp. (bacteriófagos), y *Aphelenchus* sp. (fungívoros). Entre los depredadores/omnívoros se hallaron representantes de los órdenes Mononchida y Dorylaimida. Los resultados reflejan hasta el momento una marcada variedad de la nematofauna asociada a los distintos sistemas de manejo agrícola.

IDENTIFICATION OF FOUR POPULATIONS OF PRATYLENCHUS IN PATAGONIA, ARGENTINA [IDENTIFICACIÓN DE CUATRO POBLACIONES DE PRATYLENCHUS EN LA PATAGONIA, ARGENTINA]

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The genus *Pratylenchus* has a worldwide distribution, great economic importance and is found parasitizing different crops. The purpose of this study was to identify *Pratylenchus* species using first morphological and morphometrical criteria; then these populations will be studied by molecular techniques. *Pratylenchus vulnus* was found in soil around roots of pear (*Pyrus communis*) in Chichinales, Río Negro. This species was also detected in soil and root of walnut (*Junglans regia*) in Añelo, Neuquén. *P. neglectus* was collected from the soil around alfalfa roots (*Medicago sativa*) in Senillosa, Neuquén. *P. penetrans* was collected from strawberry roots (*Fragaria ananasa*) in El Maitén, Chubut. This report presents new data on spatial distribution of *Pratylenchus* species in Argentina.

NEMATODES AS BIOINDICATORS OF SOIL FOOD WEB CONDITION IN LONG TERM CROPPING SYSTEMS [NEMATODOS COMO BIOINDICADORES DE LA CONDICIÓN DE LA CADENA ALIMENTARIA EN SISTEMAS DE CULTIVOS DE LARGO TÉRMINO]

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Nematodes are prominent in soil and useful for evaluating the soil food web, so we used nematode community indices to compare four corn production systems from 2012 to 2014. The study site was a long-term experiment on a highly productive Plano silt loam soil in southern Wisconsin. Community indices (enrichment (EI), structure (SI), maturity

(MI), and channel (CI)), taxonomic composition, and functional diversity were analyzed using uni- and multi-variate statistics. The systems included corn monoculture (CM), two organic systems of Organic Grain (OG) or Organic Forage (OF), and a high diversity Rotational Grazing (RG) system that included live cows. The systems had been in place and managed using a consistent philosophy since 1990. The organic systems had features beneficial to the soil food web such as organic inputs and crop diversity and every system had features detrimental to the food web condition; the organic systems relied on tillage practices to manage weeds, the CM system used pesticides, and cow traffic led to compaction in the RG system. Soils in the CM were similar to the RG system and better than the organic systems according to the community indices, EI, SI, MI, and CI. The two organic systems were similar for all indices. The CM and RG systems had the greatest relative abundance of k- selected functional groups, indicating a more developed soil food web. Corn in the CM system was grown with minimal tillage, which may have had a greater influence on soil food web condition than crop diversity and organic inputs.

FITONEMATODOS DE LAS PLANTAS AREACEAS EN CUBA [PHYTONEMATODES OF CACEAN PLANTS IN CUBA]

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Las areáceas son plantas de alto valor ornamental y utilitario en Cuba, que se ven afectadas por varias plagas, no obstante el conocimiento sobre los nematodos parásitos asociados no es abundante. El objetivo del trabajo fue determinar las principales especies de fitonematodos en plantas areaceas de las provincias occidentales de Cuba y la susceptibilidad de seis de ellas ante nematodos del género *Meloidogyne*. Fueron tomadas 150 muestras de suelo y raíces pertenecientes a 19 especies de plantas, que se procesaron en el laboratorio por los métodos de decantación-tamizado y embudos Baermann y Fenwick para el suelo y licuadora- tamizado más embudos Baermann para las raíces. La prueba de susceptibilidad se realizó con *M. incognita*, comparando plantas inoculadas y sin inocular por nematodos, donde se evaluaron indicadores de desarrollo y de infestación. Se identificaron 16 géneros con 28 especies de fitonematodos. Fue significativa la presencia de *Radopholus similis* en *Chrysalidocarpus lutescens* y *Cocos nucifera*, así mismo se detectó por primera vez *Meloidogyne*

incognita en *C. nucifera*, *Ptychosperma elegans* y *Veitchia merrillii*. Otros nematodos registrados por primera vez en una especie de Arecaceae en el país fueron: *Helicotylenchus dihystra*, *H. exallus*, *Quinisulcius curvus*, *Rotylenchulus reniformis*, *Tylenchorhynchus annulatus*, y *Xiphinema basiri*. Respecto a la susceptibilidad ante *M. incognita* de *Roystonea regia* (palma real), *Thrinax radiata* (yuraguana), *Syagrus roman zoffiana* (coco plumoso), *Dictyosperma album* (areca blanca), *Phoenix roebelenii* (fénix), y *Veitchia merrillii* (adonidia) se pudo determinar que *R. regia* fue afectada en indicadores de crecimiento, el resto de las especies no tuvieron diferencias significativas respecto al control. Se observaron hembras y ootecas en *P. roebelenii* y *V. merrillii* así como nódulos en ápices de raíces. En las areáceas evaluadas hubo baja reproducción de *M. incognita* en relación con las cucurbitáceas y se determinó que bajo las condiciones estudiadas fueron tolerantes a *M. incognita*, excepto *R. regia*.

NEMATODOS EN LA PROVINCIA CIENFUEGOS, HOSPEDANTES Y DISTRIBUCIÓN [NEMATODES IN CIENFUEGOS PROVINCE, HOSTS AND DISTRIBUTION]

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El presente estudio tuvo como objetivo la realización de una prospección de especies de nematodos en la provincia Cienfuegos entre el 2004 - 2014, para ello se tomaron los resultados de todas las muestras procesadas por la especialidad de nematología en el Laboratorio Provincial, empleándose los métodos recomendados por García (1979): Batidora + tamizado, Embudos Baerman, Extracción directa de las raíces y Vaso de precipitado + tamizado según fuera el caso. Para la determinación de las especies se realizaron las preparaciones necesarias empleando las claves existentes en el laboratorio. Las frecuencias de aparición absoluta y relativa se determinaron con los valores de composición y densidades poblacionales de las especies de nematodos identificados. Se determinó la presencia de 13 géneros con un total de 21 especies, de ellas cuatro constituyeron nuevos informes, para el país *Xiphinema basiri* Siddiqi en el cultivo de los cítricos (*Citrus* spp.) y para la provincia esta

especie en caña de azúcar (*Saccharum officinarum* L.), así como *Criconemoides* sp., *Macropostonia* sp., *Pratylenchus brachyurus* (Godfrey) Filipjev & Stekhoven. Se informan las 22 plantas utilizadas como hospedantes las que se agrupan en 19 familias botánicas. La mayor diversidad de especies de nematodos se determinó sobre *Saccharum officinarum* L. y los géneros *Citrus* y *Musa*. Los mayores índices de intercepción correspondieron a *Meloidogyne* sp. y *Meloidogyne incognita* (Kofoid & White) Chitwood con un 2.7 y 2.4 % respectivamente sucedidas por *Helicotylenchus* sp. y *Helicotylenchus multicinctus* (Cobb) Golden con un 1%. El género *Meloidogyne* fue el más frecuente incidiendo sobre 15 cultivos. Se observó la presencia de especies de nematodos en toda la provincia, de las cuales cuatro mostraron una amplia distribución en todos los municipios: *Helicotylenchus multicinctus* (Cobb) Golden, *Helicotylenchus* sp., *Meloidogyne incognita* (Kofoid & White) Chitwood y *Meloidogyne* sp.

claves taxonómicas cinco especies de nemátodos: *Radopholus similis* Thorne, *Pratylenchus coffeae* Filipjev y Schuurmans Stekhoven, *Helicotylenchus multicinctus* Golden, *Meloidogyne incognita* Chitwood, y *Rotylenchulus reniformis* Linford y Oliveira. No se observaron nemátodos cistógenos ni de interés cuarentenario para Cuba. Las especies *P. coffeae* y *R. similis* se detectaron en más del 70% de las muestras analizadas y fueron los fitonematodos con mayor densidad poblacional en todos los clones, seguidos de *H. multicinctus*; la especie con mayor frecuencia de aparición fue *R. similis*. *M. incognita* y *Rotylenchulus reniformis* solo aparecieron en los clones Burro CEMSA y FHIA-21, respectivamente, aunque en bajas poblaciones. En el clon Burro CEMSA se cuantificaron las mayores poblaciones en general, mientras que la densidad poblacional de nematodos más baja fue determinada en CEMSA ¾. Para el manejo se proponen acciones de lucha legal, agrotécnicas y biológicas, que deben tener en cuenta las especies presentes y sus poblaciones.

FITONEMÁTODOS ASOCIADOS A CLONES DEL CULTIVO DEL PLÁTANO (*MUSA* SPP.), Y SU MANEJO EN FINCAS DEL MUNICIPIO LAS TUNAS, CUBA [PLANT-PARASITIC NEMATODES ASSOCIATED WITH BANANA AND PLANTAIN (*MUSA* SPP.) CLONES AND THEIR MANAGEMENT IN FARMS OF LAS TUNAS MUNICIPALITY, CUBA]

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Los nematodos fitoparásitos, se encuentran presentes generalmente en el suelo y las raíces de musáceas en una gran parte del territorio nacional, aunque se encuentran particularidades, que dependen de varios factores. Con el propósito de conocer la situación en la provincia de Las Tunas de las especies de fitonemátodos, niveles poblacionales, frecuencia de aparición y distribución en los clones de plátano: FHIA – 01, FHIA – 03, FHIA – 18, FHIA – 21, Burro CEMSA, Enano Guantanamero, Macho ¾ y CEMSA ¾, se realizaron muestreos en fincas de la UEB “Almendares” de la Empresa Agropecuaria Las Tunas, de la CCS “Omar Pérez Pérez” y de la CCS “Niceto Pérez”, del municipio Las Tunas, desde enero de 2012 hasta abril de 2013. Las muestras se analizaron en la sección de Nematología del Laboratorio Provincial de Sanidad Vegetal por los procedimientos establecidos. Fueron identificadas con el uso de diferentes

XIPHINEMA BASIRI UN PELIGRO POTENCIAL PARA EL CULTIVO DEL TOMATE (*SOLANUM LYCOPERSICUM* L.) EN SISTEMAS DE CULTIVOS PROTEGIDOS [XIPHINEMA BASIRI: A POTENTIAL HAZARD OF TOMATO CROP IN PROTECTED CROP SYSTEMS]

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Las especies del género *Meloidogyne* constituyen el principal problema en los sistemas de cultivos protegidos de Cuba, mientras que *Rotylenchulus reniformis* se detecta en zonas aisladas. El objetivo del trabajo fue la determinación de los nematodos asociados con un síntoma atípico en las raíces de tomate Variedad LTM 12 en casas de cultivo de la provincia La Habana. Se tomaron muestras de suelo y raíces de plantas con marchitez y escaso desarrollo, cuyo sistema radical presentaba agallas típicas de *Meloidogyne* y otras con hinchazones en los extremos. Las raíces se observaron al estereomicroscopio y el suelo se procesó por embudos Baermann. Los nematodos extraídos se observaron en preparaciones con formaldehído al 2%; la identificación se realizó mediante una clave poltómica. En las raíces con hinchazones en los extremos, se notaron minúsculas hendiduras en la punta y no se observaron ejemplares de *Meloidogyne*. Se detectaron entre 650 y 900 individuos del

género *Xiphinema* por 100 gramos de suelo. Los ejemplares de *Xiphinema* se caracterizaron por tener las dos ramas genitales simétricas, presencia de pseudo órgano Z, cola corta, cónica y digitada, radio c' (1,12-1,18), posición de la vulva de 53 a 58%, longitud de lanza 190 a 195 µm, longitud del cuerpo entre 2,8 y 3,1 mm, cuerpo en forma de C o J, región labial separada por una constricción débil, forma de cola de juveniles similar a la hembra adulta y ausencia de machos. Esto los ubica en el grupo 5 códigos A4-B2-C4-D4-E6-F3-G2-H2-I3-J3-L1, que se corresponde con *Xiphinema basiri* Siddiqi. Dados los daños observados y sus características semejantes a simple vista con algunos de los síntomas provocados por los nematodos formadores de agallas, se recomienda continuar los estudios de distribución en otras zonas de cultivos protegidos, con vistas a alertar a los técnicos y productores.

PRELIMINARY STUDY OF NEMATODE FAUNA OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) FROM MAYABEQUE PROVINCE, CUBA [ESTUDIO PRELIMINAR DE LA FAUNA DE NEMATODOS ASOCIADA AL FRIJOL COMÚN (*PHASEOLUS VULGARIS* L.) DE LA PROVINCIA MAYABEQUE, CUBA]

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The objective of this preliminary study was to determine the nematode communities associated with beans crops in localities of Mayabeque Province. Land areas of 1ha were selected in the municipalities of Güines and San José de las Lajas and sampled using a stratified design. The collected soil samples were put into a polyethylene bag and transported to the Laboratory of Agricultural Nematology of National Center for Animal and Plant Health (CENSA). The nematodes from soil were obtained by the Baermann funnel method. The nematodes were killed in water bath at 52°C for 3 min and fixed with 1% formaldehyde. The dates were compared using Simple Analysis Variance in INFOSTAT 2.1 program. The highest diversity occurred in the localities of Güines, where nematodes belonged to the genera *Pratylenchus*, *Aphelenchoides*, *Thylenchorhynchus*, *Paratylenchus*, *Mononchus*, and *Aphelenchus* were found. In San José de las Lajas, were found the genera *Pratylenchus*, *Aphelenchus*, and *Mononchus*. The species observed in the municipality of Güines was

Pratylenchus vulnus. In Güines Municipality we found *Meloidogyne* sp. in plant with typical galls in roots.

ACTUALIZACIÓN DE LA NEMATOFUNA ASOCIADA A LA CAÑA DE AZÚCAR EN CUBA [UPGRADE OF NEMATOFUNA ASSOCIATED WITH SUGARCANE IN CUBA]

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Los nematodos influyen en la estructura y estabilidad de los agroecosistemas y además pueden causar daños al alimentarse directamente de las plantas o como vectores de enfermedades. Se asocian al cultivo de la caña de azúcar a nivel mundial más de 275 especies de 48 géneros, mientras que en Cuba se informan 83 de 25 géneros. Con el propósito de actualizar el listado de nematodos asociados al cultivo, se realizó un levantamiento nacional que abarcó 618 campos de 56 Unidades de Producción Cañeras. En cada campo se tomaron de la rizosfera 500 g de suelo y 100 de raíces. Los Laboratorios Provinciales de Sanidad Vegetal realizaron el procesamiento de las muestras por los métodos decantación-tamizado y embudos Baermann para la detección de nematodos vermiformes; Fenwick para cistógenos y plantas indicadoras para los formadores de agallas. La identificación de las especies se realizó mediante la morfología y morfometría a partir de las claves y referencias taxonómicas. Las poblaciones fueron contadas y expresadas en número de nematodos en 10 gramos de suelo y raíz fresca, a partir de las mismas se establecieron la estructura trófica y la cuantía de las comunidades, según las medidas de la abundancia o riqueza de géneros, especies e individuos. La composición trófica de la comunidad en suelo y raíces fue similar e incluyó cuatro grupos: fitófagos, bacteriófagos, micófagos y omnívoro depredador. Se identificaron 75 especies de nematodos agrupados en 32 géneros, de ellos 31 observados en suelo y 27 en raíces. Los géneros más frecuentes tanto en suelo como en raíces fueron *Helicotylenchus*, *Pratylenchus*, *Aphelenchus*, *Meloidogyne*, y *Rotylenchulus*; para las especies: *Helicotylenchus multicinctus*, *Pratylenchus zaeae*, y *Xiphinema* sp. en suelo, así como *Aphelenchoides* sp., *Pratylenchus coffeae*, y *P. zaeae* en raíces.

