

OPENING LECTURE

WHY NOT AGRICULTURE? PERCEPTIONS, REALITY, AND THE FUTURE (¿Y porqué no la Agricultura? Apreciaciones, realidad y futuro).

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With the continuing increase in world population, agriculture will be called upon more than ever to provide food and fiber to sustain the human population. Agricultural production has been augmented by a number of so-called “revolutions” including the Green Revolution of the 1940’s through 1970’s. New, yet controversial revolutions in agriculture include genetically modified organisms and RNAi technology. Even with technological breakthroughs, fewer people live in rural settings and are involved in agriculture. In developed nations generally <2% of the population is involved in agriculture. In addition, agricultural training and the number of students in graduate degree programs in agriculture continue to decline. Why are today’s students not entering the agricultural sciences? What is about agriculture and this generation of students that makes agriculture unattractive? Unfortunately agriculture has been associated with poverty and subsistence living and not growth and affluence. Agriculture is “old”, so it must necessarily be old-fashioned and out of date. The current generation of students has been taught to think of themselves as special and do not relate to “hard work”. To increase the number of students entering agricultural sciences, we must undertake several approaches. First, we need to consider re-naming some of our traditional sciences, for example, entomology has been re-labeled, Insect Science. Second, we must convince this generation of the critical importance of agriculture as a moral imperative. Third, we must teach agriculture the way today’s students learn: in groups, using multi-media, and with constant input. And finally, we must expressly teach our students that agriculture leads to prosperity.

ORAL SESSIONS

SESSION 1 NEW APPROACHES-SERGEI

A NEW ROOT-LESION NEMATODE DAMAGING *MUSA* SPP. IN GHANA (Un nuevo nematodo lesionador de raíz que afecta a *Musa* spp. en Ghana).

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Recent studies on root-lesion nematode populations recovered from damaged plantains (*Musa* spp.) in Ghana, West Africa has resulted in their description as a new species *Pratylenchus speijeri* n. sp. The nematode is morphologically similar to *Pratylenchus coffeae*, but with distinct molecular dissimilarities (D2-D3 of 28S rRNA gene). In order to assess the cryptic status of these Ghana populations within the *P. coffeae* species complex, their morphological and molecular features were compared with those of *P. coffeae* and other related amphimictic *Pratylenchus* spp. having undivided face with two lip annuli. The morphological analysis did not result in an unambiguous separation of these Ghana populations from *P. coffeae*. Only a few and often variable morphological features such as larger stylet knobs and a more frequently indented tail terminus separated these populations from *P. coffeae*. The sequence and phylogenetic analyses of D2-D3 of 28S rDNA and its rRNA genes of 60 *P. coffeae* species complex populations from different sources including an unidentified *Pratylenchus* sp. C1 from *Colocasia esculenta* roots in Japan, generated majority consensus BI trees with three major clades: i) populations from *Musa* spp. roots, in Ghana; ii) unidentified Japanese *Pratylenchus* sp. C1; iii) *P. coffeae* from different hosts and distant geographical areas. Results confirmed the Ghana populations as a new taxon and further indicated that *Pratylenchus* sp. C1, which is morphologically similar to *P. coffeae*, should also be considered a new species, even though PCR primers previously designed for *P. coffeae* by Uehara *et al.* (1998) showed high specificity for this nematode. PCR-RFLP analysis of the D2-D3 of the 28S rRNA gene revealed that the enzyme Cfr421 is diagnostic for Ghana populations, while the enzyme Bpu1102I clearly separated the species in the three major clades listed above. The study also established a PCR with a species-specific primer for the Ghana populations and PCR with a group of specific primers for the *P. coffeae* species complex. The results of this study will help facilitate breeding programs for the improvement of *Musa* germplasm and demonstrate the need for accurate diagnostics of *Pratylenchus* spp. damaging *Musa* spp. in West Africa, and other crops beyond.

DEVELOPMENT OF SPECIES-SPECIFIC SCAR MARKERS FOR IDENTIFICATION OF TWO ROOT-KNOT NEMATODES OF COFFEE, *MELOIDOGYNE ARABICIDA* AND *M. IZALCOENSIS* (Desarrollo de marcadores SCAR específicos para la identificación de nematodos asociados a café *Meloidogyne arabicida* y *M. izalcoensis*).

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In this study we developed a polymerase chain reaction (PCR)-based assay for specific detection of two root-knot nematodes *Meloidogyne arabicida* and *M. izalcoensis* (Tylenchida: Meloidogynidae), major

pathogens of coffee crops in Central America. Random amplified polymorphic DNA (RAPD) fragments specific for these two species were converted into sequence characterized amplified region (SCAR marker). PCR amplification using the SCAR primers produced a specific fragment of expected size (e.g., 300 base pairs and 670 bp) in *M. arabicida* and *M. izardoensis*, respectively, in contrast with the other coffee-associated *Meloidogyne* spp. tested. SCAR primers also allowed successful amplification of DNA from single infective juveniles, males and females. In addition, these primers were able to unambiguously detect the target species in field samples, in different isolates of a same species or when used in multiplex PCR reactions containing mixtures of species. These results demonstrate the effectiveness of these SCAR markers and their multiplex use with those previously developed for *M. exigua*, *M. incognita*, *M. paranaensis* and *M. enterolobii* may further contribute to specific diagnosis of the major root-knot nematodes infecting coffee in the Americas.

IDENTIFICATION OF *MELOIDOGYNE* SPECIES A DIFFICULT PROCESS TASK (Identificación de especies de *Meloidogyne*, un reto muy difícil).

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Root-knot nematodes are considered one of the most important plant pathogen in temperate and tropical agriculture. The nematode attack a variety of vascular plants including vegetables, fruits, fiber and ornamental plants, causing huge economic losses due to the direct effect in crop yield and product quality. It is likely that an agricultural country like Costa Rica, the yield losses caused by root-knot nematodes are higher because of above-ground symptoms are usually overlooked. In addition, the globalization and open markets for trade plants, probably new species of *Meloidogyne* may have likely been introduced. The reliable identification of *Meloidogyne* spp. is an essential step for avoiding nematode dissemination and for designing effective crop management strategies. Up today more than one hundred species of *Meloidogyne* have been described; however, an accuracy method to identify all of them has not been developed yet. The best approach for root-knot identification is to integrate morphological traits, isoenzyme and DNA analyses. On the other hand, undescribed species as well as lacking of information, sometimes represents a bottle-neck in the identification process. During two years we have conducted an intensive sampling in many agroecosystems of Costa Rica, the main goal of the project funded by CRUSA-CSIC was to identify *Meloidogyne* spp. infecting ornamental, fruit and food crops by means of esterase phenotypes, mitochondrial and ribosomal DNA markers, and morphology (mainly female perineal patterns). The results, after analysing more than 110 crops samples, showed that integrative methods were very useful to identify mayor *Meloidogyne* species. We have identified *M. incognita*, *M. javanica*, *M. arenaria*, *M. exigua*, *M. hapla*, *M. salasi*, *M. enterolobii* and most probably *M. hispanica* as a new report for Costa Rica. Molecular analysis such as PCR-RFLP using the following primers sets (C2F3 & 1108, Me1F & Me1R) and sequencing rDNA (D2A & D3B, 18S & 26S), were unable to identified other *Meloidogyne* species. Amplification products obtained with restriction enzymes (*AluI*, *DraI*, *HinfI*), were not coincident with any species previously described in the literature. These observations strongly suggest that countries like Costa Rica may have a higher biodiversity than previously estimated, and it is possible that new species of *Meloidogyne* remain undescribed.

NEMATODE BIODIVERSITY IN THE NORTH OF SINALOA, MEXICO (Biodiversidad de nematodos en la región norte del estado de Sinaloa, México).

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Mexico is considered as one of most bio-diverse countries in the world. The state of Sinaloa, in Northern Mexico is located in the transition limits of the Neotropic bio-geographical region of the American Continent. This may explain the vast biodiversity present in natural ecosystems with a wide diversity of natural and endemic vegetation. Sinaloa is also characterized by an intensive agricultural production, including agro-ecosystems where a variety of crops are produced for local and internal consumption and for exportation. With the support of UCMEXUS, CONACyT and the National Polytechnic Institute, nematode surveys were conducted during the years of 2010-2012, in several locations of the north of the state, including natural and agro ecosystems. A total of 150 soil samples from the rhizosphere of endemic and natural plants were taken along the Sinaloa River from the coast line to the slopes of the Sierra Madre Occidental. An adjacent transect on the agricultural fields was selected. Soils samples from the rhizosphere of annual and perennial crops were collected. The results showed a wide-ranging diversity of different groups of soil inhabitant nematodes, including plant parasitic nematodes, free-living nematodes (Dorylaimidae, Cephalobidae, Rhabditidae, Plectidae, etc.), marine nematodes and entomopathogenic nematodes (Diplogasteridae and Rhabditidae). Among the different groups of nematode populations identified, several are new reports for the state including (*Meloidogyne hispanica*, *Rotylenchulus reniformis*) and some may be undescribed species, including a probably new species of *Belonolaimus*, as well as other Cephalobidae and Entomopathogenic nematodes. Results of the surveys will be presented and discussed.

SESSION 2 MAJOR NEMATODES-ERWIN

ADVANCED STUDIES FOR EGYPTIAN COTTON RESISTANCE TO ROOT-KNOT NEMATODE (Estudios avanzados para la resistencia del algodón en Egipto a nematodos agalladores).

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Growth response to cotton cultivars to Root Knot Nematode, *M. incognita* race 3 was reported, two cultivars (Giza 88 and Menoufia 5) were rated as highly susceptible, while (Giza 70, Giza 83, Giza 85, Giza 89 and Giza 90) were rated as susceptible, and (Giza 45, Giza 80, Giza86 and Giza 91) were rated as resistant. The susceptible cotton cultivars were significantly reduced in growth criteria, such as number of leaves, length of both roots and shoots, fresh and dry weight. On molecular level RAPD PCR was used with ten different primers. Bands generated by each primer varied among genotypes with a range of 1 to 12 bands (250 ~to 3000bp). These bands was used to distinguished these six genotypes, primer OP-A16 gave specific markers with MW of 260 bp for G1 (Giza 70), 950bp; 700bp and 300 bp for G3 (Giza90) and 250bp for G4 (Giza80).It was noticed that OP-A16 fragment 450bp related to RKN *M. incognita* resistant and it was absence in the susceptible cultivars while fragment 1000bp found to be related to RKN susceptibility and absence in the resistance cultivars. Using the obtained data in a dendrogram shows that the two resistance genotype Giza 86 and Giza 71 had 67.6% similarity while the two susceptible genotype Giza 70

and Giza 90 showed 60.6% similarity. These Molecular Genetic studies of the six cultivars and their diversity are efficient tools for identification of different resistance and susceptible varieties of cotton plant.

DIAGNÓSTICO Y ADOPCIÓN DE PRÁCTICAS AGROECOLÓGICAS PARA EL MANEJO DE NEMATODOS DE LAS AGALLAS EN FINCAS DE LA AGRICULTURA SUBURBANA (Agroecological methods and adoption to manage root knot nematodes in suburban farms in Cuba).