FITONEMATODOS ASOCIADOS AL CULTIVO DE LA CAÑA DE AZÚCAR EN LA REGIÓN SUR ORIENTAL DE CUBA [PHYTONEMATODES ASSOCIATED TO SUGAR-CANE CULTIVATION FROM THE SOUTHEAST REGION, CUBA]

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Los nematodos parásitos se asocian a afectaciones con el desarrollo y la producción de los cultivos agrícolas, se han reconocido como uno de los factores limitantes en los rendimientos de las plantas. La necesidad de actualizar el registro de estos organismos relacionados con la caña de azúcar en la región sur oriental de Cuba constituyó el objetivo principal de esta investigación. Para ello se procesaron muestras de suelo y raíces tomadas en las diferentes zonas pluviométricas de los suelos predominantes en las cinco Unidades Empresariales de Base de la provincia Granma. Los diagnósticos y determinaciones de géneros y especies se realizaron en el Laboratorio de Nematología de la Dirección Provincial de Sanidad Vegetal de dicha provincia. Con los datos obtenidos se caracterizó la población mediante la determinación del patrón de las comunidades y el Índice de similitud entre localidades y por tipos de suelos. Se identificaron 36 especies de fitonematodos agrupados en 15 géneros con una localización del 91.66% de las especies en las áreas destinadas a producción. Los suelos mostraron las densidades más altas comparadas con las raíces. El género de mayor representación en la región resultó *Pratylenchus* con 10 especies. Se distingue la alta diversidad de géneros encontrada en las muestras de suelo de la localidad de “Andrés Cuevas” mientras que, mayor similitud de especies y géneros se identificó en las muestras de suelo de las áreas de producción de Los Chinos y Realengo y los suelos Ferralitizados cálcicos y Sialitizados cálcicos dado por los valores del Índice Cuantitativo de Sorenson. Estos resultados constituyen una contribución al conocimiento de la composición de fitonematodos asociada al cultivo de la caña de azúcar en esta región.

NEMATOFAUNA EN EL AGROECOSISTEMA CAÑERO DE LA EMPRESA AZUCARERA ARTEMISA [NEMATODE FAUNA ON THE SUGARCANE AGROECOSYSTEM OF ARTEMISA SUGAR COMPANY]

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Los nematodos fitopatógenos son causa de bajas en los rendimientos agrícolas de muchos cultivos y para mitigar sus daños resulta indispensable la implementación de medidas de manejo de la plaga. Para ello es necesario identificar la fauna parasítica del organismo y su relación con el cultivo en el escenario a estudiar. El objetivo del presente estudio fue conocer la nematofauna en el agroecosistema cañero de las Unidades Empresariales de Base (UEB) de la Empresa Azucarera Artemisa. Para los análisis, se tomaron muestras de suelo y raíces en tres campos de tres bloques en una Unidad Productora (UP) de cada UEB. Parte de la muestra de suelo fue colocada en bolsas de polietileno donde se sembraron semillas de calabaza, para la detección de *Meloidogyne* spp. La extracción y clasificación de los nematodos se realizó en el Laboratorio Provincial de Sanidad Vegetal (LAPROSAV) de la provincia de La Habana. En las muestras de raíces de las plantas de calabaza se observó la formación de agallas por *Meloidogyne incognita* hasta un grado IV de infestación. Se identificaron 11 géneros y 18 especies con mayores densidades de individuos en suelos que en raíces. El género más representado fue *Pratylenchus*. Hubo diferencias en la diversidad y riqueza de géneros y especies entre localidades, encontrándose la mayor densidad poblacional en el suelo aluvial diferenciado.

CONFIRMATION OF ROOT-KNOT NEMATODE *MELOIDOGYNE HAPLA* ON PEPPER (*CAPSICUM ANNUUM* L.) FROM IMBABURA (ECUADOR) USING MOLECULAR AND MORPHOLOGICAL TECHNIQUES [CONFIRMACIÓN DEL NEMATODO AGALLERO *MELOIDOGYNE HAPLA* EN PIMIENTO (*CAPSICUM ANNUUM* L.) DE IMBABURA (ECUADOR), UTILIZANDO TÉCNICAS MORFOLÓGICAS Y MOLECULARES]

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Root-knot nematodes are highly-adaptable, obligate, and polyphagous plant-parasites. *Meloidogyne* spp. are distributed worldwide and parasitize economically relevant crops. Infection success in the host depends on the particular interaction between a specific nematode species as well as plant variety and the crop type. Accurate diagnosis of nematode species is relevant to effective agricultural management. In the last decades, the plant health of horticultural crops such as pepper (*Capsicum annuum* L.) has experienced a progressive deterioration, characterized mainly by reduced vigor and productivity of the plant. Plant-parasitic nematodes are the most likely causal agent. In this study, we used morphological and molecular tools in order to identify a root-knot nematode, since this organism has been found on pepper crops in Imbabura Province (north of Ecuador), and are believed to be related to the production decrease. Morphological studies based on perineal patterns of root-knot nematodes female collected indicated the incidence of *Meloidogyne hapla*. The most decisive data was provided by using molecular tools like RFLP'S and PCR. Molecular analyzes were performed in sequencing the D2-D3 region of the 28S gene and then using specific SCAR primers, the mitochondrial DNA (mtDNA) region located between the 3' region of the cytochrome oxidase COII and the 5' end region of the 16 rRNA, that have shown to be useful for identification of species of plant parasitic nematodes. These results highlight the importance of taking a mul-tidimensional approach for *Meloidogyne* spp. diagnosis. This study contributes to the understanding the

variability of root-knot nematodes (*Meloidogyne* spp.) species and provides data on the identification of this parasites on pepper crops from Ecuador.

DISTRIBUCIÓN DEL NEMATODO AGALLADOR DEL AGERATO (*SUBANGUINA* SP.) EN MÉXICO [DISTRIBUTION OF GALL-NEMATODE OF AGERATUM (*SUBANGUINA* SP.) IN MÉXICO]

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El género *Subanguina* presenta 30 especies a nivel mundial, la mayoría tiene como hospedantes plantas de la familia Asteraceae, algunas de importancia ornamental y/o medicinal; en menor proporción en Poaceae, Fagaceae, Apiaceae, Plantaginaceae. Con el objetivo de determinar la distribución del nematodo agallador del agerato en México, se realizaron muestreos y colectas de plantas del género *Ageratum*, y otros especímenes de Asteraceae, en los Estados de Tlaxcala, Hidalgo, Puebla y Estado de México, en bosques de pino y oyamel; en los meses de junio a diciembre, entre los años de 2006-2007 y 2012-2014. Para realizar los recorridos y colecta de las plantas, se utilizaron mapas de municipios de los Estados Unidos Mexicanos, Google Earth, GPSmap 60CSx marca Garmin, y Cámara Digital Sony Cybershot. Los 450 sitios de muestreo con el GPS y después transferidos al Google Earth; permitió determinar la presencia del nematodo agallador del agerato, entre las altitudes de 2200-3600 msnm. y la distribución en los siguientes municipios: Tlaxco, Emiliano Zapata, Terrenate, Huamantla, Tetlanohcan, Teolocholco, Nanacamilpa y Calpulalpan, del estado de Tlaxcala; Almoloya, del estado de Hidalgo; Ixtacamaxtitlan y Chignahuapan, del estado de Puebla; y Texcoco, del Estado de México. El nematodo causa agallas en tallos, peciolas y nervaduras de las hojas, y pedúnculo de la inflorescencia en *Ageratum conyzoides*. En México, es el primer reporte de la presencia de *Subanguina* sp., en *Ageratum conyzoides*.

SPECIES OF THE GENUS *AULOLAIMUS* DE MAN, 1880 (NEMATODA, PLECTIDA, AULOLAIMIDAE) FROM THE SOUTHERN IBERIAN PENINSULA [ESPECIES DEL GÉNERO *AULOLAIMUS* DE MAN, 1880 (NEMATODA, PLECTIDA, AULOLAIMIDAE) DEL SUR DE LA PENÍNSULA IBÉRICA]

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The plectid genus *Aulolaimus* de Man, 1880 currently includes 14 valid species characterised by their bipartite pharynx consisting of long anterior tubular part and posterior arachiform bulbus. Most species were recorded from Europe and Asia, and a good number of them are only known to occur in their type localities. The study of the material collected from the Southern Iberian Peninsula reveals the existence of four species: *A. autumnalis*, *A. mowhiti*, *A. nannocephalus*, and *A. oxycephalus*. These species have been characterised morphologically and morphometrically. The main diagnostic characters for their identification are the lip region shape (from offset by distinct constriction with labial disc laterally pointed to offset by weak constriction), the number of longitudinal ridges (from 30 to 70 and equally sized), the presence or absence of cuticular pores, female tail length and shape (from elongated to filiform), as well as the proportion of the different parts of the tail (proximal cellular part, intermediate empty part and distal hyaline part) and number of male precloacal supplements (2-3). Small intraspecific variations were observed for these characters.

SYSTEMATICS AND DNA BARCODING OF FREE-LIVING MARINE NEMATODES WITH EMPHASIS ON TROPICAL DESMODORIDS USING NUCLEAR SSURDNA AND MITOCHONDRIAL COI SEQUENCES [SISTEMÁTICA Y CÓDIGO DE BARRAS DE ADN DE NEMÁTODOS MARINOS DE VIDA LIBRE CON ÉNFASIS EN DESMODORIDOS TROPICALES USANDO SECUENCIAS DE ADN NUCLEAR Y MITOCONDRIAL]

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The diversity and phylogenetic relationships of the Desmodoridae, a widespread tropical family of free-living marine nematodes, is hitherto poorly known both from molecular and taxonomic points of view. We performed a molecular phylogenetic analysis of marine nematodes to: *i*) disentangle relationships among tropical desmodorid species; and *ii*) compare the performance of the nuclear SSU rDNA and mitochondrial COI nucleotide sequences in 42 and 45 nominal species, respectively, to identify species. We generated 27 new sequences of SSU rDNA belonging to five genera not previously sequenced, and 34 new sequences of COI belonging to six genera and four families not previously sequenced. The SSU rDNA tree confirmed the Enoplida to be a monophyletic sister group to the Chromadorida. The family Comesomatidae is a sister group of the Xyalidae within the Monhysterida. Both DNA markers confirmed the congruence between the morphology- and molecular-based phylogenetic inferences for most of the families. Desmodoridae was a monophyletic group, but the relationships within the family could not be recovered; the subfamilies Desmodorinae and Spiriniinae were not monophyletic meanwhile the monophyly of Stilbonematinae was not fully supported due to a few specimens of questionable identity. COI performed better than SSU rDNA to disentangle relationships among closely related species and suggested the presence of cryptic diversity within Desmodoridae. COI is effective to explore cryptic diversity and barcode species within Nematoda,

with a possible threshold of genetic distance of 5% between conspecific and interspecific sequences, but DNA barcoding is limited by the poor knowledge of the diversity and taxonomy of the group and the lack of a good reference database of voucher COI sequences.

PATRONES DE DIVERSIDAD DE NEMÁTODOS DE VIDA LIBRE EN PASTOS MARINOS DEL ARCHIPIÉLAGO CUBANO [DIVERSITY PATTERNS OF FREE-LIVING MARINE NEMATODES IN SEAGRASS BEDS FROM CUBAN ARCHIPELAGO]

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We estimated the species richness at both local (α -diversity) and regional (γ -diversity) scales and the differentiation of diversity (β -diversity). We used seagrass bed sediment samples from four sites around the Cuban archipelago (Punta Francés, Sabana-Camagüey, Rincon de Guanabo and Gulf of Ana Maria) collected between February and October 2013. Nematodes were extracted, preserved and identified to species level. We identified 503 nematodes belonging to 114 families; the best-represented genera were *Zalonema* and *Desmodora*. Accumulation curves indicated that the species richness was similar across the four sites with an average of 144 ± 13 species. Results suggest an underestimation of the α - and γ -diversity due to high diversity and rareness of the assemblages. More individuals need to be identified in order to more accurately assess the diversity. The number of shared species between the four sites was low (16–25%) and the multivariate techniques suggest very high differentiation of diversity. Constrained dispersion of nematodes due to geographic barriers and distance may be a plausible explanation for the found patterns.

DOES MORPHOLOGY OF HOST MARINE MACROALGAE DRIVE THE ECOLOGICAL STRUCTURE OF EPIPHYTIC MEIOFAUNA? [¿LA MORFOLOGÍA DE LAS MACROALGAS REGULA LA ESTRUCTURA ECOLÓGICA DE LA MEIOFAUNA EPÍFITA?]

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We explored the relationships between the epiphytic meiofauna and the morphology of five host macroalgae species. Four morphological attributes of the macroalgae (fractal complexity, area, perimeter, and amount of trapped sediment) were measured and correlated with the density and diversity of meiofauna and nematodes. The meiofauna was similar to epiphytic communities described in other studies with harpacticoid copepods and nematodes being the two more abundant taxa. The structural complexity of the macroalgae did not correlate with the meiofauna nor with the nematode assemblages. The distribution of meiofauna was not related to the species of macroalgae and instead was probably influenced by the random recruitment induced by the hydrodynamic of the study zone. The most abundant nematode families on macroalgae were Chromadoridae, Cyatholaimidae, Oncholaimidae, and Draconematidae. Most of the nematodes were good colonizers bearing ornamented cuticle and buccal armature for grasping; these biological traits suggest adaptive advantages to waterborne transport and use of biofilm as food source.

BODY-SIZE DISTRIBUTION OF CORAL REEF NEMATODES AND THEIR RELATIONSHIP TO ABUNDANCE, LIFE STAGE AND BIOLOGICAL TRAITS [DISTRIBUCIONES DE TAMAÑO DEL CUERPO DE NEMÁTODOS DE ARRECIFES DE CORAL Y SUS RELACIONES CON LA ABUNDANCIA, ESTADO DE VIDA Y RASGOS BIOLÓGICOS]

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The body size patterns of free-living marine nematodes in tropical ecosystems are quite unknown. In addition, there is scarcity of data of

invertebrates related to body size. We measured the length and width of 7,446 nematodes of 186 species from a coral reef in Punta Francés, Cuba. Body size distribution was strongly skewed with average length of 943 μm . The body shape had a bimodal distribution with stout and slender nematodes; ecological adaptation to the life style (interstitial versus burrower) probably explains these two morphotypes. There is an evolutionary trend of body size in the phylogeny being enoplids twice larger (2,066 μm) than chromadorids (899 μm). Juveniles tend to be smaller (1,019 μm) than adults (1,426 μm). However, females tend to be wider (55 μm) than males and juveniles (48 and 40 μm , respectively). These differences by life stage in length and width hold for all the species irrespective of their absolute body size. The relationships between abundance of species and their body mass have a horseshoe shape with smallest and largest species being rare and species in the mid-range being the most abundant. This probably reflects ecological constrictions to the body size. However, the size spectrum of abundance versus body mass, irrespective to the species, suggests energetic constrictions leading to decay in the abundance of individuals in relation with the body mass. Body size and trophic groups are related but in a relaxed mode: larger nematodes tend to be predator/omnivores or non-selective deposit feeders, meanwhile smaller nematodes tend to be selective deposit feeders or epigrowth feeders.

ESTABLISHING A PERMANENT FEEDING SITE: RENIFORM NEMATODE MANIPULATION OF PLANT GENES [EL DESARROLLO DEL SITIO DE ALIMENTACIÓN PERMANENTE: LA MANIPULACIÓN DE LOS GENES DE LA PLANTA POR EL NEMATODO RENIFORME]

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Reniform nematode, *Rotelynychulus reniformis*, is a semi-endoparasite capable of infecting more than 300 host plant species in tropical, subtropical, and warm temperate regions. Female nematodes penetrate host roots and introduce effectors that lead to the formation of multinucleate feeding sites called syncytia. The objective of our study was to identify genes involved in the process of syncytium formation. We used a split root system in which half of the plant's roots were inoculated with *R. reniformis* while the rest of the root system remained uninfected. Illumina RNA-seq was used to quantify

global gene expression patterns in replicate samples of infected and uninfected root tissue at 3, 6, 9, and 12 days after inoculation. Reads were mapped to the soybean reference genome using TopHat, transcript abundances were calculated with HTSeq, and genes differentially-expressed between inoculated and uninoculated roots were identified using DESeq2. Blast2GOPro was used to annotate differentially expressed genes and to identify GO-terms over-represented in the differentially-expressed gene set. Among the differentially expressed genes include several transcription factors, cell wall modifiers, nodulation proteins, and hormone response proteins to auxin and ethylene. These genes will be examined in additional functional analyses and *in situ* hybridization studies to confirm their role in syncytium development.

EFFECTO DE LOS EXUDADOS DE LAS CÉLULAS PERIFERALES DE LA RAÍZ SOBRE EL COMPORTAMIENTO DE NEMATODOS FITOPARÁSITOS Y NO FITOPARÁSITOS [EFFECTS OF BORDER CELLS EXUDATES ON PLANT PARASITIC AND NON PLANT PARASITIC NEMATODES BEHAVIOR]

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Las células meristemáticas en el ápice de la raíz producen cientos de células periferales que envuelven a cada raíz y constituyen un componente importante de la rizosfera. El uso de suspensiones de estas células aisladas son una herramienta útil para demostrar las interacciones celulares entre plantas y patógenos. El proceso de infección en la mayoría de nematodos fitopatógenos ocurre por un mecanismo de dispersión pasiva. La infección se inicia en tejido nuevo de la zona de elongación y no en tejido maduro, esto significa que la infección ocurre entre 1 ó 2 días después de que estas células se dividieron. Por lo que cualquier intervención en el proceso de infección durante esta ventana ofrece la oportunidad de inhibir la infección antes de que esta comience. El reconocimiento de la hospedera por los nematodos involucra señales emanadas desde las raíces. La identificación y manipulación de cualquier estímulo químico necesario en cualquiera de estos estadios del proceso de reconocimiento hospedera-nematodo, pudiera potencialmente ser usado para controlar la infección. Existe evidencia

que exudados de una sola raíz pueden atraer, repeler o ser neutral en su habilidad para influenciar el comportamiento de los nematodos y esto sugiere que el comportamiento esta determinado por una combinación de señales en particular en cierto momento o lugar. En este trabajo se estudio el impacto de los exudados de plantas leguminosas y no leguminosas sobre el comportamiento de nematodos tanto fitoparásitos como no fitoparásitos. Los efectos sobre el comportamiento de los nematodos fitoparásitos y no fitoparásitos variaron de acuerdo a la fuente de estos exudados. Estos exudados afectaron dramáticamente tanto la quimiotaxis como la movilidad del nematodo. Igualmente se observaron cambios en la morfología de las células periferales del ápice de la raíz ante la presencia de nematodos fitoparásitos.

INFRARED (FTIR-ATR) CHARACTERIZATION OF GUAVA PLANTS INFECTED BY *MELOIDOGYNE ENTEROLOBII* [CARACTERIZACIÓN INFRARROJA (FTIR-ATR) DE PLANTAS DE GUAYABA INFESTADAS CON *MELOIDOGYNE ENTEROLOBII*]

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The vibrational aspects of the functional groups from the molecules of a given simple can be observed throughout infrared spectroscopy. Basically, a sample is irradiated with different wavelengths and a detector capable to register the vibrational mode of the molecules, builds a spectrum. These spectra can be compared among them and differences rise if a sample is different to another one. Infrared spectroscopy was used to compare guava plants (*Psidium guajava*) infected or not with *Meloidogyne enterolobii*. Thirty guava seed were sown in pots (5l) and after 90 days, 15 of them were inoculated with *Meloidogyne enterolobii* and the rest were used as control plants. Every week (up to 8 wk) leaf samples from both treatments were taken and let dry for 24 h at room temperature. The dried samples were placed in a Fourier

Transformed Infrared Spectrometer (FTIR) with an Attenuated Total Reflectance (ATR) accessory and the resulting spectra collected. The total data was treated to eliminate CO₂ and water interference, smoothed and a second derivative procedure was done. The main spectral contributions were assigned to proteins around 3277, 1533, and 1231 cm⁻¹ and lipids around 2924, 2855, 1737, and 1452 cm⁻¹. Carbohydrates were also detected; the major absorption was found in the 1000–1200 cm⁻¹ region. The samples from infected plants showed differences in their composition, compared to those coming from healthy plants. This technique demonstrated its usefulness for investigating many aspects of the nematode-plant interaction.

PRELIMINARY ANALYSIS OF JA-RELATED GENE EXPRESSION DURING ROOT-KNOT NEMATODE PARASITISM [ANÁLISIS PRELIMINARES DE EXPRESIÓN DE GENES JA-RELACIONADOS DURANTE EL PARASITISMO POR NEMATODOS AGALLADORES]

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Jasmonic acid (JA) is an important regulator of the plant immune system, playing a central role in the modulation of developmental processes, and signalling networks. The JA pathway is an indispensable component of plant resistance to nematodes, and is involved in wound response. Another hormone regulating the systemic wound response in tomato is systemin, an 18-amino-acid peptide signal molecule. Systemin and JA constructively interact in the same signalling pathway, coordinating wound-induced systemic expression of defence-related genes. In the present study we evaluated the expression pattern of selected genes involved in the JA pathway and the susceptibility of different tomato genotypes, including plants expressing different levels of Prosystemin, challenged with the root-knot nematode (RKN) *Meloidogyne incognita*. Six tomato genotypes: wild type, transformed lines expressing different levels of prosystemin and a homozygous Mi gene resistant tomato, were selected for the infection assay. The seedlings were inoculated 4 wk after transplanting with freshly hatched juveniles. Genotypes showing significant differences between measurements were used for

gene expression analysis. For this purpose, *in vivo* plant assays were carried out with tissues collected 5 and 6 d after RKN infection, processed for Real-Time PCR analysis (qRT-PCR). Preliminary data showed that transgenic plants overexpressing Prosystemin had significant less galls and showed milder symptoms, suggesting a Prosystemin role in inducing tolerance to RKN. The genotypes were further analysed in an expression assays with five different genes that appeared involved in the JA pathway. Comparison at two different time points showed that, in the early response, Prosystemin and LOX-Dare differentially regulated in the JA pathway. Data at 5 dpi suggested that RKNs suppress the JA pathway in wild type plants and that Prosystemin over expression could, therefore, be necessary to induce tolerance to RKNs.

THE EFFECT OF FOLIAR SPRAYS OF CHITOSAN, BION® AND METHYL JASMONATE ON THE BEHAVIOR OF THE ROOT-KNOT NEMATODE *M. INCOGNITA* IN TOMATO [EFFECTO DEL SPRAY FOLIAR DE QUITOSÁN, BION® Y METIL JASMONATO SOBRE EL COMPORTAMIENTO DEL NEMÁTODO *M. INCOGNITA* EN TOMATE]

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Plant-parasitic root-knot-nematodes, *Meloidogyne* spp., can cause major damage to their hosts, including the majority of the world's most economically important crops. Nematode management is therefore imperative. Over the recent years the use of many nematicides has been prohibited due to their harmful impact on the environment and human health. The use of resistant cultivars is an attractive alternative, but the durability of the used resistance genes remains questionable. Whereas single gene resistance is not considered sustainable in the long term, induced multigenetic resistance could offer a more sustainable solution. This objective might be obtained by the application of defense elicitors, synthetic or natural compounds, that induce systemic resistance in the host plants. In the present study, the elicitors chitosan, Bion® (active component acibenzolar-S-methyl) and methyl jasmonate have been tested for their plant defense inducing activity against *Meloidogyne*

incognita. Several experiments will be discussed which have been carried out with tomato as model plant. The plants were grown in a mixture of sand and potting soil, and then sprayed with the elicitors. Two days after spraying, the plants were inoculated with freshly hatched second-stage juveniles (J2) of *M. incognita*. At 12, 21, and 56 DAI (days after inoculation), the plants were harvested; several plant variables assessed and the root galling index determined. In addition, roots were collected and stained with acid fuchsin to visualize the nematodes microscopically or macerated and sieved to count the J2. In another experiment egg masses and number of eggs were assessed after 35 DAI. Our results show a minor influence of the tested defense elicitors on the life cycle of *M. incognita*. Only in one case, a tendency towards a higher nematode susceptibility has been observed. The implications and future perspectives for research of our results will be presented.

HERBACEOUS GRAFTING IN TOMATO (*SOLANUM LYCOPERSICUM* L.) GROWING IN PROTECTED CROP AS A TACTIC FOR MANAGEMENT OF *MELOIDOGYNE INCOGNITA* (KOFOID AND WHITE) CHITWOOD RACE 2 [EL INJERTO HERBÁCEO EN EL CULTIVO PROTEGIDO DEL TOMATE (*SOLANUM LYCOPERSICUM* L.) COMO TÁCTICA PARA EL MANEJO DE *MELOIDOGYNE INCOGNITA* (KOFOID Y WHITE) CHITWOOD RAZA 2]

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The nematode *Meloidogyne incognita* is the main pest of the protected vegetable crops in Cuba. The main objective of this work was to identify resistant rootstocks to *Meloidogyne incognita* (Kofoid and White) Chitwood race 2 for the protected crop of tomato in Cuba. Seven solanaceous genotypes were evaluated at the National Center for Animal and Plant Health (CENSA) in 2008. The experiment was set up under semi-controlled conditions. The plants were inoculated with 0.5, 1.5, 2.5, and 5 J₂ g of soil⁻¹). A randomized design with five replications per treatment was used. The selected genotypes were grafted with the hybrid tomato ‘HA 3105’. They were transplanted to a protected crop house in November 2009 and 2010, respectively, at the Horticultural Research Institute “Liliana

Dimitrova” (IIHLD) on a typical Red Ferralitic soil. In both cases, a randomized block design with three replications was used. Resistance of the ‘Rossol’, ‘Mottelle’, *Solanum torvum* Sw and *Sonulam globiferum* Dun genotypes to *M. incognita* race 2 was found in semi-controlled and field conditions. New sources of resistance to *M. incognita* race 2 in *Solanum erianthum* D. Don and the immunity in *Datura stramonium* L., reported for the first time in Cuba, were identified. A highly successful grafting and compatibility in the treatments grafted onto ‘Rossol’, ‘Mottelle’ and ‘Beaufort’ F1 were observed. The treatment ‘HA 3105’/‘Rossol’, showed a significant higher yield (148.34 and 136.71 t ha⁻¹) than the observed in the control without grafting (135.15 and 10.67 t ha⁻¹). It is supported the use of rootstock tomato ‘Rossol’ herbaceous grafting methodology and its validation for the management of *Meloidogyne* spp. in the protected tomato crop in Cuba.

NEMATODOS FORMADORES DE AGALLAS EN RAÍCES DE MANÍ [ROOT-KNOT NEMATODES IN PEANUT ROOTS]

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El cultivo de maní en Argentina es afectado por numerosas enfermedades ampliamente estudiadas, entre las que se destacan diversos hongos y virus. En los últimos años, algunos pocos artículos han señalado la presencia de fitonematodos como causantes de reducción del rendimiento. El objetivo del presente trabajo fue evaluar la presencia de nematodos en raíces y analizar las alteraciones que ocasionan en los tejidos. Se tomaron muestras de plantas de la variedad ASEM 485 INTA de un lote ubicado en la localidad de Río Tercero (Provincia de Córdoba, Argentina), durante la campaña de siembra 2013/2014. El material botánico fue fijado con una solución de FAA (formaldehído, ácido acético glacial, alcohol etílico y agua) y posteriormente se realizaron preparados histológicos permanentes con técnicas convencionales. Se hicieron observaciones con microscopio óptico y se tomaron fotomicrografías. En las raíces, fueron detectadas hembras de *Meloidogyne incognita* tanto en agallas generadas por el nematodo como en nodulaciones de *Rhizobium*. Internamente, en la zona de corteza radical, los parásitos estaban asociados a células gigantes, las que se ubicaban

próximas al tejido vascular. En los nódulos, los individuos se localizaban en el parénquima nodular. Algunos especímenes estaban acompañados por masas de huevos. Se concluye que la variedad de maní estudiada, es susceptible al ataque del nematodo citado y representa un hospedador propicio para que el parásito complete su ciclo de vida. Financiamiento: Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT, Argentina).

RELACIÓN NEMATODO – HOSPEDADOR ENTRE DOS AISLADOS DE *NACOBBUS ABERRANS* Y TRES CULTIVARES DE TOMATE (*SOLANUM LYCOPERSICUM*) [NEMATODE - HOST RELATIONSHIP BETWEEN TWO *NACOBBUS ABERRANS* ISOLATED AND THREE TOMATO (*SOLANUM LYCOPERSICUM*) CULTIVARS]

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El tomate es un cultivo hortícola muy relevante a nivel mundial. Uno de los principales problemas en su producción son los fitosanitarios, entre los que se destacan nematodos fitófagos, como *Nacobbus aberrans*. Las alteraciones ocasionadas modifican la anatomía de las raíces, provocando una disminución en el rendimiento del cultivo. El objetivo del presente estudio fue evaluar el grado de asociación entre tres cultivares de tomate y dos aislados de *N. aberrans*, a través del análisis histológico de raíces afectadas en comparación con raíces sanas. Se infectaron tres cultivares (“Superman”, “Mykonos”, y “Platense”) con dos aislados del nematodo, uno originario de Río Cuarto (Córdoba) y otro de Lules (Tucumán), ambos de Argentina; plantas sanas representaron el control negativo. Se mantuvieron en un invernáculo por 90 días; luego cada sistema radical fue fijado para realizar preparados histológicos permanentes y pruebas histoquímicas. En las raíces infectadas de todos los tratamientos se observaron agallas con masas de huevos en su superficie, y en el interior hembras asociadas a sitios de alimentación (síncitos). Los tejidos vasculares estaban desorganizados y desplazados hacia la periferia; el porcentaje de xilema disminuyó respecto a las plantas control. En Superman se observó mayor cantidad de almidón en la corteza de las agallas respecto a los otros

cultivares; en varias oportunidades, la hembra se encontraba rodeada por células que presentaban paredes muy engrosadas y taninos, esto se vio con menor frecuencia en Mykonos y no se evidenció en Platense. El porcentaje de la agalla ocupado por el síncito fue menor en Superman que en los otros tratamientos. Si bien todos los cultivares, ya sean infectados con el aislado de Lules o de Río Cuarto, sufrieron alteraciones anatómicas y establecieron una estrecha relación vegetal-parásito, las características histológicas observadas evidencian que Superman resultó más tolerante que Mykonos y Platense a los aislados evaluados.