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El programa de agricultura suburbana en Cuba concibe la producción agroecológica de cultivos alrededor de las ciudades de Cuba, mediante una red de fincas de diferentes organizaciones productoras. Las especies de *Meloidogyne*, son los principales patógenos en la mayoría de estos cultivos y dada la masividad de los nuevos productores, surgió la demanda de que estos conocieran los principios prácticos, para realizar un diagnóstico primario del grado de infestación de los suelos, que integran este programa. El trabajo se ejecutó de forma piloto en 32 fincas con varias formas de producción en la agricultura suburbana de la Habana. Mediante métodos participativos se agrupaban de 15-20 agricultores en una de las fincas para su capacitación sobre la metodología de muestreo, siembra y atención a las plantas indicadoras seleccionadas. Posteriormente realizaron el diagnóstico de cada campo de manera colectiva, empleando una escala de 0 a 5 grados. Para sistematizar las prácticas agroecológicas se realizó una propuesta base, las que se evaluaron en ejercicio realizado en equipos durante dos sesiones de trabajo. Los agricultores determinaron los niveles de infestación en sus suelos, se corroboraron en el laboratorio por especialistas y se identificó a *M. incognita*. Se detectaron distintos niveles, independiente de la forma de producción analizada y fue posible valorar como positivo el aprendizaje del método. El ejercicio sobre sistematización de prácticas agroecológicas para el manejo de estos nematodos, concluyó con la identificación de varias prácticas factibles de emplear, entre estas la biodesinfestación con rotación de cultivos e incorporación de materia orgánica al suelo, aplicación de *Trichoderma viride* en plántulas y suelo, uso de material de siembra sano y medidas preventivas. Se demostró que los métodos participativos facilitaron la realización de este diagnóstico primario por los agricultores en sus campos, a la vez que permitieron sistematizar resultados científicos y experiencias, para adoptar prácticas agroecológicas, de acuerdo a las características de sus fincas. La experiencia se encuentra en fase de generalización en el país.

EL NEMATODO DE QUISTE DE LA SOJA EN PUERTO RICO (The soybean cyst nematode in Puerto Rico).

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El nematodo de quiste de la soja (SCN, siglas en inglés), *Heterodera glycines*, es reconocido como una de las peores plagas de este cultivo en los Estados Unidos ocasionando pérdidas considerables a los agricultores. En Puerto Rico, este nematodo fue descubierto por primera vez en 1998 atacando plantas de soja en el área norte de la isla. Desde ese momento, el nematodo se ha convertido en una preocupación para las compañías productoras de semillas dedicadas al mejoramiento genético de la soja, localizadas principalmente en el área sur. El propósito de este estudio fue evaluar la población de SCN en las plantaciones de soja y determinar la densidad poblacional y la distribución del nematodo y por ende el nivel de amenaza a las empresas de semillas. Mil quinientas muestras fueron recolectadas en las áreas sembradas con soja. Los quistes se

extrajeran mediante una técnica modificada del método de Fenwick. Nuestros resultados mostraron altas poblaciones del nematodo en la misma zona donde se informó antes. De acuerdo a las densidades detectadas, la población de este nematodo ha subsistido las prácticas de control y las condiciones ambientales en la región y continúa causando daños a la soja. El número de quistes en algunas muestras superaron los 800 quistes por 300 cc de suelo (3 quistes por gramo). Al parecer, el nematodo se ha restringido a la zona donde los postes de alumbrado están permanentemente establecidos dado que aún no se han detectado en las plantaciones adyacentes. Las muestras provenientes de la zona sur de la isla han resultado negativas a la presencia de quistes. En la actualidad, hemos encontrado quistes del nematodo colonizados por bacterias. Su potencial como un agente supresor de nematodos se encuentra bajo estudio.

EXPERIENCIAS EN EL MANEJO INTEGRADO DE *MELOIDOGYNE* SPP. EN CULTIVOS INDUSTRIALES DE LA COSTA NORTE DEL PERÚ (Experiences in the integrated management of *Meloidogyne* spp. in industrial crops of the northern coast of Peru).

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El nematodo nodulador, *Meloidogyne* spp., es una de las mayores amenazas fitosanitarias de los cultivos en la costa norte del Perú. Durante este estudio se comparó el impacto del humus de lombriz (5ton/ha) y de Hunter (Extracto de plantas *Quercus falcata*, *Opuntia lindheimeri*, *Rhus aromatica* y *Rizhporia mangle*) (2L/ha) sobre *Meloidogyne* spp. en el cultivar UC-157-F1 de espárrago, en condiciones de campo. El Hunter y el Humus redujeron significativamente el número de juveniles hasta los 105 días de la aplicación. Los juveniles en el testigo fluctuaron entre 82 y 381 individuos por 100 cc de suelo. La aplicación de *Arthrobotrys dactyloides* (10^4 y 10^5 conidias/mL) en plantines del cultivar UC-157-F1 de espárrago, redujo significativamente el índice de nodulación producido por *Meloidogyne* spp. En otro experimento la aplicación de *Paecilomyces lilacinus* en combinación con diferentes proporciones de materia orgánica, al pie de plantas de vid de la var. Sultanina afectadas por *Meloidogyne* sp., se obtuvo que los tratamientos con *P. lilacinus* más 1.1 kg de materia orgánica aplicados una vez (dos meses después de la poda) y dos veces (dos y tres meses después de la poda), disminuyeron significativamente el número de huevos de *Meloidogyne* spp. *In vitro* se evaluó el impacto de productos orgánicos y biológicos sobre huevos de *Meloidogyne* spp., utilizando medio a base de agar-agua al 2%, mezclado con el producto en ensayo [Hunter 0.2%, QL Agri (Extracto de quillay -saponinas+polifenoles+ sales+azucars-) 0.05%, Nemathor (Quinoleina Fenólica), 0.75%, Ditera (*Myrothecium verrucaria* fermentado inactivo con metabolitos) 1.0%, NemaStop (*Bacillus subtilis*) 2.5%]. Sobre el agar enmendado se esparcieron uniformemente huevos del nematodo y se monitoreó durante 144 horas la eclosión. Nemathor 20L y NemaStop inhibieron totalmente la eclosión variando ésta de 6.1. a 17.7 % en el testigo.

HISTORIC CONSUMPTION OF CHEMICAL NEMATICIDES (1990-2010) AND ALTERNATIVES DEVELOPMENT ON MAIN CROPS IN COSTA RICA (Consumo histórico de nematocidas (1990-2010) y el desarrollo de alternativas en los principales cultivos de Costa Rica).

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Pesticide use in agriculture has been promoted as an important tool for development since decades, despite in many cases being an extremely hazardous technology for human health and the environment. At the same time that a number of industrialized countries are undertaking significant steps to reduce pesticide consumption, developing countries are becoming a more important marketing target. One of the main presumptions state for pesticides is that they are indispensable and, if properly handled, will not cause unreasonable harm. Collateral damage of pesticides has been attributed primarily to insufficient regulation and the ignorance of pesticide users in developing countries, and it has been assumed that strengthening of regulations and education to users would lead to an acceptable level of pesticide safety. In Costa Rica, on the last two decades, chemical nematocid importation increased even with the agricultural area still remains the same. Many imported chemical nematocides are of special environmental and human health concerns, including 1,3-D, terbufos, methyl bromide, among others. Recent studies among farmers show increased knowledge and risk awareness without substantial changes in pesticide handling. Continuous poisonings and environment contamination were reported to the surveillance systems. Alternative approaches are needed, and successful examples exist like Integrated Pest Management and biological control programs. Failures in implementation such as lack of extension and research services are avoidable. It is time to make profound changes in international and national agricultural policies and steer towards sustainable agriculture, helping the better feed of the people and environment protection.

INTERACCIÓN *FUSARIUM-MELOIDOGYNE* EN *PHYSALIS PERUVIANA* L. (*Fusarium-Meloidogyne* interaction in *Physalis peruviana* L.)

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La uchuva (*Physalis peruviana* L.) se cultiva en Colombia entre 1500 y 3000 metros de altitud; es un cultivo generador de empleo e importante fuente de divisas. *Fusarium oxysporum* se considera, actualmente en el país, el primer limitante fitosanitario del cultivo. Adicionalmente *Meloidogyne hapla*, única especie del nematodo nodulador, reportada en varias zonas productoras, reduce en 30 por ciento los parámetros de desarrollo y crecimiento de las plantas. Con el propósito de conocer si existe interacción entre ambos patógenos, se estableció un ensayo bajo un diseño completamente al azar, con 10 repeticiones y los siguientes tratamientos: *F. oxysporum*, *Meloidogyne*, *Meloidogyne* y *Fusarium* aplicados simultáneamente y testigo no inoculado. La cantidad de inóculo aplicado fue 5000 huevos de *Meloidogyne* spp. y 1×10^6 conidios de *F. oxysporum*. El inóculo de *Meloidogyne* contenía una mezcla de 40% de *M. hapla* y el resto, una especie aún no identificada. Pasados 150 días, se evaluó para la planta, diferentes parámetros de desarrollo y presencia de síntomas de la enfermedad; para el nematodo, el índice de nódulos y masas de huevos, y población de juveniles en suelo y raíces. Los parámetros de desarrollo aéreo de la planta, se redujeron porcentualmente respecto al testigo entre 20-25, 70-88 y 87-90 para los tratamientos *Meloidogyne*, *F. oxysporum* e interacción *Meloidogyne-Fusarium*, respectivamente. Las reducciones en peso fresco y seco de raíces mostraron tendencias similares. La población de juveniles (J2) del nematodo y los índices de nodulación y masas se redujeron entre 50 y 98 por ciento, en presencia de la interacción.

MANEJO DE *MELOIDOGYNE INCOGNITA* EN EL CULTIVO DE UVA DE MESA, EN UNA ZONA TROPICAL DE PERU (Management of *Meloidogyne incognita* on table grapes growing in a tropical región in Perú).

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En el Perú la uva de mesa se ha constituido en el producto agrícola más demandado para exportación. En la última campaña Octubre 2011/2012 se exportaron 131,910 TM de uva peruana a Estados Unidos (21.9%), Países Bajos (16.9%), Hong Kong (12.0%), Rusia (11.4%), China (5.9%) y otros (32.0%). La mayor incidencia de daños por *Meloidogyne incognita* se presenta en suelos de textura principalmente arenosa con irrigación en el Departamento de Piura en la Región Norte del país, cercano a la línea ecuatorial con clima tropical cálido durante todo el año, con temperaturas que pueden alcanzar hasta 40°C y una mínima de 15°C. El Objetivo de este estudio fue buscar una alternativa rápida de recuperación armónica de parrales altamente infestados por esta plaga, en un periodo importante de Pos Cosecha de la Uva Red Globe de 3 años con Patrón Harmony, y en Poda de Formación, con fines de reducir los altos niveles de infestación, en un periodo de pleno verano (Diciembre-Abril). Se aplicó el nematicida de acción por contacto Rugby (Cadusaphos) 40 Kg/ha, con aplicaciones complementarias de nematicida sistémico Neoxamilo (Oxamil) 5 L/ha aplicado por goteo en cinco ocasiones a intervalos de 15 días haciendo un total de 25 L/ha; también se aplicó quitina (Quitosan) 40 L/ha con fosfitos, aminoácidos y materia orgánica líquida. Durante 4 meses se evaluaron las poblaciones juveniles de *Meloidogyne* y la recuperación de raíces absorbentes sanas y nuevos brotamientos de planta. Se lograron controles satisfactorios de los altos niveles de infestación y un rebrotamiento en el follaje para mejoramiento progresivo de los futuros cargadores para las próximas podas de producción.