SUITABILITY OF GRAIN SORGHUM CULTIVARS TO *MELOIDOGYNE INCOGNITA* [ADECUACION DE CULTIVARES DE SORGO COMO HOSPEDANTES DE *MELOIDOGYNE INCOGNITA*]

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There has been inconsistency in the reports of reactions of grain sorghum as a host for *Meloidogyne incognita*, ranging from susceptible to very resistant. The objective of this study was to evaluate the host suitability of 29 current grain sorghum cultivars to *M. incognita*. The experiment was conducted in a greenhouse environment, where the plants were grown 60 days-long. The experiment was repeated once. One tomato cultivar was used as a susceptible control and one nematode population of *M. incognita* was used as inoculum. The plants were inoculated with 2,000 nematode eggs one week after planting. At the end of the experiment number of juveniles and eggs, and gall rating were evaluated. Different levels of susceptibility to *M. incognita* were observed among the sorghum cultivars tested. Overall the galls were very small and gall rating was not a good indicator of host suitability. According to number of eggs per gram of root, the DuPont Pioneer 83P17 was the most susceptible cultivar of the group, followed by the Monsanto cultivar DEKALB DKS 53-67. The Terral Seed Inc. cultivar REV RV9782 was the most resistant one with 27 fold less eggs of *M. incognita* than the most susceptible cultivar. In respect of number of juveniles per pot (1.6kg), the most susceptible cultivar was the Monsanto cultivar DEKALB DKS 53-67 and the most resistant Terral Seed Inc. cultivar REV RV9782, with about 26-fold fewer juveniles than the most susceptible one. Therefore the selection of a grain sorghum cultivar to be used in a crop rotation system has some

restrictions, depending on the nematodes present in the area. Further studies are necessary to evaluate the effect of *M. incognita* on grain sorghum yield.

NEMATICIDAL ACTIVITY OF *PIPER HISPIDINERVUM* ESSENTIAL OIL [ACTIVIDAD NEMATICIDA DEL ACEITE ESENCIAL DE *PIPER HISPIDINERVUM*]

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Piper hispidinervum (pimenta-longa in Brazil), is a shrub distributed throughout South America. This species is especially prominent in the state of Acre in Brazil and may extend into Amazonas. In this study we have studied the nematicidal activity of *P. hispidinervum* essential oil and its main components against the root-knot nematode *Meloidogyne javanica*. Pilot-scale steam distillations of experimentally cultivated *P. hispidinervum* fresh leaves at a pressure gradient (1, 1.5, and 2 bar) yielded three essential oils (EOs). The chemical analysis of these EOs by GC-MS showed a similar composition for the three oils, with 24 compounds, representing 98% of the total oil. Safrole was the major component (77.7 - 81.3%) followed by terpinolene (4.6 - 8.8%). *P. hispidinervum* EOs (1 µg/µl) showed strong nematicidal *in vitro* effects against *M. javanica* (J2 mortality and long-term egg hatching inhibition effects). The major components (safrole and terpinolene) were not toxic to J2, but their binary combinations at different ratios induced high nematicidal activity, suggesting a synergistic interaction for these compounds in the EO. The *in vivo* test of one selected *P. hispidinervum* EO (1 bar) on tomato seedlings showed strong effects on suppression of infection capacity of treated J2. This study demonstrates for the first time the nematicidal activity of *P. hispidinervum* EO against root-knot nematodes. *P. hispidinervum* is an important plant in Brazil, which is widely exploited as a source of safrole and the nematicidal activity of *P. hispidinervum* oil could significantly increase its commercial value.

IN-VITRO EFFECTS OF EXTRACTS OF BRASSICACEOUS GREEN MANURES ON THE MOVEMENT AND HOST LOCATION OF *GLOBODERA ROSTOCHIENSIS* SECOND-STAGE JUVENILES [EFECTO *IN-VITRO* DE ABONOS VERDES DE BRASICÁCEAS SOBRE EL MOVIMIENTO Y LOCALIZACIÓN DE HOSPEDERO DE JUVENILES DE *GLOBODERA ROSTOCHIENSIS*]

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Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, cause severe yield reduction in potato, and are the most important quarantine nematode pest for this crop in Europe. Since many decades, farmers are facing difficulties for the management of these species as their biological characteristics allow them to survive in the soil for several years. In addition, the use of chemical nematicides has been restricted in several countries, forcing farmers to search for environmentally friendly methods to control nematodes. Soil amendments with green manures received much attention as a management practice, especially with brassicaceous plants because isothiocyanate compounds are released at the breakdown of their tissues. This study evaluated the effect of root diffusates and extracts obtained from brassicaceous plants on the movement and host location of *G. rostochiensis* second-stage juveniles (J2). Root diffusates and extracts of plant tissues were obtained from yellow mustard (*Sinapis alba*), rapeseed (*Brassica napus*), and fodder radish (*Raphanus sativus*). The effect of these solutions on the movement of J2 was tested by direct exposure and in sand columns. The effect on the sensory perception was evaluated in water-agar and pluronic-gel plates with a tomato seedling as the attracting host. The movement of J2 was significantly reduced by direct exposure to extracts made from roots and from above-ground tissues, and so was the migration through sand columns. Juveniles pre-exposed to the diffusates or extracts of green manures could not readily detect the presence of a host in the agar or pluronic-gel plates, moving away from it during the first 8 hours of the experiment. These results indicate that breakdown of brassicaceous plant tissues have disruptive

effects on the mobility and sensory perception of *G. rostochiensis* juveniles, making these plants interesting for application in environmentally friendly management options.

ANTHELMINTIC ACTIVITY *IN VITRO* OF *MELALEUCA QUINQUENERVIA*, *CITRUS SINENSIS*, AND *PIPER ADUNCUM* ESSENTIAL OIL ON *HAEMONCHUS CONTORTUS* [ACTIVIDAD ANTIHELMINTICA *IN VITRO* DE LOS ACEITES ESENCIALES *MELALEUCA QUINQUENERVIA*, *CITRUS SINENSIS*, Y *PIPER ADUNCUM* SOBRE *HAEMONCHUS CONTORTUS*]

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Plant-derived compounds are being studied for their potential use against gastrointestinal nematodes. *Melaleuca quinquenervia*, *Citrus sinensis*, and *Piper aduncum* essential oils, were evaluated against *Haemonchus contortus* (Embrapa 2010) resistant isolate through the egg hatch test (EHT) and larval development test (LDT). Essential oils were tested in EHT and LDT at concentrations ranging from 0.02 mg/mL to 50 mg/mL and from 0.04 mg/mL to 3.12 mg/mL, respectively. All concentrations, positive control (Thiabendazole on EHT or Ivermectin on LDT), and negative controls (Tween 80 at 2% on EHT or DMSO at 0.5% on LDT) were performed in six replicates and in three independent experiments. Significant differences ($P \leq 0.01$) among means of estimates inhibition percentages of the EHT and LDT were identified by one-way analysis of variance followed by Tukey's multiple comparison test. LC_{50} and LC_{90} were analyzed statistically by the Probit procedure of the SAS software. The results showed in the EHT, the LC_{50} and LC_{90} of the essential oils were respectively as follows: 1.52 and 5.63 mg/mL for *M. quinquenervia*, 0.27 and 0.99 mg/mL for *C. sinensis*, 1.75 and 6.69 mg/mL for *P. aduncum*. In the LDT, the LC_{50} and LC_{90} were respectively: 0.44 and 0.94 mg/mL for *M. quinquenervia*, 0.97 and 2.32 mg/mL for *C. sinensis*, 0.19 and 0.51 for *P. aduncum*. The anthelmintic activity of essential

oils followed the same pattern in all *in vitro* tests, *C. sinensis* essential oils could be interesting candidates for nematode control, although *in vivo* studies are necessary to validate the anthelmintic properties of this oil.

NEMATICIDAL ACTIVITY OF NAPHTHOQUINONES FROM WALNUT AGAINST THE ROOT-LESION NEMATODE *PRATYLENCHUS THORNEI* [ACTIVIDAD NEMATICIDA DE LAS NAFTOQUINONAS DE NUEZ CONTRA EL NEMATODO DE LAS LESIONES RADICULARES *PRATYLENCHUS THORNEI*]

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Pratylenchus is among the top 10 plant-parasitic nematodes in plant pathology with major ecological and economic impact worldwide. *Pratylenchus thornei* is a widely distributed common root-lesion nematode species and an important parasite of cereals and legumes. Symptoms associated to the presence of *P. thornei* usually involve stunting, lack of vigour and leaf nutritional deficiencies, such as chlorosis. These nematodes are difficult to control and new, effective, and eco-friendly sustainable management strategies are still needed. The aim of this work was to assess the effect of naphthoquinones (juglone, 1,4-naphthoquinone and plumbagin) from walnut hulls on the mortality of *P. thornei*. Twenty mixed stages of *P. thornei* were placed in 1 mL of each compound concentration (500, 250, and 150 ppm) and nematode mortality monitored during 72 h. Each treatment consisted of five replicates and tap water and Triton X-100 5000 ppm as controls. The compound 1,4-naphthoquinone was generally more active than juglone and plumbagin at 500 ppm, however, juglone caused 57% *P. thornei* mortality at 150 ppm, within 72 h of exposure. 100% mortality was achieved after 24 h of exposure to 1,4-naphthoquinone 500 ppm. After 72 h, more than 70% of nematode mortality was achieved at 1,4-naphthoquinone 250 ppm and juglone and plumbagin 500 ppm. Plumbagin was less effective causing 40% mortality at 150 ppm. Estimated values of lethal concentration necessary to result in

50% *P. thornei* mortality ranged from 135 (juglone) to 179 ppm (plumbagin) at 72 h of exposure. These phytochemicals have potential to be used as natural nematicides in the management of *P. thornei* by application on natural habitats. Additionally, walnut residues can be considered as high-value residues and thus, valorized by the extraction of their bioactive/nematicide compounds.

1,4-NAPHTHOQUINONE BIONEMATICIDE – ECOTOXICOLOGICAL CHARACTERIZATION USING STANDARD AND NEMATODE COMMUNITY TESTS [1,4-NAFTOQUINONA BIONEMATICIDA – CARACTERIZACIÓN ECOTOXICOLÓGICA UTILIZANDO ENFOQUES ESTÁNDAR Y EFECTO SOBRE LA COMUNIDAD DE NEMATODOS]

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Phytoparasitic nematodes, such as *Meloidogyne* and *Pratylenchus*, cause important crop losses worldwide and are difficult to control, despite the profusion of chemical tools available. A project is ongoing, aiming to develop an eco-friendly nematicide, with origin in agricultural residues. To assure its environmental safety, potential risks for non-target nematodes and other soil organisms need to be evaluated. The aim of this study was to characterize the ecotoxicity of 1,4-naphthoquinone (NTQ), polyphenolic compound with nematicidal activity, naturally found in walnut residues, using standard test-species and a new approach based on the evaluation of effects on the soil nematode community. Standard ISO tests included seed emergence and plant growth with *Zea mays* and *Brassica napus*; avoidance with *Eisenia andrei* and *Folsonia candida* plus reproduction with the latter species and *Enchytraeus crypticus*. For the community tests, uncontaminated soil samples and local nematode community were used. Part of the soil was defaunated and the remaining used to extract nematodes (adaptation of Tray method).

Defaunated soil was spiked with a range of NTQ concentrations and further inoculated with the original nematode community (≈ 400 nematodes/replicate). After 7 and 14 days of exposure, nematode abundance and richness (feeding groups and family level) were evaluated. Toxic effects of NTQ were detected in all test-species, with higher sensitivity for *F. candida* and *E. andrei*; plants were clearly less sensitive. Nematode abundance decreased along the concentration gradient after 7 days, although with a partial recovery after 14 days, at NTQ concentrations < 48 mg/kg soil, while the number of families consistently decreased in both sampling periods. Important changes were also observed in the community composition in terms of feeding groups at 7 and 14 days, with effects starting from 96 mg/kg soil. These results highlight the use of soil nematode communities as toxicity indicators, in complement to standard ecotoxicological tests.

COMPOSITIONS OF CRUDE GLYCERIN AND UREA AS SOIL AMENDMENTS FOR THE MANAGEMENT OF NEMATODES [PREPARACIONES DE GLICERINA CRUDA Y UREA COMO MEJORADORES ORGÁNICOS PARA EL MANEJO DE NEMATODOS]

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Amending soil with organic matter has been proven to be an effective method of managing plant parasitic nematodes. Since soil fumigants result in drastic reduction of beneficial soil microbial populations and are at risk of decreased availability due to regulatory agencies, it is vital to develop more effective cropping systems utilizing soil amendments. A soil amendment composed of crude glycerin, urea, propionic acid, potassium hydroxide, and phosphoric acid was evaluated in a series of tests in a greenhouse, microplots, and on polyethylene-covered raised vegetable beds at the E. V. Smith Research Center in Shorter, Alabama, U.S.A. Positive growth response and increased yield of yellow squash (*Cucurbita pepo*), strawberry (*Fragaria x ananassa*), and tomato (*Lycopersicon esculentum*) occurred in amended plots compared to water treated control plots. Root knot nematode (*Meloidogyne incognita*) populations and number of galls decreased in amended plots, while saprophytic nematode

(Rhabditidae and Acrobelidae families) populations increased. Soil from amended plots was found to have increased urease, protease, alpha-glucosidase, beta-glucosidase, and dehydrogenase activity. Increased enzyme activity is related to increased microbial activity in soil. This was confirmed by plating soil dilutions on selective media; fungi, bacteria, and actinomycetes populations increased in amended soil. This soil amendment has great potential for commercialization as nematodes are controlled as effectively as soil fumigants without reducing other soil microbial populations and activity.

EFFECTO DE LA BIODESINFECCIÓN CON RESIDUOS ORGÁNICOS Y EL USO DE COBERTURA VEGETAL PARA EL MANEJO DE *MELOIDOGYNE* SPP. [EFFECT OF BIODISINFECTION WITH ORGANIC RESIDUES AND THE VEGETABLE SCREEN USE FOR THE MANAGEMENT OF *MELOIDOGYNE* SPP.]

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En la actualidad, se están utilizando compuestos de origen orgánico para manejar los patógenos de los vegetales de origen edáfico como bacterias, hongos, virus, nematodos, y también para la flora arvensis. Se evaluó el efecto de la biodesinfección del suelo utilizando bagazo, bagazo + gallinaza; residuos de caña – maíz kingrass, residuos de caña – maíz kingrass +gallinaza, estiércol vacuno+gallinaza (con y sin cobertura), en un suelo con alto índice de infestación de *Meloidogyne* spp. Los residuos vegetales se incorporaron al suelo antes del establecimiento del cultivo de la col. Para determinar el efecto de la biodesinfección sobre las poblaciones de *Meloidogyne* spp. en el suelo, se determinó el índice de infestación antes y después de la biodesinfección, el número de microorganismos totales y número de nemátodos saprobióticos, así como las plantas arvenses y la composición química y orgánica del suelo. Al término de la cosecha se extrajeron las raíces en su totalidad y se determinó el índice de agallamiento y el rendimiento del cultivo, expresados en kg/ha. El índice de infestación en suelo por *Meloidogyne* spp., después de la biodesinfección disminuyó de grado 5 a inferior a 2. Los resultados obtenidos demostraron la viabilidad del empleo de la técnica logrando una disminución de la incidencia de patógenos vegetales y aumentando la presencia de

nematodos saprófagos. La composición química y orgánica del suelo mostró diferencias entre los tratamientos estudiados.

EFFECTO ALELOPATICO DE *TAGETES ERECTA* L. AL NEMATODO *MELOIDOGYNE INCOGNITA* [ALLELOPATHIC EFFECT OF *TAGETES ERECTA* L. TO *MELOIDOGYNE INCOGNITA*]

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Entre las principales plagas que afectan a los cultivos hortícolas en Cuba, se encuentran los nematodos formadores de nódulos (*Meloidogyne* sp.). El manejo de nematodos actualmente se realiza con medidas de exclusión, prácticas agronómicas, utilización de agentes de control biológico y variedades resistentes, pero se requieren otras alternativas que contribuyan al manejo integrado como la aplicación de sustancias extraídas de plantas. El objetivo del trabajo fue evaluar el efecto de extractos acuosos y exudados de las raíces de *Tagetes erecta* L. *in vitro* y en el suelo, sobre *M. incognita*. Esta planta se siembra en Cuba, como ornamental y repelente de insectos plaga en la agricultura urbana, suburbana, y familiar. Bajo condiciones *in vitro* se evaluaron los efectos de distintas concentraciones de los extractos acuosos de la parte foliar y radical sobre la vitalidad de juveniles de *M. incognita*. En condiciones semicontroladas se estudió la influencia de la siembra de *T. erecta* para reducir la infestación en suelo, así como los efectos de su establecimiento a distintas distancias de siembra sobre la infestación en habichuela lina (*Vigna unguicula* sp. *sesquipedalis* L.). El extracto acuoso y los exudados de las raíces de *T. erecta* mostraron efecto alelopático sobre juveniles de *M. incognita* y produjeron la muerte de los nematodos; sin embargo, los extractos acuosos de las hojas y flores de la planta no produjeron ningún efecto sobre los juveniles de *M. incognita*. En el suelo se apreciaron reducciones significativas del nivel de infestación de los nematodos cuando *T. erecta* era sembrada en suelo infestado. Igualmente su establecimiento a distancias de 0-10 cm de la habichuela, produjo disminución de la infestación por nematodos en dicha planta. Los resultados indican las potencialidades de esta especie para ser introducida como alternativa viable dentro del manejo de los nematodos formadores de gallas.

MECHANISMS OF NEMATODE SUPPRESSION IN INUNDATED AMENDED WASTE SOIL CONTAMINATED WITH POTATO CYST NEMATODES, *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA* [MECANISMOS DE SUPPRESSION DE NEMATODOS EN SUELOS CON ENMIENDAS DE RESIDUOS E INUNDADOS Y CONTAMINADOS CON LOS NEMATODOS DE QUISTES DE LA PAPA, *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA*]

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Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida* are a significant threat to potato production. PCN can persist in soil for long periods of time and are easily spread by farm machinery, waste soil and residues from potato processing industries. Inundation is receiving more attention as an alternative to soil fumigants for disinfestation of waste soil. We investigated if agricultural and industrial waste products accelerate the disinfestation process in inundated conditions by studying PCN survival over time, as well as the mechanisms of this observed nematode suppression. Experiments were conducted in 2-L closed containers filled with 1.5 L non-amended soil (1), or soil amended with (2) steamed potato peels, (3) fresh potato peels, or (4) green top parts of leek. In each container, 25 cysts of *G. rostochiensis* or *G. pallida* were buried in the soil in retrievable nylon mesh bags and 400 ml tap water was added. The viability, hatching, and infectivity of the eggs and juveniles retrieved from the containers as well as concentrations of volatile fatty acids (VFA) and gases (O₂, CO₂, H₂S, CH₄, and N₂) were determined 14, 17, 21, 24, and 28 d after inundation. After 28 d, inundation of non-amended soil caused 50% reduction in PCN survival while in amended soil survival was reduced up to 99.5%. Hatching and infectivity of juveniles were reduced one week after inundation in amended soil compared with

non-amended inundated soil. Higher concentrations of VFA and carbon dioxide and lower levels of oxygen were detected in amended treatments. Hydrogen sulphide was occasionally detected in the leek amended treatment. Our study showed that application of amendments improved the effect of inundation as a disinfection practice and that depletion of oxygen and generation of H₂S but also formation of acetic, butyric, and propionic acids are involved in nematode suppression.

AISLAMIENTO, CARACTERIZACIÓN Y VIRULENCIA DE HONGOS NEMATÓFAGOS CONTRA *MELOIDOGYNE* SPP., EN EL VALLE DEL FUERTE SINALOA, MÉXICO [ISOLATION, CHARACTERIZATION AND VIRULENCE OF NEMATOPHAGOUS FUNGI AGAINST *MELOIDOGYNE* SPP., IN THE NORTH OF SINALOA, MEXICO]

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La producción de diversos cultivos hortícolas en el Norte de Sinaloa, está siendo limitada por el ataque del “Nematodo Nodulador” *Meloidogyne* spp. La búsqueda de alternativas para el manejo de este fitoparásito surge como respuesta a esta problemática, entre estas se encuentra el aislamiento e identificación de hongos nematófagos nativos del Valle del Fuerte. El objetivo de la presente investigación es identificar hongos nematófagos existentes en suelos donde se producen cultivos en condiciones protegidas y susceptibles a *Meloidogyne* spp. El estudio se realizó entre los meses de enero del 2013 a julio 2014. Muestras de suelo provenientes de tres regiones productoras de chile bell pepper del Valle del Fuerte, fueron procesadas mediante el método de espolvoreado en placa (Agua-agar). Para purificar y seleccionar hongos nematófagos, los aislamientos fueron transferidos a placas con maíz-agar (Corn-meal-agar), identificándose las estructuras morfológicas para el diagnóstico a nivel género. Se identificaron: *Paecilomyces* sp., *Dactylella* sp., *Arthrobotrys* sp., *Nematoctonus* sp., y otros. Pruebas *in vitro* de patogenicidad están siendo actualmente conducidas para evaluar su efectividad como nematófagos y determinar su potencial como agentes de control biológico utilizado nematodos de vida libre de la familia Rhabditidae. Los resultados sugieren una

diversidad de hongos nematófagos con potencial para ser utilizados como agentes de control biológico.

GROWTH KINETICS OF *DACTYLELLA OVIPARASITICA* STRAINS IN A PEAT CARRIER [CINÉTICA DE CRECIMIENTO DE CEPAS DE *DACTYLELLA OVIPARASITICA* EN UN PORTADOR DE TURBA]

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The ascomycete *Dactylella oviparasitica* (syn. *Brachyphoris oviparasitica*) is a hyperparasite of several endoparasitic nematodes. The fungus is considered the primary agent responsible for a decades-long population suppression of the sugar beet cyst nematode *Heterodera schachtii* at a field site at Agricultural Operations, University of California, Riverside. Three genetically different strains of *D. oviparasitica* (DO50, ARF, DOST) show activity as potential biocontrol agents against *H. schachtii*. A commercial preparation of reed sedge peat (BioApt[®]) proved to be a suitable carrier medium for soil infestation with DO50. The strain grown aseptically on BioApt[®] remained viable for at least 2 yr when stored at room temperature or 4°C. The objective of this project was to evaluate growth kinetics of DO50, ARF, and DOST in BioApt[®]. Dry BioApt[®] (-30 microfines) was mixed 1:1 (DW/V) with deionized water and allowed to hydrate overnight. Moist peat was autoclaved for 30 min and 600 cm³ distributed to 1 L Pyrex wide mouthed laboratory bottles. Lidded bottles containing moist peat were autoclaved for 30 min and allowed to cool overnight prior to addition of fungal cultures. Three 1 cm² sub-samples of PDA from 1-mon-old cultures of DO50, ARF, or DOST were added aseptically to bottles. They were stored in the dark at 22°C and monitored for fungal growth for 8 wk. Every 2 wk peat cultures were mixed gently by rotating bottles and 0.5 g were removed from each bottle to determine CFU/gDW peat. Fungal growth increased rapidly between 2 and 6 weeks then leveled off for all three fungi. DO50 grew best, reaching 3.2 x 10⁶ CFU/gDW followed by DOST (1.4 x 10⁶) and ARF (0.5 x 10⁶). Growth of the fungal strains in peat reflected their growth on water agar, suggesting that peat does not provide an appreciable source of nutrients.

AN EFFICIENT WAY FOR BIOLOGICAL CONTROL OF THE ROOT-KNOT NEMATODE ON TOMATO AT PROTECTED FIELD [UNA MEJOR FORMA DE CONTROL DEL NEMATODO AGALLERO *MELOIDOGYNE INCOGNITA* EN CAMPOS PROTEGIDOS]

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The tomato disease caused by root knot nematode *Meloidogyne incognita* was developed in China last decade and chemical pesticides are used to treat the soil mainly causing negative influence on the environment. Application of biological pesticides is concerned by scientists and farmers. A fungi strain of *Aspergillus niger*-Y61 and its fermented broth have demonstrated a killing function to the root knot nematode when applied to treat the soil. When the broth was used at 5, 10, and 20 times by concentrated, the number of *M. incognita* J2 and egg mass/100ml were reduced by 52.5-94%, 71.3-88.1%, and 92.3-99.6%, respectively, at the spring season. The broth was concentrated at 5, 10, and 20 times and the number of *M. incognita* J2 and egg mass/100ml were reduced by 55.5-74.6%, 69.3-78.1%, and 89.7-96.7%, respectively, at the fall season. The nematicidal substance produced by *A. niger*-Y61 displayed a higher ability to inhibit *M. incognita* at the low root knot index area of tomato. The addition of natural products such as sesame dregs is a way of reducing the infectious and a killing ability to root-knot nematode when treated with soil, and the egg mass or J2 could be killed up to 75% treated by 10% sesame dregs in protected and low root knot index area. But no good control result appears on the high root-knot index area of tomato plant. *A. niger*-Y61 broth in combination of sesame dregs with different matching, treated the soil before transplanting, and the results showed that the root-knot number was reduced from 61% to 87%, the J2 numbers were reduced from 85.33% to 87.11%, and the egg masses were also reduced over 81.47%. The assay indicated that the sesame dregs plus *A. niger*-Y61 broth is an efficient way to control the root knot nematode of *M. incognita*.

PARASITISM BY *CATENARIA VERMICOLA* BIRCHFIELD, 1950 (BLASTOCLADIOMYCOTA) IN NEMATODES OF THE GENUS *DORYLAIMUS DUJARDIN*, 1845 (NEMATODA, DORYLAIMIDA) IN A CAVE OF THE SOUTHERN IBERIAN PENINSULA [PARASITISMO POR *CATENARIA VERMICOLA* BIRCHFIELD, 1950 (BLASTOCLADIOMYCOTA) EN NEMATÓDOS DEL GÉNERO *DORYLAIMUS DUJARDIN*, 1845 (NEMATODA, DORYLAIMIDA) EN UNA CUEVA DEL SUR DE LA PENÍNSULA IBERICA]

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Fungi are saprophytic or parasitic organisms, usually associated to aquatic or wet habitats. In certain cases, some edaphic microfauna, as nematodes, living in wet soils, may be affected by the parasitic action of these organisms, which feed and reproduce on them. Depending on the fungi species, they might have different mechanisms to trap or to infect nematodes. Nematode-fungus interactions have frequently been studied, especially due to their applied interest as fungi may be used as biocontrol agents. During the study of nematode fauna of the Farallón cave (Riópar, Albacete province, Spain), infections by endoparasitic fungi were observed. Thus, among the thirteen nematode species collected from this cave, several specimens belonging to the genus *Dorylaimus* were found to be parasitized by the fungus *Catenaria vermicola*. Different life cycle stages of this parasite have been observed in the specimens examined. This study shows that the infection starts through the nematode mouth and progressively extends along its digestive tube. Final infection stage containing well developed sporangia and discharge tubes breaking through the cuticle are often observed.

BASES CIENTÍFICO TÉCNICAS PARA EL ESTABLECIMIENTO DE *POCHONIA CHLAMYDOSPORIA* VAR. *CATENULATA* EN EL MANEJO DE *MELOIDOGYNE* SPP., EN SISTEMAS DE PRODUCCIÓN PROTEGIDOS DE HORTALIZAS [TECHNICAL SCIENTIFIC BASES FOR ESTABLISHING OF *POCHONIA CHLAMYDOSPORIA* VAR. *CATENULATA* IN THE MANAGEMENT OF *MELOIDOGYNE* SPP., IN PROTECTED SYSTEMS OF VEGETABLE PRODUCTION]

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En Sistemas de Producción Protegidos de Hortalizas (SPPH), *Meloidogyne* spp., constituye una importante plaga del suelo. Dentro de las alternativas biológicas para su manejo, se encuentra el hongo parásito facultativo de huevos de nematodos *Pochonia chlamydosporia*, del cual se realizaron aislamientos en Cuba y se seleccionó la cepa IMI SD 187 de la variedad *catenulata*, de esta se obtuvo y registró un producto bionemático, denominado KamiC®. Se requiere conocer, la receptividad de suelos a esta cepa, determinar la habilidad de colonización endofítica en especies solanáceas y cucurbitáceas; así como, los efectos que causa el Cloruro de Sodio sobre la actividad parasítica del hongo y la compatibilidad de este con productos plaguicidas y biostimulantes vegetales, con el objetivo de sentar bases científicas para un manejo efectivo de *Meloidogyne* spp., en SPPH. Para análisis de receptividad de suelos, se usó la técnica soil-membrane; la colonización endofítica se determinó en segmentos radicales, desinfectados superficialmente y sembrados en medio Semiselectivo; la actividad parasítica frente a *Meloidogyne incognita* se realizó en condiciones *in vivo*, en presencia de NaCl, y se usó el método de incorporación de productos al medio de cultivo *in vitro*, para determinar la compatibilidad con la especie fúngica. Se demostró que, los suelos más receptivos son Fluvisol y Ferralítico, en relación al Pardo y representantes de la microbiota nativa y el pH, son factores determinantes para el establecimiento fúngico. El hongo, colonizó endofíticamente especies solanáceas y cucurbitáceas, en rango de 3-65%, y estimuló el desarrollo vegetal. En presencia de NaCl, mostró adecuada actividad parasítica sobre *Meloidogyne incognita*. Resultó compatible a

los productos FitoMas E, Amidor, Cipermetrina, Karate, Mitigan, Benomilo, y Zineb. Se informan resultados de efectividad en condiciones de campo. Las cualidades demostradas por este hongo, sientan bases científicas para realizar un manejo efectivo de *Meloidogyne* spp., en SPPH.