***NACOBBUS ABERRANS*: DISTRIBUCIÓN, DAÑO EN CULTIVOS Y CONTROL EN LA REGIÓN DEL BAJÍO, MÉXICO (Nacobbus aberrans: Distribution, crop damages and control in the Bajío region, Mexico).**

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Nacobbus aberrans es un fitonematodo importante de plantas, objeto de regulaciones carentenarias en varios países del mundo, debido al daño que causa en varios hospedantes por su alta capacidad reproductiva que lo convierten en un patógeno importante y difícil de manejar. El primer reporte de *N. aberrans* en México fue hecho por Brunner (1967) en plantas de chile, denominándolo *N. serendipiticus* (= *N. aberrans*). Actualmente esta presente en los estados de Coahuila, Distrito Federal, Guanajuato, Hidalgo, Estado de México, Michoacán, Morelos, Puebla, Oaxaca, San Luis Potosí, Tlaxcala y Zacatecas, asociado principalmente a los cultivos de chile, tomate y frijol. Sin embargo, poca información sobre el daño en cultivos, biología y ecología se ha publicado a pesar de su presencia en zonas agrícolas diversas de México donde han reportado su presencia. En 1973 y 1992 fue reportado en el cultivo de Jitomate en Guanajuato y Michoacán, respectivamente. Por lo anterior y a la enorme importancia agrícola de Guanajuato y Michoacán en la producción de cultivos hortícolas de alto valor comercial, se efectuó una prospección espacial de *N. aberrans* en la región, encontrándolo en tres municipios de Guanajuato asociados a los cultivos de zanahoria, tomate y chile, y tres municipios de Michoacán en los cultivos de jitomate y pepino, con daños superiores al 60% de agallamiento. Se elaboraron mapas de riesgo y se asoció la presencia del patógeno con algunas características edafoclimáticas. En cuanto al manejo de este fitonematodo, se evaluó un nematicida químico no fumigante del grupo de los fluoroalkenilos, como una alternativa al uso de bromuro de metilo en los sistemas agrícolas.

NEMATODOS ASOCIADOS CON *RUBUS GLAUCUS* BENTH. EN COLOMBIA (Nematodes associated to *Rubus glaucus* Benth. in Colombia).

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En Colombia se siembran 14,458 hectáreas con mora (*Rubus glaucus* Benth.), destinada principalmente para jugos y dulces. Este es un cultivo de economía campesina en el trópico alto, que genera 3.7 millones de empleos por año, de los cuales cerca del 60 por ciento corresponden a mano de obra familiar. Los nematodos son uno de los limitantes sanitarios del cultivo. En el año 2000 se reportaron dos y seis géneros de nematodos fitoparásitos en raíces y suelo de mora, respectivamente, en cuatro municipios del Oriente Antioqueño. Con el objetivo de conocer la situación nematológica actual, el muestreo se extendió a 16 municipios de seis departamentos (Antioquia, Caldas, Cundinamarca, Huila, Santander y Tolima) y se realizaron pruebas de parasitismo en suelo proveniente de las localidades. En raíces se encontró *Aphelenchus*, *Aphelenchoides*, *Criconea*, *Helicotylenchus*, *Hemicylichophora*, *Pratylenchus*, *Pratylenchus* y Trichodoridae en poblaciones promedio de 8, 4, 1, 9, 51, 3, 12 y 10 nematodos por 1g de raíces; en suelo de la rizosfera se encontró *Aphelenchus*, *Aphelenchoides*, *Criconea*, *Helicotylenchus*, *Hemicylichophora*, *Meloidogyne*, *Pratylenchus*, *Pratylenchus*, *Scutellonema*, *Xiphinema* y Trichodoridae en poblaciones máximas de 54, 54, 121, 145, 240, 150, 441, 156, 20, 193 y 167 nematodos por 100 cc de suelo. Las máximas poblaciones de nematodos fitoparásitos se encontraron en Antioquia, excepto, para los géneros *Hemicylichophora*, *Trichodorus* y *Helicotylenchus* que mostraron poblaciones superiores en los departamentos de Caldas, Cundinamarca y Huila, respectivamente. En pruebas de parasitismo se encontraron los más altos índices de reproducción para *Hemicylichophora* seguido de *Pratylenchus* y Trichodoridae.

PINE WILT DISEASE AND RED RING DISEASE OF COCONUT: OPPORTUNITIES AND PERSPECTIVES IN THE STUDY OF TWO NEMATODES *BURSAPHELENCHUS XYLOPHILUS* AND *B. COCOPHILUS* (La enfermedad del marchitamiento de los pinos y el anillo rojo del cocotero: Oportunidades y perspectivas en el estudio de dos nematodos, *Bursaphelenchus xylophilus* and *B. cocophilus*).

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The xylophagous nematodes *Bursaphelenchus cocophilus* and *B. xylophilus* (Nematoda: Parasitaphelenchidae), are causative agents of two serious diseases, “red ring disease” (RRD) with great importance to coconut and certain oil palm species in Central and South America, and in “pine wilt disease” (PWD), which has wreaked havoc in forest areas in the Far East and now more recently in Europe (Portugal and Spain). PWD has not yet been detected in Brasil or any other country in Central or South America, but there is great risk of introduction due to worldwide movement of wood and wood products through major sea ports. Recently, for example, wood shipments originating from Brasil, infested with *B. xylophilus*, have been intercepted in China. Due to the taxonomical proximity between these two nematodes (the only two plant pathogenic species within the genus *Bursaphelenchus*, containing nearly 100 species), the similarity of life cycles (with the insect vectors *Rhynchophorus palmarum* for *B. cocophilus* and *Monochamus* spp. for *B. xylophilus*) and the likely genomic and proteomic similarities, a comparative study between these two nematodes may provide important insights into understanding RRD, in particular through the genome sequencing of *B. cocophilus*, and comparison with the recently sequenced genome of *B. xylophilus*. Knowledge on specific pathogenicity genes, involved in the RRD process, combined with past and present knowledge, should be helpful in designing new and effective control methods for this major tropical disease of coconut and palm.

PLANT-PARASITIC NEMATODES AFFECTING TEMPERATE FRUIT TREES IN CHILE (Nematodos fitoparásitos que afectan a frutales de clima templado en Chile).

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Some of the major temperate fruit trees grown in Chile are apples, pears, grapes, olives, walnuts, avocados, pomegranates and citrus. Most of these fruit trees, with the exception of avocados, have populations of plant-parasitic nematodes that affect their development and productivity and force producers to take management measures to reduce the population levels. Several species of plant-parasitic nematodes of the genera *Xiphinema*, *Pratylenchus*, *Meloidogyne* and the species *Tylenchulus semipenetrans* are very common in orchards and in many opportunities also associated with secondary pathological problems such as the “black foot” on vines or the gall crown on cherry trees and vines. The management of these problems has been done mainly using chemical control measures, such as soil fumigants or nematicides organophosphates and carbamates, being the use of resistant rootstocks very limited and focused mainly on grapevines and citrus plantations. In the rest of the fruit trees, rootstocks used are sensitive to the majority of the species found in the orchards. The tighter control over the nursery is one of the permanent and more effective measurements preventing further spread of PPN, while the introduction of some nematicides based on microorganisms has not been a frequent and efficient tool used by farmers.

THE ROLE OF *BURSAPHELENCHUS XYLOPHILUS* ASSOCIATED BACTERIA IN PINE WILT DISEASE (Rol de *Bursaphelenchus xylophilus* asociado a una bacteria en la enfermedad del marchitamiento de los pinos).

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Pine wilt disease (PWD) is a complex disease integrating three major factors: the causal agent, the pinewood nematode *Bursaphelenchus xylophilus*; the insect-vector *Monochamus* spp.; and the host pine tree, *Pinus* sp. Since the early 80's, the notion that another pathogenic agent involved, namely bacteria, may play a role in PWD has been gaining traction, however the role of bacteria in PWD is still unknown. The present work suggests the intriguing possibility that some *B. xylophilus*-associated bacteria may play a significant role in the development of this disease. This is inferred as a consequence of: (i) the phenotypic characterization, of a collection of 35 isolates of *B. xylophilus*-associated bacteria, in different tests broadly used to test plant pathogenic and plant growth promoting bacteria, and (ii) greenhouse experiments that infer pathogenicity of these bacteria in maritime pine, *Pinus pinaster*. The results illustrate the presence of a heterogeneous microbial community associated with *B. xylophilus* and the traits exhibited by at least some of these bacteria appear to be related to PWD symptoms. The inoculation of four specific *B. xylophilus*-associated bacteria in *P. pinaster* seedlings resulted in the development of some PWD symptoms suggesting that these bacteria likely play an active role with *B. xylophilus* in PWD.

SESSION 3 PHYSIOL PLANT DEFENSE-YUJI

INDUCED RESISTANCE TO NEMATODES IN COTTON: A NOVEL CONTRIBUTION TO NEMATODE MANAGEMENT (Resistencia inducida a los nematodos en algodón: una aportación novedosa al manejo de nematodos).

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Induced resistance against plant-parasitic nematodes has not previously been shown in cotton. We tested whether co-infection of cotton by *Meloidogyne incognita* and *Rotylenchulus reniformis* affected population levels of either nematode compared to single-species infection. In split-root experiments, single plants were inoculated with 1) *R. reniformis* only, 2) *M. incognita* only, 3) both species, or 4) a non-treated control. Half of the root system of 6-week-old plants was inoculated with *R. reniformis* on day 0 and the other half with *M. incognita* on day 0 or day 14 depending on the experiment. Experiments were conducted on DP 0935 B2RF (susceptible to both nematodes) and LONREN-1 (resistant to *R. reniformis*). Root galling and total nematode levels (vermiform and eggs) were determined from each half of the root system 8 weeks after inoculation with challenge inoculum. When *M. incognita* was introduced 14 days after *R. reniformis*, reductions in galling (36 % on DP 0935 and 33 % on LONREN-1) and *M. incognita* levels (34 % on DP 0935 and 45 % on LONREN-1) occurred compared to plants inoculated only with *M. incognita*; differences were not seen with simultaneous inoculation. Activities of enzymes (P-peroxidase, G-peroxidase, and catalase) involved in systemic acquired resistance (SAR) were greater ($P \leq 0.05$) in leaves of *M. incognita*, *R. reniformis*, and acibenzolar-S-methyl (Actigard) treated plants than in the control. These studies documented for the first time that infection of cotton by a nematode can induce SAR against another nematode species. Preliminary studies have found that potassium silicate can reduce *M. incognita* levels on cotton; ongoing work will determine if this is an SAR response.

SESSION 4 NOVEL SOURCES-PHIL

GENETIC MAPPING AND BREEDING FOR ROOT-KNOT NEMATODE RESISTANCE USING HIGH-THROUGHPUT SNP GENOTYPING (Mapeo genético y mejoramiento para la resistencia a nematodos agalladores usando PNS genotipificados de alto rendimiento).

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Conventional breeding for root-knot nematode (*Meloidogyne* spp.) resistance is time-consuming and expensive due to screening progenies for resistance response. Screens for resistance rely on assays of nematode reproduction or indices of root-gall symptoms and are often variable even under controlled conditions. Development and application of large-scale molecular marker genotyping

approaches for indirect selection of resistance loci can expedite the breeding process and the private sector is applying these systems in major crops such as corn, cotton, and soybean. In public programs these innovations are often unavailable. We developed a comprehensive molecular breeding (MB) system for cowpea (*Vigna unguiculata*), an important grain legume and fodder crop, and targeted resistance to *M. incognita* and *M. javanica* and the associated vascular wilt fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum*. An Illumina 1536-SNP GoldenGate genotyping platform was developed with genome-wide EST-derived SNP markers and this was converted to a more flexible KBioscience KASPar system. The genotyping assay was applied to recombinant inbred line (RIL) populations to construct individual and consensus genetic maps incorporating 1107 markers. RIL populations segregating for nematode and wilt resistance were screened in field and growth-pouch assays, followed by QTL mapping to locate resistance loci. The genome-wide SNP marker coverage enables targeted resistance loci to be combined with favorable growth, yield and other stress tolerance traits. Large numbers of individuals can be genotyped simultaneously at hundreds of loci in marker-assisted backcrossing and recurrent selection breeding strategies.

GENOME ANALYSIS OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* AND OTHER PATHOGENS CLUSTERED ON CHROMOSOME 11 IN COTTON (Análisis genómico de la resistencia a *Meloidogyne incognita* y otros patógenos agrupados en el cromosoma 11 en algodón).

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In tetraploid cotton, chromosome 11 and its homoeologous chromosome 21 have been found to carry many resistance (R) or pathogen-induced R genes underlying quantitative trait loci (QTLs) involved in resistance to root-knot nematode (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*), Fusarium wilt, Verticillium wilt, and black root rot resistance. These resistance genes operate either singly or in combination with other resistance QTL which map to other parts of the cotton genome. Commonly, combinations of QTL contributed by resistant and susceptible parents confer very high resistance levels in segregating progenies. Genetic and QTL mapping of nematode and pathogen resistance trait determinants, coupled with physical alignment of BACs into chromosomal maps are being employed to analyze this cotton genome region. Two cotton (*Gossypium hirsutum*) BAC libraries are being used for genetic and physical mapping of these two chromosomes. BAC-end derived SSR markers which mapped to chromosomes 11 and 21 were used with the root-knot nematode susceptible cv. Acala Maxxa library to identify 26 BAC clones, which were sequenced and the sequences annotated. The root-knot nematode resistant N901 (cv. Acala NemX) BAC library was screened with SSR markers associated with determinants of root-knot nematode resistance which mapped on the two chromosomes, and 48 N901 BAC clones were identified and sequenced. The assembled BAC sequences from both libraries were annotated with a focus on resistance and defense gene and related classes. Annotation also was made of all the other markers which mapped to these chromosome regions. Sequence information and genetic mapping are providing an enhanced genetic and physical framework of the resistance gene-rich region. This will aid resistance gene discovery and whole genome assembly efforts, as well as breeding for resistance.

KIP-RELATED PROTEINS CONTROL THE DEVELOPMENT OF FEEDING SITES INDUCED BY ROOT-KNOT NEMATODES (Proteínas relacionadas al KIP para controlar el desarrollo del sitio de alimentación inducido por nematodos agalladores).

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Root-knot nematodes (RKN) are considered as one of the most specialized and harmful plant-parasitic nematodes. The capacity of manipulating the host cell cycle machinery is a fundamental process for the successful reproduction of these plant pathogens. Understanding the molecular mechanism how RKN manipulate the host cell cycle can bring new opportunities to exploit diverse and novel forms of resistance. In plants, the regulation of the cell cycle is driven by the activation of cyclin-dependent kinases (CDKs) through multiple transcriptional and post-translational mechanisms that control their activity. Their binding to CDK inhibitors provides one level of regulation of these CDKs complexes. In this scenario, plant Kip-Related Proteins (KRP inhibitors) are regulators of the CDKs, and modulating KRP activity can be envisaged to block nematode feeding site development. Here we investigate the influence of this gene family during plant-nematode interaction and our data reveal that two KRP members control gall development in *Arabidopsis*. Given that alterations in concentration levels of CDK inhibitors can induce cell cycle transitions, our results show that by intensifying CDK inhibition through over expression of some members of the KRP family dramatically affected nematode feeding site development and compromised the nematode's life cycle. A promising manner to control nematode propagation could be achieved by modulating cell cycle regulators in host crop species.

RESISTANCE OF IRRIGATED-RICE CULTIVARS TO THE ROOT-LESION NEMATODE *PRATYLENCHUS ZEA* (Resistencia de variedades de arroz bajo riego al nematodo lesionador *Pratylenchus zae*).

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Considering the economic importance of the rice production in Southern Brazil, the reaction of eight commercial irrigated rice genotypes, currently cultivated in the Rio Grande do Sul state, were evaluated for resistance to *Pratylenchus zae*. Seedlings of the different cultivars maintained in pots with sterilized soil, were inoculated with 800 *P. zae*/plant. The experiment was carried out under greenhouse conditions in a completely randomized design with six replications and sorghum '5067' plants were used as control. Ninety days after inoculation, the number of nematodes in the roots and soil of each replication was determined in order to calculate the nematode reproduction factor (RF=final population/initial population) in the different genetic materials. 'BR-IRGA 417', 'BR-IRGA 422CL', 'BR-IRGA 420' and 'PUITA SL' rice cultivars were susceptible (FR>1,00) to *P. zae* while 'BRS QUERÊNCIA', 'BRS7 TAIM', 'BRS PELOTAS' and 'BR-IRGA 410' were considered resistant to the nematode (FR<1,00) as compared to the control (FR=71,32).

RESPUESTA DE ACCESIONES DE PAPA SILVESTRE A *MELOIDOGYNE JAVANICA* (Response of wild potato accessions to root-knot nematode *Meloidogyne javanica*).

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Problemas de Meloidogynosis son observados en diferentes cultivos de importancia económica. En el cultivo de papa la especie *M. javanica* es considerada la más patogénica en diferentes variedades cultivadas en el mundo. Especies silvestres del género *Solanum* aun son consideradas como una reserva natural en relación a su diversidad genética, que pueden ser utilizadas para resistencia a plagas. El presente trabajo tuvo como objetivo evaluar la resistencia de 24 accesiones del Banco de Germoplasma de papa da Embrapa, incluyendo especies silvestres, a *M. javanica*. Plantas individuales de papa mantenidas en macetas con suelo esterilizado, fueron inoculadas con 5 000 huevos + J2 de *M. javanica*, en invernadero. Después de 55 días, se evaluó el número de agallas y huevos en las raíces de cada planta, verificándose el factor de reproducción (FR= pob. final/pob. inicial) y el grado de resistencia, en relación con el testigo (papa cv. BRS Ana y tomate 'Santa Cruz'). De las diferentes accesiones de papa en estudio, 22 fueron susceptibles (61-8, 55-5, SCH-68, 46-10, 68-8, 513, 55-7, 546, 676, 56-8, 525, *S. calvescens*, 51-9, 664, 63-2, 543, 545, NYL-235, 45-4, 511, 499, 44-7) y dos fueron resistentes (675-*S. tuberosum* x *S. berthaultii* y 68-16- *S. chacoense*) (FR<1,00) a *M. javanica*.

SESSION 5 ENTOMOPATOGENOS

EXPERIMENTAL INFECTION OF Aedes Aegypti MOSQUITO LARVAE WITH NEMATODES ENTOMOPATHOGENIC (*STEINERNEMA CARPOCAPSAE*) FOR BIOLOGICAL CONTROL IN YUCATAN MEXICO (Infección experimental de larvas del mosquito *Aedes aegypti* con nematodos (*Steinernema carpocapsae*) para control biológico en Yucatán México).

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Dengue is currently the most important arbovirosis in the world, affecting over 120 countries of the world, in tropical and sub-tropical areas, with 2,500 million people in tin risk. Due to global warming, it is expected that *Aedes aegypti* will be able to survive at higher latitudes, increasing the number of people in risk. Therefore, it is crucial to develop control strategies of the mosquito preventing environmental impact. Nowadays a significant development opportunity is the incorporation of entomopathogenic nematodes in the programs of integrated pest management. In laboratory tests, *Steinernema carpocapsae* infects more than 250 species of insects and has potential as a biological control tool. When the nematode comes into contact with host insect larvae, penetrates them by their natural orifices (mouth, anus, spiracles) and finally the body cavity releasing the bacteria *Xenorhabdus nematophila* in there. The bacteria has toxin known as Txp40. This toxin has been shown to be active against a variety of insect species growing rapidly, causing septicemia and death of the larvae. The objective of this paper was to determine the concentration of nematodes producing the highest percentage of mortality of *A. aegypti* larvae under normal environmental conditions of water for human consumption in Yucatan (temperature 25.9- 31.5°C, 6.9 - 8.7 mg/L; pH 7.20 - 8.10). The experimental design was a 96 h static bioassay and consisted on exposing 10 L2 larvae per experimental unit (400 ml flask and five replicates per treatment) to four nematode concentrations (500,000, 1,000,000, 1, 250,000 and 1,500,000/ml plus controls). The bioassay was undertaken at room temperature

and basic physicochemical parameters (O₂, pH, temperature, conductivity). The best treatment was that of 500,000/ml produced the best results with 65% of mortality with respect to controls after 72 h (ONE-WAY ANOVA; F5.72, 0.05; p< 0.04). We conclude that *S. carpocapsae* is a promising biological control tool for Yucatán, but it is still necessary to determine its performance at higher volume scales.

LIFE CYCLE OF ENTOMOPATHOGENIC NEMATODES ON MICRO-HOSTS (Ciclo biológico de nematodos entomopatógenos en micro-hospedantes).

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The enormous amounts of insects (both individuals and species) that inhabit the soil, make these potential hosts to entomopathogenic nematodes practically infinite, but still, the absence of nematodes in many soils could not be explained. One of the aspects about these organisms which remains unknown is if they have the ability to complete their life cycle and produce progeny into small insects (<5 mm); to assess this question, we evaluated four *Steinernema* species: *S. carpocapsae*, *S. riobrave*, *S. feltiae* and *S. glaseri* on *Tribolium castaneum*, *D. melanogaster*, *S. rubrocinctus* and *F. schultzei*. Infective juveniles (IJ) of every species were tested in Petri dishes with the chosen hosts and dissections were performed at 4 days to determine the number of nematodes capable of invading the insect and their sex ratio, and another set was dissected after 7 days to determine the offspring production. The results indicated that the genus *Steinernema* nematodes were capable of completing their life cycle in hosts with a size about 5 mm, however, *S. glaseri* could only produce a very limited number of progeny. When host were shorter, (about 2mm) the nematodes could not complete their cycle, showing low infection rates without progeny, only *S. riobrave* was capable to produce some offspring via *Endotokia matricida* in few thrips. The sex ratio of the invading nematode was skewed towards males, demonstrating that they were the first sex performing colonization. This could indicate that the size of the nematode in relation to the size of the host can affect the nematode life cycle and that the initial number of nematodes available to achieve effective infection is greater than the number of offspring produced, resulting in negative population growth.

MOSQUITO CONTROL WITH MERMITHID NEMATODES (Control de mosquitos con nematodos mermitidos).

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Los nematodos mermithidos parásitos de mosquitos constituyen una alternativa para reducir las poblaciones de mosquitos en comunidades endémicas de malaria. En la "Planta de producción masiva de nematodos *Romanomermis iyengari*", normalmente se usan 500 bandejas de plástico de 47 x 35 x 12cm, para infestar 1000 larvas de mosquito *Culex quinquefasciatus* (especie hospedante) en cada una de ellas, con este sistema se generan mensualmente un mínimo de 360 cultivos de nematodos en los que se producen 150 millones de nematodos infectivos (juveniles 2, J2) para su aplicación en criaderos naturales de larvas de mosquitos. Previo a la aplicación se realizaron mapas de las comunidades para ubicar todos los criaderos naturales, también se determinó la densidad larvaria pre-tratamiento (densidad de larvas de mosquito antes de los tratamientos). La aplicación de nematodos se realizó en todos los criaderos naturales de larvas de mosquitos, los cuales variaron entre 80-8240m². Tres días después se tomaron datos de porcentajes de infestación (PINF) e intensidad de infestación (IINF). Siete días después de la aplicación se tomaron datos de densidad larvaria pos-tratamiento. La aplicación de 3000 nematodos/m² causó PINF de 46-100%, y la reducción poblacional de larvas de mosquito varió de 79-88%. En 60% de los criaderos los nematodos reciclaron biológicamente y persistieron, por lo tanto la aplicación de nematodos controló las poblaciones de larvas *An. pseudopunctipennis*.