EFFECTO DE *POCHONIA CHLAMYDOSPORIA* VAR. *CATENULATA*, *GLOMUS CUBENSE*, Y HUMUS DE LOMBRIZ, EN EL CONTROL DE *MELOIDOGYNE INCOGNITA* EN TOMATE EN CULTIVO PROTEGIDO [EFFECTS OF *POCHONIA CHLAMYDOSPORIA* VAR. *CATENULATA* AND WORM CASTING ON THE CONTROL OF *MELOIDOGYNE INCOGNITA* IN TOMATO IN A PROTECTED CROP]

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Pochonia chlamydosporia var. *catenulata* es un parásito facultativo de huevos de nematodos formadores de agallas. La interacción de la cepa IMI SD 187 de *Pochonia chlamydosporia* var. *catenulata* con *Glomus cubense* y humus de lombriz fueron empleados en el cultivo de tomate (*Solanum lycopersicum* L.) híbrido HA 3108 en condición de microplot para controlar la incidencia de *Meloidogyne incognita* en cultivo protegido. Las evaluaciones se realizaron al finalizar el ciclo del cultivo a los 90 días: la masa fresca y seca de la raíz (g), masa seca de la parte aérea (g), y rendimiento (kg.planta⁻¹), determinación foliar de los contenidos de macronutrientes, el índice de agallamiento (IA), porcentaje de colonización de HMA, colonización del sustrato, raíces, ootecas y parasitismo de huevos por *P. chlamydosporia* y en combinación con los demás factores. Para determinar la influencia de los tratamientos en los parámetros evaluados los datos se sometieron a un análisis de varianza simple (ANOVA) y la comparación entre las medias se realizó mediante la prueba de rangos múltiples de Tukey ($P \leq 0,05$) a través del programa Infostat (2002); previa transformación de los datos expresados en porcentaje, según \sqrt{x} y para las UFC, $\ln(x+1)$. Los resultados alcanzados en este trabajo, representa un acercamiento a la compleja interacción planta - nematodo - micorriza

– humus de lombriz - *Pochonia*, demostrándose, el efecto sinérgico de los HMA, humus de lombriz y el agente de control biológico, en la disminución del índice de agallamiento y el número de juveniles infectivos del cultivo del tomate, híbrido HA 3108 con una mayor % de colonización de ootecas y % de parasitismo de huevos, lo que representa una menor población de *M. incognita*, que sin duda está relacionado con el aumento de los rendimientos en aquellos tratamientos donde interactúan los microorganismos y el humus de lombriz con el agente de control biológico.

USO DE KLAMIC® EN ESTRATEGIAS DE MANEJO DE MELOIDOGYNE INCOGNITA EN DIFERENTES SISTEMAS HORTICOLAS DE CUBA [USE OF KLAMIC® IN MANAGEMENT STRATEGIES OF MELOIDOGYNE INCOGNITA AT DIFFERENT VEGETABLES PRODUCTION SYSTEMS IN CUBA]

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KlamiC® es un bionematicida desarrollado en el CENSA, a base de la cepa seleccionada IMI SD 187 de *Pochonia chlamydosporia* var. *catenulata*, el cual ha mostrado ser un potencial Agente de Control Biológico de nematodos formadores de agallas del genero *Meloidogyne* spp. en cultivos horticolas. Se tuvo como objetivo evaluar en campo la efectividad técnica, compatibilidad, y estabilidad del bionematicida para su uso dentro de estrategias de manejo de nematodos. Durante varios años se desarrollaron experimentos en diferentes escenarios agrícolas de cuatro provincias del país, empleando estrategias inundativas e inoculativas combinadas con otras tácticas biológicas y agronómicas, comparadas en algunos casos con tratamientos de nematicidas químicos. Se evaluó la colonización del hongo en los suelos, la rizosferas, y parasitismo de huevos en condiciones edafoclimáticas diferentes. Los resultados demostraron la capacidad de establecimiento y persistencia del hongo nematófago en los suelos y la rizosferas de varias especies de plantas; así como, la compatibilidad con aplicaciones conjuntas de bioproductos compuestos por *Tsukamurella paurometabola* (Steinhaus), *Trichoderma asperellum*, y *Glomus mosseae*. El uso combinado de KlamiC® con plantas trampas, genotipos tolerantes y rotaciones de cultivos susceptibles, no hospedantes y pobres hospedantes a nematodos, potencia su efectividad cuando las

poblaciones de nematodos son altas. En sentido general el índice de agallamiento disminuyó a partir del segundo ciclo de los cultivos, se logra reducir el nivel poblacional de *M. incognita* en los suelos y daños en las plantas, las cuales tuvieron mayor biomasa foliar, cumplieron su ciclo vegetativo y reproductivo con rendimientos superiores a los tratamientos control. Las aplicaciones en campo de Agrocelhone® (1,3-dicloropropeno+c loropirina) influyeron negativamente sobre el desarrollo y concentraciones de *P. chlamydosporia*. Las estrategias fueron socializadas con actores involucrados en la producción. Se muestran resultados de diferentes casos de estudio.

EVALUACIÓN IN VITRO DEL EFECTO OVICIDA DE POCHONIA CHLAMYDOSPORIA VAR. CATENULATA (KLAMIC®) SOBRE FASCIOLA HEPÁTICA [IN VITRO EVALUATION OF THE OVICIDE EFFECT OF POCHONIA CHLAMYDOSPORIA VAR. CATENULATA (KLAMIC®) ON FASCIOLA HEPATICA]

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La Fasciolosis es una zoonosis causada por el trematodo *Fasciola hepatica*. Su control se hace cada día más difícil además de presentarse grandes problemas de resistencia a los productos químicos utilizados como fasciolicidas. La búsqueda de alternativas terapéuticas para su control se ha puesto a prueba con disímiles productos de origen natural y biológico con propiedades para candidatos antiparasitarios, entre ellos podemos citar el hongo *Pochonia chlamydosporia* el cual ha sido bastante estudiada su acción, la cual está basada en la formación de hifas indiferenciadas que permiten la colonización y penetración por acción mecánica y enzimática sobre el agente. El CENSA cuenta con un producto registrado como KlamiC® a partir de una cepa autóctona de *Pochonia chlamydosporia* var. *catenulata* (IMI SD 187), parásito facultativo de huevos de nematodos y el objetivo es evaluar el efecto sobre huevos de *Fasciola hepatica*. Para evaluar la acción ovicida in vitro de este hongo sobre *Fasciola hepatica*, se trabajó con huevos obtenidos mediante la oviposición in vitro de adultas recolectadas de mataderos de bovinos y se utilizó una muestra de un lote de KlamiC® para obtener la suspensión de clamidosporas del hongo.

Se realizó un bioensayo, con dos tratamientos Control negativo (1000 huevos *F. hepatica*) y Tratamiento con KlamiC® (10⁴ clamidosporas + 1000 huevos *F. hepatica*), con 10 réplicas por tratamiento. La lectura se realizó a los 3, 7, 14, y 21 días, el efecto ovicida del hongo se realizó en tres niveles de efecto: tipo 1; tipo 2; tipo 3. Se observó el efecto ovicida del hongo en los huevos de *Fasciola* llegando a la destrucción total del huevo a los 21 días. Se recomienda realizar experimentos *in vivo* para evaluar las potencialidades de *Pochonia chlamidosporia* var. *catenulata* IMI SD 187, para el control de *Fasciola hepatica* en especies animales de interés pecuario.

POTENCIALIDADES DEL NEMACID PARA EL MANEJO DE MELOIDOGYNE INCOGNITA (KOFOID Y WHITE) CHITWOOD EN HORTALIZAS [POTENTIALITIES OF NEMACID FOR MELOIDOGYNE INCOGNITA (KOFOID AND WHITE) CHITWOOD MANAGEMENT IN VEGETABLES]

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NEMACID®, constituido por los metabolitos de los efluentes de la fermentación líquida de *Lecanicillium* sp., efectivo para el control de nematodos. Este trabajo tuvo como objetivo realizar una síntesis de los principales resultados alcanzados con la aplicación del NEMACID® sobre huevos y juveniles de segundo estado (J2) de una población pura de *M. incognita* raza 2, así como su Efectividad Técnica (ET) en Casas de Cultivos Protegidos de diferentes áreas agrícolas y cultivos, con dosis de (15 Kg. ha⁻¹ para niveles de infestación por debajo de grado 2 y 30 Kg. ha⁻¹ para niveles por encima de grado 3), 4 aplicaciones con frecuencia de 7 días. Los efectos nematocidas y nematostáticos fueron demostrados *in vitro*, al causar necrosis en las ootecas e impedir la ocurrencia de la eclosión en el 100% de los huevos y la pérdida irreversible de la movilidad de los juveniles (J2). En condiciones de producción, se logró alta (ET) entre (90-95 %) contra *M. incognita*, con un óptimo desarrollo vegetativo y con una disminución del grado de infestación de 5 a grados de 0 y 1, obteniéndose rendimientos de 6 Kg.m² para el cultivo de pepino,

5Kg.m² para la remolacha y 10 Kg.m² en tomate, con incrementos de producción entre 30 y 40%. Se demuestra que NEMACID® puede utilizarse como una alternativa, dentro de un programa de manejo integrado, en la producción protegida de hortalizas.

EVALUACIÓN DE LA HABILIDAD PARASÍTICA DE PURPUREOCILLIUM LILACINUM IN MELOIDOGYNE SPP. [ASSESSMENT OF THE PARASITIC ABILITY OF PURPUREOCILLIUM LILACINUM ON MELOIDOGYNE SPP.]

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Meloidogyne spp. está ampliamente diseminado en Chavimochic (La Libertad, Perú) y la utilización de *Paecilomyces lilacinus*, para su control, se ha generalizado en nuestro medio. Observaciones de campo acreditan que la eficacia de este controlador ha disminuido, siendoya cuestionada la rentabilidad de su uso. En esta investigación se evaluó, en condiciones controladas, la habilidad parasítica de una cepa de *P. lilacinus*, cuyo uso es masivo en campo. Así, de *Paecilomyces lilacinus* (cepa Ag. Chira) se obtuvo una suspensión de conidias que fue filtrada y calibrada a tres concentraciones: 10⁴, 10⁵, y 10⁶ ufc/mL y los huevos de *Meloidogyne* spp. fueron extraídos de raíces de apio severamente infestadas. Los enfrentamientos de *P. lilacinus* con huevos de *Meloidogyne* se efectuaron en: a) placas de Petri con agar-agua, b) placas de Petri con agar-agua enmendado con 100 ppm de ampicilina, cloranfenicol, captan, y pentacloronitrobenceno, y c) terrinas con 100cc de tierra agrícola autoclavizada. Cada placa recibió 50 y cada terrina 1,000 huevos de *Meloidogyne* y 10⁴, 10⁵, y 10⁶ ufcde *P. lilacinus*/mL, según el tratamiento, los cuales fueron dispuestos en un diseño completamente al azar con 10 repeticiones. La población de J-2 de *Meloidogyne* se evaluó durante 7 días y a los 10 días en terrinas. No se encontró significación estadística entre tratamientos en agar-agar y agar enmendado, pero si cuando *P. lilacinus* se aplicó en terrinas, siendo este impacto del 47.05, 43.92, y 80.39% cuando se aplicó a 10⁴, 10⁵, y 10⁶ conidias/mL, respectivamente.

EFFECT OF *TRICHODERMA ASPERELLUM* STRAINS ON TOMATO DEVELOPMENT AND *MELOIDOGYNE INCOGNITA* POPULATION [EFECTO DE CEPAS DE *TRICHODERMA ASPERELLUM* SOBRE EL DESARROLLO DEL TOMATE Y POBLACIÓN DE *MELOIDOGYNE INCOGNITA*]

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The objective of this study was to determine the potential of strains of *Trichoderma asperellum* Lieckfeldt & Nirenberg, to control *M. incognita*, (Kofoid and White) Chitwood. Under laboratory conditions (*in vitro*), six native strains of *T. asperellum* (*Ta.25*, *Ta.1*, *Ta.90*, *Ta.78*, *Ta.79*, and *T.13*) were selected for their high antagonistic capability, production of various metabolites, and adaptation to different environmental conditions and substrates. For the *in vitro* trial, 1/10, 1/25, and 1/50 dilutions and the pure filtrate of each strain were tested, including a control treatment with sterile water. For the study under semi-controlled conditions, tomato (*Solanum lycopersicum* L.) plants were inoculated with 2.5 second instar juveniles (J) per gram of soil, and after 72 hours, 10⁷ CFU of the strain *Ta.90* were added per pot. The treatments were: healthy plants (absolute control), plants + *Ta.90*, plants + *Ta.90* + nematodes, plants + nematodes, and nematodes + *Ta.90* (without plants). The parameters evaluated after 35 days were: galling index (IA), number of eggs per root system, plant height, stem diameter, root length, number of leaves, and weight of fresh root. High mortality levels were shown by all the *T. asperellum* strain filtrates, highlighting the strain *Ta.90* with 90% mortality at 24 hours, and this strain has shown egg parasitism. In the pot trial this latter strain significantly reduced the number of eggs per female (near 100 eggs), compared with the unapplied control (220-250 eggs), and attenuated the effect of the nematode on parameters such as plant height, stem diameter, number of leaves, and root fresh weight, among others, that have shown statistic differences with untreated plants.

EFFECTOS DE *TRICHODERMA HARZIANUM* Y *AZADIRACHTA INDICA* SOBRE UNA POBLACIÓN DE *MELOIDOGYNE* SPP. EN PLÁNTULAS DE *SOLANUM LYCOPERSICUM* [EFFECT OF *TRICHODERMA HARZIANUM* AND *AZADIRACHTA INDICA* ON A POPULATION OF *MELOIDOGYNE* SPP. IN SEEDLINGS OF *SOLANUM LYCOPERSICUM*]

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Los nematodos formadores de agallas constituyen un problema para el cultivo de hortalizas como el tomate, lo que motiva la búsqueda de alternativas eficientes para su manejo. Por ello se desarrolló un experimento en áreas de investigación de la Universidad de Pinar del Río, Cuba, para evaluar el efecto de *T. harzianum* (cepa A-34) y extracto acuoso de nim sobre una población de *Meloidogyne* spp. en plántulas de tomate "PR-92". Se utilizaron 50 bolsas de nailon con capacidad de 900 g de sustrato, el cual se conformó mediante una mezcla de suelo, turba, y cascarilla de arroz, a una proporción de 70 + 20 + 10. Una vez llenadas las bolsas con sustrato fueron seleccionadas 10 al azar para el tratamiento control absoluto (sin *Meloidogyne* spp.). En las 40 restantes se inoculó 0.5 J₂-huevos.g de suelo⁻¹ de *Meloidogyne* spp, distribuyendo en las mismas los tratamientos *Trichoderma*, *nim*, *Trichoderma+nim*, y control (con *Meloidogyne* spp.). Los resultados mostraron una reducción del índice de agallamiento en 53.5%, 38.5%, y 61.5% para los tratamientos *Trichoderma*, *nim*, y su combinación, respectivamente, con diferencias significativas respecto al control 35 días después de la siembra. Se puso de manifiesto que un incremento del índice de agallamiento reduce la masa fresca y seca radical y la longitud del tallo, mientras que la reducción del mismo favorece el crecimiento de las plántulas de tomate. También se lograron incrementos significativos en la longitud y diámetro del tallo y la masa seca de las plántulas de tomate con la aplicación de *T. harzianum* y su combinación con *nim*.