SUSCEPTIBILITY OF LARVAE OF *SPODOPTERA FRUGIPERDA* AND *HELICOVERPA GELOTOPOEON* TO THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA DIAPREPESI* UNDER LABORATORY CONDITIONS (Susceptibilidad de larvas de *Spodoptera frugiperda* y *Helicoverpa gelotopoeon* al nematodo entomopatógeno *Steinernema diaprepesi* en condiciones de laboratorio).

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Spodoptera frugiperda and *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) are polyphagous species that cause severe losses to different crops. Susceptibility of last-instar larvae of both insects to infective juveniles (IJ) of a *Steinernema diaprepesi* isolate from the locality of Santa Rosa de Calchines (province of Santa Fe, Argentina) was evaluated under laboratory conditions. The insects were individually placed in Petri dishes (35 mm diameter) and the following IJ doses were applied: 0 (control), 50, and 100 (n=15); dishes were maintained at 25°C. Mortality (expressed as percentage) was evaluated every 24 h for 6 days. Four days after the insect death, the cadaver was put on a White trap; emerging IJ were extracted during 10 days and further counted. At the end of the experiment, mortality of *S. frugiperda* was 93% (at a 50 IJ dose) and 100% (at a 100 IJ dose), whereas in *H. gelotopoeon*, mortality was 87% (50 IJ) and 93% (100 IJ). No mortality was registered in the untreated insects. IJ production was significantly different between doses for *S. frugiperda* (11329 at 50 IJ vs. 27150 at 100 IJ); however, it was not significant for *H. gelotopoeon* (19830 at 50 IJ vs. 26361 at 100 IJ). No differences were observed between species subjected to the same IJ dose. These results showed the susceptibility of both lepidopterans to the isolate of *S. diaprepesi*.

SESSION 6 NOVEL STRATEGIES RODRIGO

ADVANCES IN NEMATODE MANAGEMENT USING *IN VITRO* *PASTEURIA* SPP. (Avances en el manejo de nematodos utilizando *Pasteuria* spp. en condiciones *in vitro*).

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Innovations within the last decade have allowed for the practical and economical *in vitro* production of *Pasteuria* spp. spores for the biological control of plant parasitic nematodes. A product utilizing *Pasteuria* spp. known to parasitize *Belonolaimus longicaudatus* has U.S. EPA registration and has been commercially available for use on turfgrass in the U.S. since January 2010. Furthermore, EPA has allowed an exemption from the requirement of a tolerance for residues when applied pre-harvest, supporting the use and safety of *Pasteuria*-based products in food production. Root-knot nematodes, *Meloidogyne* spp., continue to be a major pest in food production globally. Field and greenhouse studies conducted in 2011 using *Meloidogyne* spp.-specific *Pasteuria* have demonstrated significant pesticidal efficacy. Continuing greenhouse and field studies conducted in 2012 confirm results from 2011 using *Pasteuria* applied to cucumber and tomato crops. Further development and registration of *Pasteuria*-based products will offer growers novel biological products for *Meloidogyne* spp. control.

INDUCED SYSTEMIC RESISTANCE (ISR) TO CONTROL *ROTYLENCHULUS RENIFORMIS* IN PINEAPPLE (Resistencia sistémica inducida (RSI) para controlar *Rotylenchulus reniformis* en piña).

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Pineapple infestations by the reniform nematode *Rotylenchulus reniformis* cannot be managed anymore in the French West Indies. The new European regulations forbid the use of nematicides therefore we looked for a possible ISR contribution to nematode biocontrol in pineapple. In greenhouse trials, methyljasmonate (10-4M) or water (control) treatments were applied over the root system of pineapple planted either in 1L single pot or in twin-pots (split-root system). In the single pot trial, a monospecific inoculation of 5,000 juveniles of *R. reniformis* has been made and population increase and the fecundity were monitored after 2 months. In the second experiment 25,000 juveniles were inoculated in one side of the twin-pots. On the other side, enzymatic indicators of stress (LOX, SOD, PAL, and CHI) were measured at 0, 1, 4, 10, and 14 hours after treatments on non-inoculated plants. The ISR 'priming' effect was measured with the same indicators 10 days after the inoculation of treated plants. In the first trial, the fecundity was strongly reduced by the methyljasmonate (50 to 70%, $p=0.003$). In the split-root trial, the data support the evidence of an ISR set up by the plants as we observed a systemic and transient stress response of the plant to the methyljasmonate. We also observed a 'priming' effect on the plant defense against the nematodes characterized by a significant increase of the enzymatic indicators. These results support the idea of a possible ISR contribution to a *R. reniformis* biocontrol.

OVERVIEW OF FLUENSULFONE, A NEW NEMATICIDE FROM THE FLUOROALKENYL GROUP (Revisión del fluensulfone, un nuevo nematocida del grupo fluoroalkenil).

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Fluensulfone, a fluoroalkenyl with favourable toxicity and ecotoxicity profile, been possesses distinct nematocidal activity compared with those of currently available nematicides. In-vitro studies revealed that the nematocidal activity of fluensulfone was irreversible against second-stage juveniles of *M. javanica* in contrast to fenamiphos or cadusafos. *Meloidogyne javanica* juveniles exposed to fluensulfone lost their infectivity, even after rinsing in water while still active. Another remarkable feature of fluensulfone is its systemic activity via foliar spray. A single spray of peppers with a fluensulfone solution at 3.0 g/L prior to inoculation with *M. incognita* reduced the galling index by 80% and the number of nematode eggs by 73 to 82% of controls. This systemic activity was much higher than that of oxamyl at the same concentration; however, like oxamyl and fenamiphos, foliar spray or soil drenching with fluensulfone did not kill *M. incognita* within pepper roots. Repeated soil application of fluensulfone to sandy alkaline soil did not result in enhanced degradation unlike fenamiphos or cadusafos. Repeated applications of fenamiphos or cadusafos did not lower the nematocidal activity of a subsequent application of fluensulfone. Results from laboratory and performance in field trials indicate that this new nematicide, currently under registration process in several countries, is very promising and will be an important part of nematode control strategies in the near future.

SITE-SPECIFIC MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* IN COTTON (Manejo localizado de *Rotylenchulus reniformis* en Algodón).

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Site specific management of *Rotylenchulus reniformis* utilizing management zones delineated by soil attributes is an emerging practice in cotton production. Two trials to determine differential effects of soil attributes used for management zone delineation on nematicide efficacy were conducted from 2009–2011. A heterogeneous 26 hectare field was delineated into zones using apparent soil electrical conductivity, elevation, and initial nematode populations. Nematicides were applied in 6 row strips and replicated 5 times within zones and randomized between zones. The nematicides 1, 3-dichloropropene, aldicarb, and the abamectin seed treatment exhibited the largest yield increases in the zones where EC values and seasonal soil moisture were the lowest. The foliar nematicide oxamyl exhibited efficacy in the management zone where moisture was not limiting. In the homogeneous 15 hectare field, no zones were indicated thus the nematicides were applied across the field as large strips in a RCDB with 5 replications. The nematicides aldicarb, oxamyl, and the seed treatment thiodicarb were evaluated alone and in combinations with an untreated control for efficacy to *R. reniformis*. Although no significant increases in cotton yield were observed compared to the untreated control, all nematicides numerically increased seed cotton yield in at least one of three seasons with one exception. Results suggest that specific nematicide selection in homogeneous soils is of much less importance for management of *R. reniformis* than in the heterogeneous soils. In heterogeneous soils, yield increases by the nematicides were the largest in areas of lower EC, elevation, and seasonal moisture. The knowledge of how these nematicides perform across management zones will allow producers to make a more informed decision when choosing nematicides for site specific management of *Rotylenchulus reniformis*.

SUGARCANE BLACKSTRAP MOLASSES AND BIOGLYCERIN FROM BIODIESEL PRODUCTION AS ORGANIC AMENDMENTS FOR NEMATODE CONTROL (Melaza de caña de azúcar y bioglicerina de la producción de biodiesel como mejoradores orgánicos para el control de nematodos).

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The pesticidal efficacy of formulations of sugarcane black strap molasses [BSM] and bioglycerin [BG] from biodiesel production was studied in greenhouse experiments. Formulations contained N, P, and K to provide for rapid enhancement of microbial activity and plant nutrients. Soil for the experiment was a sandy loam from a cotton [*Gossypium hirsutum*] field infested with the reniform nematode [*Rotylenchulus reniformis*]. The materials were applied by drenching into soil contained in 1L pots which were covered immediately after application with transparent low density polyethylene bags for 10 days. The bags were removed 10 days after treatment and soil samples were taken for nematode analysis with the salad bowl incubation technique [SBIT]. Each pot was planted with 5 seeds of ‘Marketmore – 76’ cucumber [*Cucumis sativus*] and the resulting plants were grown for 6 weeks when they were removed and final soil samples for nematode analysis were collected. Shoot heights and the weights of fresh shoots and roots were recorded and roots were incubated [SBIT] to determine nematode populations. All formulations of BSM and BG applied in the range of 2 – 5 gm/Kg soil resulted in drastic reductions in populations of *R. reniformis*. Populations of microbivorous nematodes were increased exponentially by the BG treatments but to a much lesser degree by those with BSM. Numbers of dorylaimida were unaffected by BG applications at < 3 gm; the nematodes were eliminated by all BSM rates and by BG at > 2 gms. Values for shoot height, and fresh weights of shoots and roots were improved proportionately to application rates of BSM and BG formulations. Results indicated that the form of C and N used significantly determined plant response and degree of nematicidal activity. The concept of C/N ratio as a predictor of nematicidal activity for soil amendments is questionable and subject to interpretation.

USE OF CROPPING SYSTEMS AND ORGANIC AMENDMENTS FOR THE MANAGEMENT OF PLANT PATHOGENIC NEMATODES AND OTHER SOILBORNE PESTS: SCIENTIFIC, ECONOMIC, AND STATISTICAL CONSIDERATIONS (Sistemas de producción y enmiendas orgánicas para el manejo de fitonematodos y otras plagas de origen edáfico: consideraciones científicas, económicas y estadísticas).

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There are numerous cropping systems that result in increased crop yield and suppression of weeds, insects, plant pathogenic nematodes and other soil-borne plant pests. The most effective systems are those that result in long-term sustainable yields while increasing soil organic matter and enhanced microbiological activity against pests. These suppressive systems engender soils resistant to pests through a form of “biological buffering” based on the presence broad diversity of microbial species. Component plants in the systems may produce pest suppressive compounds with specially associated microbiology-active plants. The feasibility of establishing such systems depends *inter alia* on: soil properties, climatology, and the presence of environmental conditions appropriate for development of activities by desirable microbial species. The relative value of various components of a suppressive system can be determined using standard techniques for quantitative analyses. The value of non phytopathogenic nematodes has been precisely determined for several long-term cropping systems. It is possible to do the same for other components of the soil biota. Although many suppressive systems have been identified and studied in detail, relatively few have been implemented or accepted by producers. Key to the adoption of the systems is logistical and economical considerations. One impediment to adoption is a mindset on eliminating pests, rather than a mindset on practices that promote beneficial that directly or indirectly lead to pest suppression. Econometric (regression) analyses of both field and greenhouse experiments at Auburn show that in many instances, beneficials have a significant positive relationship with crop yield. Moreover, the positive effect of beneficials on crop yield is often of more economic significance than the negative effect of pests. A second impediment to adoption arises when several cropping cycles are required to fully realize benefits. The age of uncertainty we now live in favors profits now, with little concern for future profits. As a result, many farmers are reluctant to adopt systems that will not be profitable immediately.