INOCULANTES FUNGICOS CON ACTIVIDAD ANTAGÓNICA DE NEMATODOS EN PLANTAS DE CHILE HABANERO [FUNGI INOCULANTS WITH ANTAGONISTIC ACTIVITY ON NEMATODES IN HABANERO PEPPER PLANTS]

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En un diseño completamente al azar, se evaluaron en condiciones protegidas, especies nativas de *Trichoderma* en el control de *Meloidogyne incognita*, en plantas de chile habanero (*Capsicum chinense* Jacq.). Los tratamientos consistieron en la aplicación de cinco cepas nativas de *Trichoderma* spp. (Th02-01, Th07-05, Th09-06, Th43-13, y Th43-14), un testigo comercial (Fithán®) y un testigo control sin inoculantes microbianos. Cada tratamiento constó de 10 plantas como unidad experimental. Las plantas del cultivo se inocularon tres ocasiones con 1×10^6 esporas por mL, la primera se realizó al momento de la siembra, la segunda al trasplante, y la tercera a los ocho días posteriores a éste. Las plantas se desarrollaron en macetas de 7 kg de capacidad con suelo sin antecedentes del nematodo e inoculado para el ensayo con 2 500 huevos larvados distribuidos alrededor del cuello de la planta, al momento del trasplante. Al término del ciclo del cultivo se consideraron como estimadores de intensidad de control del nematodo: el índice de agallamiento, el número de huevos por un g de raíz licuada y el número de hembras por g de raíz teñida. El análisis de varianza sobre las variables de intensidad de control del nematodo mostraron diferencias significativas ($P \leq 0.01$). La cepa nativa de *Trichoderma* sp.(Th43-14) logró reducir 79.43% la formación de agallas, 96.70% la reducción en el número de huevos por g de raíz licuada, y 70% de disminución en el número de hembras por g de raíz teñida, en relación al testigo sin inoculantes microbianos, que mostró alto índice de infestación de nematodos. La cepa nativa de *Trichoderma* sp. (Th43-14) aislada de la rizosfera sin uso agrícola es potencialmente prometedora para reducir los daños de *M. incognita*.

PHYTONEMATODE CONTROL ACTIVITY OF SEVERAL SOLID FORMULATIONS OF THE BIOPRODUCT HEBERNEM-S WITH DIFFERENT LEVELS OF WETTABILITY [ACTIVIDAD DE CONTROL DE FITONEMATODOS DE DIVERSAS FORMULACIONES SÓLIDAS DEL BIOPRODUCTO HEBERNEM-S CON DIFERENTES NIVELES DE HUMECTABILIDAD]

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HeberNem-S (HN-S) is a registered bioproduct with proven phytonematode control activity, especially against *Meloidogyne* sp. infestations in protected crops. It is based on the bacterium *Tsukamurella paurometabola* C-924 which displays its biopesticide action, among others, by means of hydrogen sulphur, chitinase, and protease production affecting the nematode egg hatching as well as the larval survival. In the way to improve the properties of the product to facilitate its application, recent works have been carried out focused to obtain formulations with low wettability times. As result, a group of formulations containing the bacterium with different components were obtained either by spray drying or by further solid-solid mixture. The present work describes the evaluation of the effectiveness on nematode control of the best formulations selected in terms of wettability time requirements. Several experiments have been performed both in pots and in houses for protected crops. Tomato plants have been used as indicators of nematode attack. Root damages were evaluated according to the Bridge and Page reported scale and the infestation grades achieved after 35 days of interactions between plants, nematodes and different HN-S formulations were statistically compared. As controls, a batch of the product already tested against plant parasitic nematodes under field conditions and untreated plants were used. All kind of product formulations under study showed similar biological activity effects, comparable with the product batch used as positive control and at the same time with statistically significant differences regarding the damages observed in roots of the untreated control plants. These results suggest that product composition used with the aim to lower the wettability time of HN-S does not affect its activity against nematodes.

**POTENCIALIDADES DEL BIOPRODUCTO
HEBERNEM® COMO PROMOTOR
DEL CRECIMIENTO VEGETAL
[POTENTIALITIES OF BIOPRODUCT
HEBERNEM® AS VEGETABLE GROW
PROMOTING]**

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En el Centro de Ingeniería Genética y Biotecnología de Camagüey se produce el inoculante microbiano HeberNem®, cuyo principio activo es la cepa *Tsukamurella paurometabola* C-924, aislada a partir de rizosfera de plátano como antagonista de nematodos fitoparásitos; en experimentos previos se observó que además de su actividad nematicida ejercía otros efectos sobre las plantas. El objetivo del trabajo fue determinar las potencialidades de *T. paurometabola* C-924 como promotora del crecimiento vegetal. Se confirmó que la cepa posee actividad estimuladora sobre los cultivos a través de diferentes mecanismos de acción. Bajo las condiciones ensayadas produce ácido indolacético, es capaz de solubilizar fosfatos y produce amoniaco a partir de materia orgánica. Se comprobó que produce exoenzimas líticas que pueden proteger las plantas contra el ataque de patógenos. Se estudió su compatibilidad con otros microorganismos del suelo empleados como biofertilizantes (*Rhizobium leguminosarum*, *Pseudomonas fluorescens*, y *Azotobacter chroococcum*) y se observó que favorece el proceso de colonización de hongos micorrizógenos, estimulando la formación de micorrizas arbusculares. Se determinó además que estimula el desarrollo de cultivos de interés económico como: frijol, maíz, plátano, y lechuga. Se logró con su aplicación plantas de mayor altura, mayor desarrollo del sistema radical, mayor follaje, y peso seco. Este trabajo informó por primera vez para la ciencia la actividad promotora del crecimiento vegetal para la especie *T. paurometabola* y los mecanismos involucrados en este proceso. *T. paurometabola* C-924 tiene potencialidades para su empleo como biofertilizante en diferentes sistemas agrícolas.

**EVALUACIÓN TOXICOLÓGICA DEL
NEMATICIDA BIOLÓGICO HEBERNEM-S
EN MAMÍFEROS. ESTUDIOS DEL
PRIMER SEGMENTO [TOXICOLOGICAL
EVALUATION OF BIOLOGICAL
NEMATICIDE HEBERNEM-S IN MAMMALS.
ASSAYS OF THE FIRST TIER]**

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En la agricultura una de las especies más fitopatogénicas son los nemátodos. Para el control de los mismos pueden usarse nematicidas químicos o de origen biológico, siendo estos últimos más eficaces. El HeberNem-S es un bionematicida, con gran efectividad en el control de esta plaga, por lo que en el presente estudio como parte de la estimación de su seguridad, evaluamos su toxicidad en mamíferos. Utilizando cuatro de los ensayos estipulados por las guías que regulan la evaluación toxicológica de los bioplaguicidas, donde se tuvieron en consideración los efectos tóxicos y la capacidad de provocar infectividad o patogenicidad. Estos ensayos fueron: toxicidad aguda dérmica en ratas; irritabilidad/infectividad/patogenicidad dérmica en conejos; irritabilidad/infectividad/patogenicidad oftálmica en conejos; sensibilización en curieles. En estos estudios se utilizaron dosis de 1000 a 10000 veces superiores de la dosis letal media para el control del fitonemátodo en el suelo (10^6 a 10^7 unidades formadoras de colonia (ufc)/mL de la solución final del producto que se aplica al suelo). De manera general no se presentaron signos tóxicos ni alteraciones en el comportamiento de los animales, que mantuvieron actividad somatomotora, y repuestas a estímulos acorde a lo reportado para las distintas especies. En todos los casos existió un adecuado incremento del peso corporal, lo cual demuestra la inocuidad del producto. Las determinaciones de infectividad del microorganismo demostraron la inexistencia de ufc de *Tsukamurella paurometabolla* lo que demuestra que el microorganismo no fue capaz de invadir las barreras naturales del huésped para infectarlo ni provocar patogenicidad. La administración del HeberNem, no provocó signos clínicos, reacciones tóxicas, infectivas o patogénicas, irritabilidad dérmica u oftálmica ni sensibilización en los animales de experimentación.

EVALUACIÓN ECOTOXICOLÓGICA DEL HEBERNEM-S [ECOTOXICOLOGICAL EVALUATION OF HEBERNEM-S]

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Los nemátodos constituyen una de las plagas más fitopatogénicas en muchos cultivos de importancia económica. Para su control son empleados plaguicidas de origen químico y biológico, convirtiéndose estos últimos en una opción más inocua para el medio ambiente. No obstante, es indiscutible la necesidad de evaluar los posibles efectos tóxicos de estos productos sobre diferentes especies bioindicadoras de daños al ecosistema. En el presente estudio se evaluó la ecotoxicidad del bionematicida HeberNem-S en especies representativas de los ecosistemas acuático y terrestre: renacuajos de *Osteopilus septentrionalis*, alevines de *Poecilia reticulata*, lombriz de tierra *Eisenia andrei*, el insecto benéfico *Chrysopa* exterior y las plantas terrestres *Zea mays* (maíz) y *Phaseolus vulgaris* (frijol negro). Se evaluaron dosis dos y/o tres órdenes por encima de la aplicación en la agricultura (10^8 ufc/mL) y la exposición fue por inmersión, contacto e ingestión de la sustancia de ensayo. Se determinaron los efectos tóxicos en dependencia de la especie utilizada (mortalidad, alteraciones conductuales, etc) y la infectividad de este producto. No se observaron signos de toxicidad significativos en ninguno de los grupos expuestos. La evaluación de la infectividad demostró que no existió colonización bacteriana en los organismos evaluados. HeberNem-S no fue tóxico ni infectivo en las especies estudiadas bajo nuestras condiciones de ensayo.

EFFECT OF FLUENSULFONE ON THE MOBILITY, REPRODUCTION AND ROOT GALLING OF *N. ABERRANS* UNDER *IN VITRO* AND MICROPLOTS CONDITIONS [EFECTO DEL FLUENSULFONE SOBRE LA MOVILIDAD, REPRODUCCIÓN Y AGALLAMIENTO DE *NACOBBUS ABERRANS* EN CONDICIONES *IN VITRO* Y MICROPARCELAS]

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The effect of fluensulfone on juveniles (J2) of *N. aberrans in vitro* and greenhouse conditions in tomato and cucumber crops was evaluated. Within assays *in vitro*, nine concentrations of fluensulfone and one of oxamyl were assessed, and the percentage immobile nematodes at 24 hours after exposure was determined. In greenhouse bioassay, fluensulfone dosages of 1 and 2 L.ha⁻¹, oxamyl at 4 L.ha⁻¹, and one of dichloropropene + chloropicrin (DC+CP) (300 L.ha⁻¹) were evaluated under microplots of both tomato and cucumber. All treatments were distributed under a completely randomized design with three replications. At 60 days after transplanting (DAT), nematode population density, reproduction rate, invasion, galling, and five agronomic variables (growth, yield, etc.) were assessed. The fluensulfone had a significant effect ($P = 0.0001$) on the mobility of juveniles of *N. aberrans*. At 24 hours, 100% of nematodes were immobilized at a concentration of 288 ppm, with an EC50 of 96 ppm. Nematode population density, reproduction rate, and root galling of *N. aberrans* were significantly reduced by fluensulfone applications in tomato and cucumber, with values similar to those observed in those treated plants with DC+CP. Less root galling was obtained with the applications of DC+CP and fluensulfone (2 L.ha⁻¹), with a control of 98 and 91%, respectively compared to the untreated plants. The pre-transplant nematicides application had a significant effect on growth and development of tomato and cucumber plants ($P = 0.0001$). Tomato fruit yield (total weight fruits/plant) increased by 38 and 48.8% with DC+CP and fluensulfone (2 L.ha⁻¹) treatments as compared to untreated microplots.

In cucumber, fruit yield increased favorably with oxamyl applications and fluensulfone (1 L.ha⁻¹). A discussion is included considering fluensulfone use in tomato and cucumber crops affected by *N. aberrans* as good alternative of methyl bromide and already other non-fumigant nematicides.

MANEJO AGROECOLÓGICO DE NEMATODOS EN LA AGRICULTURA URBANA [AGROECOLOGICAL MANAGEMENT OF NEMATODES IN URBAN AGRICULTURE]

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Los nematodos del género *Meloidogyne* constituyen uno de los principales problemas desde el punto de vista fitosanitario que enfrenta la Agricultura Urbana en Cuba. El manejo de sus poblaciones se enfoca simultáneamente para disminuir sus daños y proteger el medio ambiente. Con este objetivo se valoró la eficacia de diferentes tratamientos en condiciones semicontroladas y en campo (cámaras de organopónicos y casas de cultivo), en áreas de agricultura urbana. Se determinó el efecto de diferentes concentraciones de tabaquina (50%, 25%, y 12,5%), cachaza (50%, 25%, y 12,5%, 8% y 4%), y *Trichoderma* (1e⁷, 1e⁸, y 1e⁹ conidios. gramo⁻¹). Los mejores resultados fueron tabaquina 12,5%, cachaza 4% y *Trichoderma* 1e⁹ conidios. gramo⁻¹. La efectividad de *Trichoderma* osciló entre 60% y 100%; con la aplicación de tabaquina se lograron reducciones de un 93,6% de la infestación y rendimientos de 30,41 Kg/m² lo que representa el 152 % de la norma nacional (20 Kg./m²) y con la adición de cachaza se obtuvieron disminuciones del nivel de infestación entre el 50 y 74,8%, con un rendimiento de 42,43 Kg./m² que representa el 212% de la norma nacional. De forma general estas opciones para el manejo agroecológico de nematodos, tuvieron un efecto positivo sobre la reducción de las poblaciones y los rendimientos en los cultivos, materializado en una reducción del grado de contaminación mayor de un 70% y un incremento de los rendimientos agrícolas que alcanzó más de un 50%.

STEINERNEMA PAPILLATUM N. SP. (RHABDITIDA: STEINERNEMATIDAE), A NEW ENTOMOPATHOGENIC NEMATODE FROM VENEZUELA [STEINERNEMA PAPILLATUM N. SP. (RHABDITIDA: STEINERNEMATIDAE), NUEVO NEMATODO ENTOMOPATÓGENO DE VENEZUELA]

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During a survey of entomopathogenic nematodes in Zulia and Falcón states (northwestern Venezuela), two nematodes from the genus *Steinernema* were isolated from soil samples. Morphological data indicate that the new species belong to the 'bicornutum-group' of *Steinernema*. For taxonomic studies, 10 *G. mellonella* were exposed to IJ, first and second generation males and females were collected. Morphological, morphometric, and molecular studies were performed. We describe these two populations and designed them as *Steinernema papillatum* n.sp. due to their prominent deirids located at the base of the basal bulb in males of both generations.

VARIABILIDAD MOLECULAR DE AISLAMIENOS VENEZOLANOS DE NEMATODOS ENTOMOPATÓGENOS Y SUS BACTERIAS SIMBIOTES [MOLECULAR VARIABILITY OF VENEZUELAN ISOLATES OF ENTOMOPATHOGENIC NEMATODES AND THEIR SYMBIOTIC BACTERIA]

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Los nematodos entomopatógenos representan efectivos agentes de control biológico de insectos

plagas. El objetivo del trabajo fue conocer la variabilidad molecular en una colección de aislamientos venezolanos de nematodos entomopatógenos y sus bacterias simbiotas a través de la aplicación de los marcadores RAPD. A partir de trabajos de caracterización previos, realizados con la colección de nematodos entomopatógenos del laboratorio de Nematología de la Unidad de Protección Vegetal del Instituto Nacional de Investigaciones Agrícolas de Venezuela (INIA), se analizaron 12 aislamientos provenientes de 5 estados de Venezuela. Los ADN de los nematodos y bacterias fueron extraídos siguiendo una modificación del método de Dellaporta descrita por Salazar *et al.* Los RAPD se desarrollaron con los cebadores de los juegos de reactivos OPA y OPB. Se determinaron las huellas genéticas de los aislados del nematodo y de la bacteria simbiota. El total de bandas amplificadas para nematodos fue de 493 y para bacterias 496, con 99,18, y 100% de polimorfismo, respectivamente. El agrupamiento obtenido para los nematodos estuvo relacionado con la altitud sobre el nivel del mar a la que fueron colectados, mientras que para la bacteria se encontró tendencia al agrupamiento según el tipo de suelo del que provenían.