SESSION 7 CONTROL DE NEMATODOS

ALTERNATIVE METHODS OF FUMIGANTS OF SOIL (Métodos alternativos de fumigantes de suelo).

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Diversity of plantations is an agricultural activity quite important for the east of Antioquia region and Colombia as well. At the same time the presence of plagues and diseases on the soil and its handling call the attention on friendly practices with the humanity, environmental cost and production of plantations. Available measurements among organics, biological, physical, genetic, agrochemical ones that are effectively and selectively tasted, those on low toxicity, allow us to obtain healthier products and free of residues through the usage of control strategies without contamination. In the green chamber at the facility of vegetal health UNIVERSIDAD CATOLICA DE ORIENTE trying to create a knowledge on the effects of bio fumigations presenting on the handling of pathogens on the soil and development of the plants under controlled conditions and in a design completely lined by side, with seven treatments and three repetitions and the following products were evaluated: Mustard, cabbage, grass, gallinaza (natural fertilizer), DAZOMET, in soil infested with nematodes and using as a responding plant tomato var Rutgers. Results indicate that bio fumigations obtained from the mixture G+M+C+G (Gallinaza, mustard, cabbage, grass),

G+M (Gallinaza, mustard), G+C (Gallinaza, cabbage) and even C+G (Cabbage, grass) in combination with sun taken help the development of tomato VAR Rutgers plants making meaningful differences on BAS (Dazomet) treatments, Ts (sterilized soil) and Tc (Soil field).

LA BIOFUMIGACION Y AGREGADOS ORGÁNICOS (Biofumigation and organic amendments).

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La biofumigación esta basada en la liberación de sustancias volátiles resultantes de la descomposición de agregados orgánicos y residuos agroindustriales, que actúan como fumigantes, en el control de organismos fitopatógenos. La biofumigación además de tener un efecto negativo contra los organismos fitopatógenos, mejora el estado nutricional del suelo, incrementa el contenido de materia orgánica y revitaliza la actividad microbiana. En base a lo anterior la biofumigación fue aplicada en un suelo altamente infestado con *Meloidogyne incognita*, aplicando los siguientes tratamientos: Sorgo+estiércol, maíz+estiércol, maíz+gallinaza, sorgo +gallinaza a dosis de 4.32 y 8.64 ton/ha y el testigo; los tratamientos se dejaron por 3 semanas y posterior a este tiempo se evaluó cada 30 días la población de nematodos del suelo y el índice de agallamiento. Las plántulas de jitomate previo a la plantación se inocularon con una sepa mexicana de *Pochonia clamydosporea*, la que también fue aplicada a través del riego al área experimental cada 30 días, con excepción de los testigos. Después de 30 días y posterior a este tiempo la población de nematodos bacteriófagos, micofagos y omnívoros se incremento y el índice de agallamiento desapareció. Concluimos que el uso de la materia orgánica es una magnífica alternativa barata y capaz de controlar microorganismos fitopatógenos y mejora la fertilidad de los suelos.

THE USE OF THE ACC DEAMINASE PRODUCING BACTERIUM *PSEUDOMONAS PUTIDA* UW4 AS A BIOCONTROL AGENT FOR PINE WILT DISEASE (El uso de la bacteria productora de ACC deaminase *Pseudomonas putida* UW4 como un agente de biocontrol para la enfermedad de marchitamiento de los pinos).

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Pine wilt disease, caused by the nematode *Bursaphelenchus xylophilus*, is responsible for the devastation of worldwide pine forestlands. Until today, the only effective solution to this serious threat resides on the destruction of infected trees, which is both economic and ecologically unacceptable. The use of ACC deaminase-producing plant growth promoting bacteria has been shown to be a useful strategy in order to reduce biotic and abiotic constraints that affect plant health and development. In this sense, we report the use of the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 as a potential biological control agent for pine wilt disease. An inoculation assay was performed in 3-4 months *Pinus pinaster* (maritime pine) seedlings obtained from a nursery in Portugal. The bacteria *P. putida* UW4 wild-type and ACC deaminase mutant strains were inoculated in the roots of pine seedlings followed by stem inoculation of *B. xylophilus*. The inoculation of the *P. putida* UW4 wild-type strain lead to a significant reduction of *B. xylophilus* induced symptoms. Moreover, regardless the inoculation with *B. xylophilus*, seedlings inoculated with *P. putida* UW4 also demonstrated an increased root and shoot development. In addition, *P. putida* UW4 ACC deaminase knockout mutant was unable to promote pine seedling growth or to decrease

B. xylophilus induced symptoms. The results obtained indicate that the inoculation of ACC deaminase-producing bacteria in pine seedlings growing in a nursery system might constitute a novel strategy to obtain *B. xylophilus* resistant pine trees. This is the first report on the use of ACC deaminase-producing bacteria as potential biological control agents for tree diseases.

VOLATILE ORGANIC COMPOUNDS TOXIC TO *MELOIDOGYNE INCOGNITA* EMITTED BY SOIL INCORPORATED WITH CASTOR BEAN CAKE (Compuestos orgánicos volátiles tóxicos a *Meloidogyne incognita* emitidos en el suelo cuando se incorpora pasta de fríjol de Hguerilla).

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Organic residues incorporated into soils affect microbiota through water soluble molecules (WSM) as well as volatile organic compounds (VOC). Although studies about WSMs are abundant, researches on VOCs are scarce, especially those toxic to plant parasitic nematodes. Therefore, we aimed to evaluate the toxicity effect on second stage juveniles (J2) and eggs of *Meloidogyne incognita* (MI) of VOCs emitted by different substrates (sterile soil or not and artificial substrate) incorporated with doses (1.5 and 3.0%) of castor bean cake (CBC). Then, substrate incorporated with CBC was placed in the plastic cup and two microtubes were half digged into it which was used for eggs and J2 exposure to VOCs. The cup was hermetically sealed with Parafilm®. The J2 and eggs were injected with a syringe into the microtubes within the formed gas chambers by 10 days and 20 days after sealing. Doses of CBC incorporated into soil and in artificial substrate emitted toxic VOCs which caused J2 immobility by 92.5% to 100% in the gas chambers (10 and 20 days). However, the J2 mortality varied from 13 to 80% within three substrates in all gas chambers. It is worthy to mention that the effect on J2 mortality of highest compared to the lowest BCD doses incorporated into sterile soil was 2.3 to 4 times greater, respectively, in 10 days and 20 days formed gas chambers. J2 hatching was not affected by VOCs.

SESSION 8 SOIL HEALTH

DENSITY-INDEPENDENT NEMATODE REGULATION MECHANISMS OF SOIL FUNGI WITH SAPROTROPHIC-PARASITIC BEHAVIOURS (Mecanismos de regulación densidad-independiente de nematodos con hongos edáficos con comportamiento saprofitico-parasítico).

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Several nematode parasitic fungi like *Pochonia chlamydosporia* have different trophic behaviours in soil, ranging from parasitism to endophytism or saprotrophic growth. We explored the potentialities of non-parasitic fungal populations, when switching towards parasitism. Models describing the tri-trophic nematode, fungus and root interactions were developed to simulate six-months stable population dynamics of *Meloidogyne incognita*, in presence or absence of the fungus. The model used hourly constants estimated on the basis of known lifetimes of nematode stages, roots and *P. chlamydosporia*. Modelling yielded discrete density data for all nematode life-stages, as well as root and fungal fractions, with nematode suboptimal cycles lasting around 6 months, fitting subtropical field conditions. When the fungus was present in the microcosm, the density of the virulent propagule fraction increased due to transmission efficiency and,

in a host independent way, as a function of the recruitment of parasitic propagules from the saprotrophic population. This switch, coupled with the saprotrophic growth rate, significantly affected the *M. incognita* egg densities, with local extinctions expected at saprotrophic growth rates higher than 2.2%. This property may have technical and practical implications in real systems, and can provide a possible framework for the nematode suppressive conditions reported in some studies.

METHODS TO INCREASE SOIL HEALTH AND THEIR INCIDENCE ON DAMAGE CAUSED BY PLANT-PARASITIC NEMATODES IN VINEYARDS (Medidas para incrementar la sanidad del suelo y su incidencia en daños causados por nematodos fitoparásitos en viñas).

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The term “soil health” involves many biotic and abiotic factors and their interactions, and is also related to the concept of soil quality. In crop production, it can be associated specifically to plants health, defining a soil with limited disease outbreaks as healthy. To maintain or improve soil health, considering healthy crops as a goal, different tools must be developed enabling the management of soil biotic and abiotic factors increasing its suppressiveness to plant-parasitic nematodes. In vineyards, some cultural practices applied by farmers exert a strong influence on soil health, specifically on its biological components, in order to manage the populations or reduce the damages caused by plant parasitic species. These practices include rotations, the application of different organic amendments such as manure, compost, cover crops or reduced tillage. These methods have been used more intensively in established plantations to improve crops health and roots development. Incorporation of chitin-based products or liquid compost is also a frequent practice, improving the overall roots health. Finally, the introduction in soil of selected microorganisms such as root rhizobacteria isolated from plants with low nematode infestation levels represent innovative alternatives, actually under study.

NATURE OF SOIL HEALTH: WITH SPECIAL REFERENCE TO NEMATODES (Naturaleza de la salud del suelo: con especial referencia a nematodos).

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Soil is a regenerative habitat for organisms. It has solid, liquid and gaseous components and undergoes three system phases: development, dynamic equilibrium and senescence. The domain of Soil Health evolved during the past two decades. A healthy soil resists degradation and responds to management in a predictable manner. Healthy soil is imperative for a sustainable high quality of human life. There are many examples of soil quality degradation catalyzing erosion of civilizations. In addition to nematodes that cause infectious diseases of plants, animals and humans, it is well known that many species play significant roles soil nutrient mineralization and can be used as indicators of soil health. This contribution briefly summarizes team research on: 1) nematode diversity and population density in natural and managed ecosystems, 2) vertical distribution of trophic groups of nematodes in orchards, 3) impact of orchard management on nutrient mineralization potential, 4) relationships between soil health (Cornell University Soil Health Assessment System) and potato early-die (*Pratylenchus penetrans* and *Verticillium dahliae*) and 5) status of *Globodera* spp. in Central Asia. Nematode species richness was highest in a pristine forest system, compared to early secondary succession or agricultural systems. Population densities, however, were highest under secondary succession. Orchard population densities of bacterial-feeding nematodes, flagellates, ciliates amoebae and endomycorrhizal spores were significantly greater in the O-horizon, compared to soil depths of 0-15 or 15-30 cm. Population densities of these organisms were significantly greater in organic, compared to conventional orchards. In an analysis of the soil health of twenty-five potato fields with histories of

early-die, phosphorus, potassium, minor elements and surface hardness were optimal whereas aggregate stability, available water capacity, subsurface hardness, organic matter, active carbon, and mineralizable nitrogen were poor. Many agricultural districts in Central Asia have undergone millennia of extensive soil health degradation, including sites with current *Globodera* spp. infestations. An alternative hypothesis to infectious diseases caused by phytopathogenic nematodes is that true root cause is unhealthy soil. It is now time for interdisciplinary teams to test this hypothesis.

OCURRENCE OF MICROBIAL BIOLOGICAL CONTROL AGENTS IN MEXICAN TROPIC SOILS (Hallazgo de agentes microbianos de control biológico en suelos del trópico mexicano).

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Both *Pasteuria* spp. (Bacillaceae) and the fungus *Pochonia chlamydosporia* have been used as biological control agents due to parasitism on nematodes of economic importance. The occurrence and interactions with plant parasitic nematodes of these microorganisms can provide an indication about soil conditions and health. Soil samples were taken in the buffer zone of the Biosphere Reserve “Los Tuxtlas”, in the southeast of Mexico, and examined for the occurrence of *Pasteuria* endospores and of *P. chlamydosporia* native isolates. The samples were collected from locations with different land uses (i.e. natural forest, secondary forest, pastures and maize fields) and were processed in order to detect both biological control agents in soil. One hundred nematodes killed, fixed and dehydrated from each sampling point were randomly examined to detect the occurrence of *Pasteuria* on their cuticle. The fungus was detected using a selective medium for each soil sample. *Pasteuria* endospores adhering to nematodes (*Helicotylenchus*, *Pratylenchus*, *Criconebella*, *Tylenchus*, *Plectus* and *Aporcelaimium* spp.) were observed in eight of 120 sampling sites (two from secondary forest, two from maize fields, one from White lily and three from pasture fields). Two varieties of the fungus (alone or in combination), were found in 30 soil samples (25 samples were positives to *P. chlamydosporia* var. *chlamydosporia* and ten to *P. chlamydosporia* var. *catenulata*). Six isolates were present in pasture fields, four in maize fields, 13 in secondary forest and 12 in natural forest. The ability to colonize roots and to parasitize eggs by all isolates of the fungus were tested and results varied among isolates.

POCHONIA CHLAMYDOSPORIA: A ‘MULTIPLE’ APPROACH (BIOLOGY, BIOTECHNOLOGY, AGRIBUSINESS) FOR DEVELOPMENT OF BIOLOGICAL CONTROL AGENTS AS BIOPESTICIDES THAT ARE BENEFICIAL TO SOIL HEALTH (*Pochonia chlamydosporia*: un enfoque ‘múltiple’ (biología, biotecnología, agronegocios) para el desarrollo de agentes biológicos como biopesticidas benéficos a la salud del suelo).

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The nematophagous fungus *Pochonia chlamydosporia* is one of the most studied biological control agents against sedentary plant-parasitic nematodes. Its worldwide distribution, easy laboratory culturing, access to isolates (fungal collections), saprophytic and parasitic behaviours, effectiveness in nematode control as

well as the need to find alternatives to nematicides, have led to intensive screening and testing of numerous isolates as potential biocontrol agents against root-knot (*Meloidogyne*), cyst (*Heterodera*, *Globodera*), false root-knot (*Nacobbus*) and reniform (*Rotylenchulus*) nematodes. However, studies on the biology of the fungus are few in comparison to those on the ecological aspects of the plant-fungus-nematode (tritrophic) interaction. Nevertheless, most ecology studies have focused on a few selected isolates and problems associated with fungal survival and establishment in soil after inoculation with chlamydospores, colonisation of the rhizosphere and parasitism of nematode eggs. The application of biotechnology (the exploitation of biological processes for industrial and other purposes) has resulted in mass production of *P. chlamydosporia* and chlamydospore-based products are available for soil application in both developed and developing countries. Although only a few isolates are commercially available, different isolates are currently produced in the Americas, Africa and Europe. From an agribusiness perspective, facilities and resources for fungal production vary from basic to sophisticated, depending on country and, crucially, they are not always subjected to quality control and standard operation procedures. These factors probably account for the lack of consistency in nematode control products and thereby affect commercial potential and effectiveness. Therefore, there is a need to properly characterize and fingerprint isolates and to improve fungus production (i.e., commercial vs local, cottage industry) and formulation. Other factors to be considered include: soil receptivity to non-native isolates, soil multitrophic interactions, local regulations such as biosecurity and legislation and, through newly developed molecular methods, the monitoring of isolates after their introduction and establishment in the soil/rhizosphere. Only when these factors are applied in an appropriate and integrated manner in crop and pest management programmes can the full biocontrol potential of *P. chlamydosporia* be realised and thereby benefit soil health.

SESSION 9 NEMATODES ECOLOGY

A POTENTIAL CASE OF MUTUALISM BETWEEN *SCLERORHABDITIS NEOTROPICALIS* N. SP (NEMATODA), *AZTECA CONSTRUCTOR* ANTS AND *CECROPIA OBTUSIFOLIA* TREES IN THE TROPICAL RAIN FOREST (Un caso potencial de mutualismo entre *Sclerorhabditis neotropicalis* n. sp (Nematoda), la hormiga *Azteca constructor* y el árbol *Cecropia obtusifolia* en la selva tropical).

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Sclerorhabditis neotropicalis Esquivel *et al.* was recently described from the tropical rainforests of Costa Rica. Noteworthy is an apparent mutualistic relationship between the nematode, the *Azteca constructor* ant colony and the *Cecropia obtusifolia* tree. Several authors have described the mutualistic relationship between *Cecropia* and *Azteca*. The *Cecropia* tree provides food and a nesting site (domatia), while the ants protect the tree from herbivores. The question arises: What is the role of nematodes in this association? Many of the symbiotic associations between ants and plants, previously considered as bipartite associations, could in fact be tripartite or multipartite involving microorganisms such as fungi, bacteria and nematodes. Certain species of *Azteca* ants are obligatory inhabitants of *Cecropia* trees, and their cloistral behavior could explain the abundance and incidence of *S. neotropicalis* in all nests of *A. constructor*. Worker ants act as phoretic carriers inside the plant and they establish nematode cultures in the internodes used for ant reproduction. It is therefore possible that the new nematode species represents a third partner in the mutualism, although its role is unknown. The nematodes are not associated with ant garbage dumps, but rather with mounds of plant tissue scraped by ants from inner walls of the stem, and generally located near the ant brood. Together with bacteria and fungi, the nematodes could be acting as nutrient recyclers of wastes produced by the ant colony, transforming them into nutrients for the plant. Samples from several localities of Costa Rica, show that the new nematode species is present in all *Azteca/Cecropia* associations thus far examined. These observations strongly suggest that the association between the nematodes and the *Azteca* ants inhabiting *Cecropia* stems is more than casual.

HABITAT EFFECTS ON ENTOMOPATHOGENIC NEMATODE SPECIES, COMMUNITIES AND INDIVIDUALS: WHAT CONTRIBUTES MOST TO BIOLOGICAL CONTROL? (Comunidades o individuos ¿qué contribuye más en el control biológico?: Caso de estudio mediante nematodos entomopatógenos en distintos tipos de hábitat).

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We are investigating whether and how entomopathogenic nematode (EPN) species richness, dominance and behavior in different soils affect the regional spatial patterns of the weevil pest *Diaprepes abbreviatus*. Among 7 EPN species detected by qPCR in citrus orchards, *Steinernema diaprepesi* and *Heterorhabditis zealandica* are spatially associated with the central ridge eco-region, *Steinernema* sp. *glaseri*-group with the flatwoods eco-region and *H. indica* inhabits both regions, dominating flatwoods communities. Numbers of weevils are higher and EPN diversity lower in the flatwoods compared to the central ridge, but numbers of EPNs are similar in both regions. In controlled studies, weevil larvae were killed at significantly higher rates by *Steinernema diaprepesi* than by *Heterorhabditis indica* (the two dominant species). Both species killed weevils more effectively in a central ridge soil than one from the flatwoods. The effects of species and soils on weevil mortality measured after 7 days are small compared to weevil demographics; however, over longer intervals (weevils reside in soil for several months) these rates would result in large regional differences in weevil survivorship. EPN species richness/diversity was recently reported to be positively related to insecticidal efficacy. Ongoing research will be described that tests this hypothesis for the dominant EPN species in Florida citrus orchards.

MODELLING APPROACHES IN NEMATODE ECOLOGY STUDIES (Métodos de modelación aplicados a estudios de ecología de nematodos).

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Modelling the interactions of multiple organisms in soil has a limited predictive value but can provide important information about fundamental mechanisms underpinning several ecological services. In the case of nematodes, modelling approaches were initially developed to describe the life-cycle of important plant parasitic species, as well as their interactions with the host plant roots or their role in soil trophic webs. More recent approaches were applied to the study of microbial biocontrol agents of ectoparasitic or sedentary nematodes, to the analysis of the spatial spreading of species and to the evaluation of climate change effects on nematode life-cycles or plant damage. Quantitative data used in modelling require the identification of several biological constants or functions related mainly to the organisms' life-cycles, biology, or mutual interactions. Future trends in nematode ecological modelling will consider the inclusion of biochemical signals affecting their interactions with roots and microorganisms in soil, and the quantitative impact of their effects in the rhizosphere. Several modelling approaches were thus far developed to analyse, under different perspectives, nematode trophic behaviours in soil, including density effects, pathogenicity, saprophytism or predation, each one tailored to study specific ecosystems. In this review the implications of some modelling approaches and their specific needs will be discussed in the light of the information they can produce and their practical exploitation in experimental validation and crop protection assays.

SOIL DISTURBANCE IN THE MEXICAN TROPICS AND ITS EFFECT ON SOIL NEMATODE DIVERSITY (La perturbación del suelo en el trópico mexicano y su efecto en la diversidad de nematodos edáficos).

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A study was conducted at the Biosphere Reserve "Los Tuxtlas", Mexico, to determine the effect of different land use intensities in the Mexican tropics on nematode diversity. Three localities in the reserve were chosen as replicates and, from each of them, different sites under four land use intensities (natural forest, secondary forest, pasture and maize fields) were selected. Total abundance of nematodes, genera estimates, diversity indices and structure (relative abundance) of different trophic groups and other indices based on microbial feeding nematodes were assessed and compared. Fifty-three families and 124 genera of nematodes were identified from the study area. The dominant families were Criconeematidae, Hoplolaimidae, Cephalobidae and Tylenchidae, and the most abundant genera were *Helicotylenchus*, *Discocriconebella*, *Tylenchus* and *Steinernema*. Plant feeders and bacterial feeders were the most abundant trophic groups followed by fungal feeders, omnivores and predators. The highest number of bacterial feeders was extracted from pastures and maize, fungal feeders from maize, omnivores and predators from natural forest and secondary forest, and plant feeders from secondary forest. The highest nematode abundance, genera richness and diversity were found in natural forest, closely followed by secondary forest. Intensive agricultural systems, represented by maize fields and pastures, had low genera richness and significantly less diversity than non-disturbed systems. Most of the population estimates and indices were useful in establishing the significance of effects of different land uses on soil nematodes in the study area.

SESSION 10 NACOBBUS

DEFENSE RESPONSES IN CHILLI CM-334 INFECTED BY *NACOBBUS ABERRANS* (Respuestas de defensa en chilli CM-334 a la infección por *Nacobbus aberrans*).

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Chilli CM-334 (*Capsicum annuum*) is resistant to *Phytophthora capsici*, but this resistance is broken down by the infection of *Nacobbus aberrans*. Transcript accumulation of 3-hydroxy-3-methylglutaryl CoA reductase (HMG1, HMG2 and HMG3 isoforms), farnesyl pyrophosphate synthase (FPPS), squalene synthase (SS) and 5-epi-aristolochene synthase (EAS) genes, as well as the enzymatic activities of HMGCoA-r and EAS in roots of chilli CM-334 infected and non-infected by *N. aberrans* were determined. Forty plants were inoculated with 2000 second stage juveniles of the nematode. At 0, 2, 7, 14 and 21 days after inoculation (dai) roots were harvested. Accumulation of HMG1 transcripts was lower than that of HMG2 at 2 dai in plants infected by the nematode, although at 21 dai HMG1 transcripts were higher than those of HMG2; the largest accumulation of HMG1 corresponded to a higher HMGCoAr activity and the presence of the immature females of the nematode inside the roots. A high activity of EAS was related with EAS transcript accumulation in infected roots although the relationship was not proportional; at 14 and 21 dai the highest activity of EAS was observed. No changes in the accumulation of HMG3 and SS were observed. FPPS mRNA levels increased in infected plants at 21 dai. Results suggest that *N. aberrans* modifies the expression of some genes of the mevalonate pathway in order to create suitable conditions for its establishment and development.