SELECCIÓN DE AISLAMIENTOS DE NEMATÓDOS ENTOMOPATÓGENOS NATIVOS PARA EL CONTROL DE CABRITO DEL CIRUELO (*AEGORHINUS NODIPENNIS*) EN HUERTOS CHILENOS DE ARÁNDANOS [SELECTION OF ISOLATES OF NATIVE ENTOMOPATHOGENIC NEMATODES TO CONTROL PLUM WEEVIL (*AEGORHINUS NODIPENNIS*) ON CHILEAN BLUEBERRY ORCHARDS]

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En Chile, el cultivo de berries, especialmente arándanos, es afectado por el curculiónido cabrito del ciruelo, *Aegorhinus nodipennis*. Su control es difícil por los hábitos crípticos de la larva, que queda fuera del alcance de insecticidas convencionales. Como alternativa de control, están los nemátodos entomopatógenos (NEP), que ya han sido probados para control de *Aegorhinus superciliosus*, con bastante éxito. El objetivo de esta investigación es determinar si alguno de los aislamientos presentes en la colección de nematodos entomopatógenos

nativos del Banco de Recursos Microbianos de INIA, es capaz de producir parasitismo, y patogenicidad contra *A. nodipennis*. Para esto se realizó screening con 21 cepas, colocando una larva de tercer o cuarto estadio en contenedores con mezcla húmeda de aserrín y suelo pasteurizado en proporción 1:1, inoculando con una concentración de 100 dauers, manteniéndolos en cámara de incubación a $15 \pm 2^\circ\text{C}$, registrando mortalidad diaria durante 10 días. Para la cepa que alcanzó mayor mortalidad se determinó comportamiento, mediante ensayo de movilidad, utilizando tubos de pvc de 50*5 cm, los cuales se llenaron con mezcla húmeda de suelo: arena; 3:1. Cada 5 cm se colocaron larvas de *Galleria mellonella* en contenedores porosos y una suspensión de nematodo en uno de los extremos. Los tubos se mantuvieron en posición vertical y horizontal durante 5 días a 18°C . De los aislamientos evaluados, todos mostraron tener algún grado de patogenicidad sobre las larvas, 5 de ellas superaron el 60% de mortalidad, destacándose QU-N61 de la especie *Steinernema unicornium*, con 87%. Este aislamiento alcanzó una movilidad en sentido horizontal de 4,25 cm y vertical de 3,5 cm diarios, lo que demuestra su capacidad de perseguidor. Estos datos son alentadores y abren una posibilidad para la utilización de este nematodo en el control de esta plaga.

AMMONIA AND UREA KINETIC IN *GALLERIA MELLONELLA* LARVAE INFECTED BY *HETERORHABDITIS* SPP. [CINETICA DE LA UREA Y EL AMONIO EN LARVAS DE *GALLERIA MELLONELLA* INFECTADAS CON *HETERORHABDITIS* SPP.]

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The life cycle of entomopathogenic nematodes occurs inside an insect cadaver. Once, infective Juveniles (IJ) have entered in the host, their symbiotic bacteria kill the insect and degrade its tissue for nematodes to feed. The nematodes start feeding and develop into adults which lay eggs. Thousands of new nematodes then hatch and grow in the cadaver. Due to the nematodes defecation, the levels of ammonia and urea increase and the food resource lost some of its quality. In this work, the levels of those 2 compounds produced by 2 *Heterorhabditis* species were monitored along the infection process. *Heterorhabditis amazonensis* and *H. indica* were inoculated into *Galleria mellonella*

larvae and their urea and ammonia accumulated in the cadavers were measured every 24 h for 11 d using colorimetric techniques. At the same time, *G. mellonella* larvae were infected with the symbiotic bacteria (positive control) and freeze-killed *G. mellonella* larvae were used as negative control. In another experiments, *G. mellonella* were infected with the same nematodes and after 7 d boric acid, sterile water, urea and ammonia were injected into the cadavers and the emergence of the new IJ was monitored. The results suggest that urea and ammonia levels increase as the life cycle of the nematodes occurs, the emergence of new IJ varies depending on the urea and ammonia concentration and is retarded when boric acid is applied (because inactivate urea and ammonia).

DOSIS DE *HETERORHABDITIS INDICA* POINAT CEPA P₂M EN EL MANEJO DE LA BROCA DEL CAFÉ (*HYPOTHENEMUS HAMPEI* FERRARI) [DOSE OF *HETERORHABDITIS INDICA* POINAR STRAIN P₂M IN THE MANAGEMENT OF COFFEE BERRY BORER (*HYPOTHENEMUS HAMPEI* FERRARI)]

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Con el objetivo de evaluar el efecto de *Heterorhaditis indica* cepa P₂M en el manejo de la broca del café (*Hypothenemus hampei* Ferrari) se realizó la investigación en condiciones controladas en el Laboratorio Provincial de Sanidad Vegetal de Sancti Spíritus con cinco dosis del patógeno (20, 45, 75, 100, y 200 iji/adultos). Se diseñaron dos experimentos: variante 1 con aplicaciones directa sobre los adultos del insecto en palcas Petri con siete repeticiones y variante 2 con la aplicación al suelo en bolsas de nylon con granos de la variedad Robusta con presencia de larvas y adultos del insecto. Se determinó la susceptibilidad de los adultos del insecto a las 24, 48, y 72 horas y efecto de las dosis sobre el porcentaje de mortalidad de las larvas y adultos. Como resultado relevante de la variante 1 se destaca que los adultos tuvieron una susceptibilidad por encima del 50% en 24 horas con las dosis superiores al 20 iji/adultos y en la variante 2 lo valores de mortalidad de las larvas fueron superiores a los adultos del insecto a las 72 horas. El nematodo constituye una alternativa viable en

control de la broca con valores de mortalidad de 50% en 24 horas.

EVALUATION OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS AMAZONENSIS* IN THE BIOLOGICAL CONTROL OF *MACONELLYCOCCUS HIRSUTUS* (HEMIPTERA: PSEUDOCOCCIDAE) AND *CAPULINIA* SP. (HEMIPTERA: ERIOCOCCIDAE) [EVALUACION DEL NEMATODO ENTOMOPATOGENO *HETERORHABDITIS AMAZONENSIS* EN EL CONTROL BIOLÓGICO DE *MACONELLYCOCCUS HIRSUTUS* Y *CAPULINIA* SP. (HEMIPTERA)]

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In the last 10 yr two hemipterans have increased their presence in Venezuela. *Maconellycooccus hirsutus* arrived in the country late in the 90s, affecting more than 200 species (especially ornamentals and fruits); besides, *Capulinia* sp. has become one of the most important insect pests of guava orchards in the last decade. The biological control potential of *Heterorhaditis amazonensis* was evaluated in both insect pests in laboratory conditions. Virulence, pathogenicity and efficiency were evaluated in different instars. In both cases, the females adults of resulted *M. hirsutus* and *Capulinia* sp. more susceptible to *H. amazonensis* (60 and 70% respectively), compared to the 2nd and 3rd instars (19 and 16%). The first instar of both insects is known as the “walker instar” and is the colonizer stage of both species but there was no infection at all by the nematodes. In semi field experiments, *M. hirsutus* was grown in Christmas palms (*Adonidia merrillii*) and *Capulinia* sp. in guava plants (*Psidium guajava*). The results indicated a reduction of the population between 30 to 50% after 15 days post application. *H. amazonensis* could be considered as a possible biological control agent for both *M. hirsutus* and *Capulinia* sp. but to be effective, the nematodes should be applied to control adult females (breaking the life cycle of the insects).

EMPLEO DE *HETERORHABDITIS INDICA* POINAR PARA EL CONTROL DE *TYOPHORUS NIGRITUS* (F.) (COLEOPTERA: CRHYSOMELIDAE) EN PLANTACIONES DE BONIATO [USED OF *HETERORHABDITIS INDICA* POINAR FOR *TYOPHORUS NIGRITUS* (F.) (COLEOPTERA: CRHYSOMELIDAE) CONTROL IN SWET POTATO PLANTATION]

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A partir del período 2002-2003 se observaron afectaciones a la calidad comercial del boniato ocasionadas por *T. nigrinus*. Por tal motivo se hizo necesario el estudio de alternativas de control biológico que contribuyeran a disminuir las poblaciones del insecto. El trabajo se realizó en el laboratorio de Entomología del Instituto de Investigaciones de Viandas Tropicales (INIVIT), donde se estudiaron concentraciones de ij3 sobre larvas y pupas del insecto. En campo se establecieron las mejores concentraciones del nemátodo, resultantes del ensayo anterior (797 760 ij3·parcela⁻¹ y 2 659 200 ij3·parcela⁻¹), en dos momentos de aplicación (plantación y a los 40 días), y en dos épocas del año. Las larvas y pupas de *T. nigrinus* mostraron susceptibilidad a *H. indica* en condiciones de laboratorio. Se observó que el porcentaje de mortalidad para ambos estados de desarrollo aumentó según se incrementó la concentración de ij3·suelo⁻¹. Diez días después a la inoculación, los nematodos (ij3) emergieron del interior de los cadáveres, y se observaron sobre la superficie de los mismos. En época de primavera el menor grado de ataque y las menores pérdidas al rendimiento se lograron con la aplicación de los ij3 a la mayor concentración en el momento de la plantación y posteriormente a los 40 días, con diferencias significativas con los restantes tratamientos. En época de frío se encontró que independientemente del tratamiento aplicado, las pérdidas fueron menores en comparación con los resultados obtenidos en primavera, de igual forma los tratamientos con mejores resultados se correspondieron con aquellos en que se aplicaron las mayores concentraciones de nemátodos siendo nulo el grado de ataque en cada uno de estos. Estos resultados confirmaron la efectividad de los nemátodos entomopatógenos en altas

concentraciones para el control de plagas del suelo.

CONTROL DE *CYCLOCEPHALA* SP. Y *ANOMALA* SP. CON *HETERORHABDITIS* SP. EN ARÁNDANO [CONTROL OF *CYCLOCEPHALA* SP. AND *ANOMALA* SP. USING *HETERORHABDITIS* SP. ON BLUEBERRIES]

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En el cultivo de arándano la presencia de *Cyclocephala* sp. y *Anomala* sp. (gallinas ciegas), cuyos larvas se alimentan de raicillas, representa un grave riesgo, puesno existe una medida de control que llegue hasta donde se ubica la plaga y que no deje residuos. Por ello, utilizando un diseño completamente aleatorizado con cuatro repeticiones, se evaluaron dos poblaciones nativas de *Hetherorhabditis* sp. aisladas de maní y palto en Virú y Chao, La Libertad-Perú, en su patogenicidad, desplazamiento, sobrevivencia y cantidad de cadáveres de *G. mellonella* parasitados con *Hetherorhabditis* por planta. Se registró una mortalidad de 98%, el desplazamiento horizontal fue de 65 cm y 40 cm de profundidad. La mayor población de juveniles infectivos en el suelo se alcanzó a los 60 días descendiendo a partir de los 120 días. El tratamiento de 3 cadáveres por planta redujo de 14 a 3 larvas de gallinas ciegas por metro cuadrado.

PRIMER REPORTE DE UNA POBLACIÓN DE *BELONOLAIMUS* EN LA COSTA DE SINALOA, MÉXICO [FIRST REPORT OF *BELONOLAIMUS* IN THE COAST OF SINALOA, MEXICO]

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Entre las especies identificadas en un estudio para conocer la diversidad nematológica, a lo largo del río Sinaloa, en el norte del estado, se clasifico una población perteneciente al genero *Belonolaimus*.

El análisis comparativo de los caracteres diagnósticos morfológicos, muestran una similitud con *B. longicaudatus*, aunque existen algunas diferencias que podrían indicar la existencia de una nueva especie. Análisis de secuencias moleculares preliminares (segmentos d2 d3 de la subunidad ribosomal 28S) relacionan esta población con *B. longicaudatus*; sin embargo, datos moleculares de otros estudios indican que poblaciones de diferentes áreas geográficas de *B. longicaudatus* no son monofiléticas, Gozel *et al.* (2006). Por lo que se sugiere realizar análisis adicionales para un diagnóstico molecular más confiable. *Belonolaimus longicaudatus*, es considerado como un nematodo fitoparásito de considerable importancia debido a su capacidad de causar daños extensos en diversos cultivos agrícolas de importancia económica, Crow and Han (2005). Aunque en Sinaloa se encuentra asociado a plantas naturales en la región costera es necesario determinar su patogenicidad considerando la posible amenaza que representa para esta región agrícola. Por el momento, esta población se asocia con el complejo de *B. longicaudatus*. Datos morfológicos, ecológicos y moleculares adicionales, permitirán confirmar la posición específica de esta población. En este reporte se ilustran caracteres morfológicos diagnósticos y se describe y discute la importancia de la presencia única de *B. longicaudatus* en esta región de la costa del pacífico en Sinaloa, México. Crow, W. T., and H. Han, 2005. Sting nematode. The Plant Health Instructor. DOI: 10.1094/PHI-I-2005-1208-01. Gozel, U., B. J. Adams, K. B. Nguyen, R. N. Inserra, R. M. Giblin-Davis, and L. W. Duncan. 2006. A phylogeny of *Belonolaimus* populations in Florida inferred from DNA sequences. *Nematologica* 36:155 -171.

POSIBLES AGENTES DE CONTROL DE MELOIDOGYNE SP. PARA EL MANEJO AGRONÓMICO DE SACHA INCHI EN COLOMBIA [POSSIBLE MELOIDOGYNE SP. CONTROL AGENTS FOR AGRONOMIC MANAGEMENT OF SACHA INCHI IN COLOMBIA]

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El cultivo agrícola de *Plukenetia volubilis* o sachá inchi es altamente promisorio por sus altos contenidos de aceites esenciales en sus almendras,

los cuales son aptos para el consumo humano. No obstante su productividad se ve afectada por el ataque de nematodos del género *Meloidogyne* spp. Con el fin de obtener posibles estrategias de manejo integral de esta plaga en sachá inchi, se evaluó la eficacia *in vitro* de diferentes productos químicos, biológicos, y extractos vegetales registrados en Colombia frente a diferentes estadios del nematodo. Adicionalmente se evaluaron extractos bacterianos de cepas bacterianas pertenecientes al género *Bacillus* spp. y *Pseudomonas* spp. Para las evaluaciones se determinó el porcentaje de eclosión de huevos y la movilidad de juveniles para cada producto comercial u extracto bacteriano con relación a un control no tratado, teniendo 4 réplicas por tratamiento y dos repeticiones en tiempos independientes. Los productos químicos con ingredientes activos Cadusafos y Thiodicarb, disminuyeron significativamente el porcentaje de eclosión de los huevos en un 96 y 54% respecto al control; mientras que los productos a base de extractos de quillay, ajo y ruda, lo disminuyeron en un 53, 34, y 25 con relación al control respectivamente. Para los productos biológicos aquel que tiene combinación de diferentes agentes biocontroladores (*Trichoderma* sp. y *Paecilomyces* –reclasificado *Purpureocillium*-sp.), tuvo mayor efecto en la disminución de la eclosión de huevos. La evaluación de los extractos bacterianos así como el efecto de los productos comerciales sobre los estadios juveniles han arrojado datos preliminares promisorios para el control de *Meloidogyne* sp., evaluaciones que siguen siendo llevadas a cabo. Estos resultados sugieren que dentro de los productos registrados en Colombia y los extractos bacterianos pertenecientes al cepario del grupo CIBIOP, se pueden tener potenciales agentes controladores que ayuden a la elaboración de un plan estratégico de manejo del cultivo de sachá inchi en la región del Bajo Cauca Antioqueño. Colombia