DNA-CHARACTER BASED ANALYSIS: AN APPROACH TOWARDS THE SPECIES DIAGNOSIS AND IDENTIFICATION OF THE FALSE ROOT-KNOT NEMATODE *NACOBBUS ABERRANS* SENSU LATO (Análisis basado en caracteres de ADN: un enfoque hacia el diagnóstico e identificación de especies del nematodo agallador *Nacobbus aberrans* sensu lato).

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The thresholds defined by genetic distances have been widely used to determine the identity of organisms; however, there are no clear criteria for setting those thresholds making it difficult the determination of the taxonomic situation of a particular species. To solve this issue, the use of DNA character-based diagnosis is proposed as alternative method during the species diagnostics and/or identification process. Due to that *Nacobbus aberrans* s.l. has an enormous physiological, ecological and molecular variation, this bioinformatics tool was applied to DNA sequences of three molecular markers (ITS1, D2-D3 expansion segments, and the mitochondrial COII/16S) to demonstrate, how the DNA character-based would be able to detect unique and discrete attributes or characters that allow the species diagnostics of *Nacobbus* species complex. The Characteristic Attributes Organization System (CAOS) algorithm was used to identify unique “simple pure characters” (sPu) to DNA sequences of phylogenetic groups of *Nacobbus* defined a priori (I. Nebraska, II. Central Mexican Region, III. Northern Mexican Region and IV. Argentinian Lowlands). This approach allowed detecting several unique characters in each phylogenetic group that ranged from 2-20 sPu (ITS1), 5-22 sPu (D2-D3) and 14-23 sPu (COII/16S). These results improved the taxonomic resolution level for the determination of *Nacobbus* species.

EFFICIENCY OF SYMBIOTIC BACTERIA OF ENTOMOPATHOGENIC NEMATODES TO CONTROL A POPULATION OF *NACOBBUS ABERRANS* (Eficiencia de bacterias simbiotes de nematodos entomopatógenos para el control de una población de *Nacobbus aberrans*).

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In the last years special efforts have been made to identify natural antagonists for the integrated control of plant-parasitic nematodes of agricultural importance. *Nacobbus aberrans* is widely distributed in Argentina and parasitizes several crops and weeds. Recent studies conducted by our research group with isolates of entomopathogenic nematodes (EPN) (families Steinernematidae and Heterorhabditidae) showed a clear antagonistic activity on *N. aberrans*, which would be caused by the EPN-symbiotic bacteria (SB) complex. In the present work, the effect of SB extracted from EPN on this plant-parasitic nematode was evaluated *in vitro* and on tomato plants. A nematocidal effect (under *in vitro* conditions) on second-stage juveniles of the nematode was observed, producing 100% mortality in some cases. The application SB and their metabolites to the soil significantly reduced the number of galls (33% to 67%) and nematode reproduction (55% to 80%) on the host. These results show that SB and their metabolites might be used as potential biological control agents of local *N. aberrans* populations.

EXPERIENCES IN THE BIOLOGICAL CONTROL OF *NACOBBUS ABERRANS* USING *POCHONIA CHLAMYDOSPORIA* IN MEXICO: PROGRESS, SCOPE AND THINGS TO DO (Experiencias en el control biológico de *Nacobbus aberrans* con *Pochonia chlamydosporia* en México: avances, alcances y pendientes).

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The false root-knot nematode, *Nacobbus aberrans*, is an important plant-parasitic nematode in Mexico, as it damages crops such as tomato, chili and bean. This nematode has been detected from ten states of Mexico causing significant yield losses, tomato being the most affected crop. *N. aberrans* has become an important and difficult pest to control, because of its wide host range, including weeds between host crops, and it has a high reproductive capacity under field conditions. In Mexico, the biological control of nematodes using nematophagous fungi has been little studied; nevertheless, some surveys to find soil nematophagous fungi potentially useful as control agents of plant-parasitic nematodes have been done. *Pochonia chlamydosporia* has been isolated in Mexico and screened against *N. aberrans* to find and incorporate the most effective isolates into integrated pest management strategies of this nematode. In this study, a global view is given about the advances and perspectives around the use of this fungus as biological control agent of *N. aberrans*. The subjects that have been tested were: 1) biological management of the false root-knot nematode with the fungus under greenhouse and field conditions, 2) fungus application to seedbeds, 3) new isolates and testing for their parasitic potential, and 4) incorporation of the fungus into an Integrated Control scheme of *N. aberrans*. The Mexican isolates must be studied further in order to corroborate results and then they can be used in a large scale; their characterization so deeper by molecular methods must be done too.

“WARTY BEETROOT” (*BETA VULGARIS*) A RECENT DISEASE IN MEXICO, CAUSED BY THE FALSE ROOT-KNOT NEMATODE *NACOBBUS ABERRANS* (El “grano del betabel” (*Beta vulgaris*) una reciente enfermedad en México, causada por el nematodo agallador *Nacobbus aberrans*).

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In Mexico, the first report on *Nacobbus aberrans* was made in 1967, causing a disease known as “Jicamilla del chile”(root galling) in Chapingo, State of Mexico. Since then the nematode has been found in fields parasiting a wide host range of plants such as tomato (*Solanum lycopersicum*), chili (*Capsicum annuum*), and bean (*Phaseolus vulgaris*), producing considerable losses and representing a serious problem for horticultural production in the country. It has also been found in a number of weeds growing in the fields. In 2009, it was found on beetroot (*Beta vulgaris*) in the Valley of Tepeaca, Puebla (Mexico). *N. aberrans* induces galls on the taproot of beetroot and lesions on the head of the root, the disease being known by farmers as “warty beetroot”. The present study deals with the distribution and incidence of this disease and describes the histological changes caused by *N. aberrans* on the taproot and the head of the root of this vegetable. Twenty eight fields planted with beetroot were sampled in the study area of Tepeaca Valley during 2008-2009. Ten subsamples were randomly taken per field sampled and 100-150 g soil and 15 plants were collected at each sampling point. All subsamples per field were mixed to make a composite sample. Disease incidence was determined as the percentage of plants per sample with the disease symptoms. Galled roots were selected from the taproot and wart-like lesions on the head of the root for histological study. All roots were fixed in FAA, embedded in paraffin, and histological sections (10-15 µm thick) were made.

Histological sections were stained with fuchsin-fast green and mounted in synthetic resin for analysis. Results showed that the “wart-like beetroot” disease is distributed in the municipalities of Los Reyes de Juárez, Palmar de Bravo and Quecholac in 50% of fields sampled. Disease incidence reached up to 100% in some infested fields. Histological examination showed that in the galls of the taproot, the nematode induced the formation of feeding sites (syncytium) in the vascular cylinder. Syncytium cells cytoplasm was dense and granular, with one or two nuclei, hypertrophied nuclei and nucleoli, and thickened or dissolved cell walls. In contrast, wart-like lesions on the head of the root showed juvenile stages of the nematode that were found within cavities surrounded by lignified cells. When young females invaded the storage parenchyma, they induced a circumscribed syncytium-like lesion. Mature females with egg masses were also found in both the main root galls and wart-like lesions.

SESSION 11 NEMATODES AS INDICATORS

FUNCTIONAL CONNECTANCE AND ECOSYSTEM SERVICES (Conectividad funcional y servicios ecosistémicos).

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Connectance is the proportion of potential trophic or behavioral links in an ecosystem that is actually realized. The abundant and diverse organisms of the soil ecosystem are assembled in highly aggregated patches. The characteristics of the assemblage in each patch are determined by the availability and nature of resources, organism feeding adaptations and specializations, their tolerance or adaptation to ambient microsite conditions, and the auto-regulatory dynamics of predator-prey relationships. Also influenced by ambient microsite conditions, assemblages may differ in phenological synchrony among patches. Connectance within patches is facilitated by the aggregation; connectance among patches may be a function of organism motility, phoresy and emigration in response to resource depletion. Although connectance and interaction strength among patches may be conceptually intuitive, they are difficult to measure. Emerging technologies, including bar-coding and high-throughput sequencing, hold promise for further advances in this area. However, when desired ecosystem services of agricultural or environmental importance would be enhanced by increased functional connectance, it may be possible to promote such connectance by engineering or cultural practices that facilitate co-location and interaction of organisms. In agricultural production systems, for example, desired ecosystem services such as mineralization of nutrients and predator regulation of pest species may be facilitated by drip irrigation and reduced tillage to localize root and resource distribution and by minimizing environmental constraints for key participants.

MODELING FUNCTIONAL AND STRUCTURAL ATTRIBUTES OF NEMATODE COMMUNITIES: DIVERSITY, METABOLIC FOOTPRINTS AND FOOD WEB TOPOLOGY IN RESPONSE TO AGRICULTURAL MANAGEMENT (Modelaje de atributos funcionales y estructurales de comunidades de nematodos: diversidad, huellas metabólicas y topología de la red trófica en respuesta al manejo agrario).

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Soil nematode communities have been widely used to assess soil condition in natural and agricultural ecosystems. This paper presents some applications of the main families of ecological indices - diversity indices, soil food web indices, and metabolic footprints – as indicators of the effects of agricultural management on the agro-ecosystem. Nematode diversity clearly responds to agricultural intensification and

soil use both at experimental and landscape scales, while food web indices are indicators of soil food web functions such as nutrient mineralization and soil suppressiveness. Metabolic footprints, indicators of the magnitude of the ecological roles developed by nematodes, react significantly to a number of categorical and continuous management and environmental variables. Finally, new tools of network analyses such as the study of nematode-based food web topology and inference of connectance and centrality measures of nematode taxa in the soil food web have been rarely used in nematode ecology, but present an opportunity to explore the structure of soil biodiversity in response to agricultural management.

NEMATODE DIVERSITY IN NATIVE AND CULTIVATED VEGETATION IN BRAZILIAN SAVANNAS (Diversidad de nematodos en la vegetación nativa y cultivada de las sabanas brasileñas).

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Nematode assemblages were studied in two regions in Brazilian Savannas. The first study was in three locations in Sao Carlos, State of Sao Paulo, Brazil: native vegetation of Brazilian Savanna (Cerrado), a perennial crop (guava, *Psidium guajava*) and an annual crop (corn, *Zea mays*). The most abundant genus in Cerrado was *Discocriconemella* and in cultivated areas was *Helicotylenchus*. With some exceptions, the most abundant nematode taxa also had the greatest frequencies and predominance values. The replacement of the native Cerrado vegetation with guava and corn crops influenced nematode communities, resulting in: reduction in abundance of Criconematoidea, as well as absence of *Discocriconemella* and *Tylenchulus* (taxonomic groups very abundant in the original vegetation of Cerrado); greater abundances of *Helicotylenchus* and *Pratylenchus*; decreased genera richness, trophic diversity and relative abundance of predators and omnivorous nematodes. The nematode assemblages associated with corn were most dissimilar to the original Cerrado. Corn cultivation resulted in reduction in abundance of *Dorylaimellus* and absence of *Dorylaimoides* and *Labronema* (genera very abundant in the original vegetation). The second group of studies was in long-term crop rotation and tillage experiments and in native Cerrado in the Federal District of Brazil. In all studies, *Criconematoidea* and *Pratylenchus* were potential indicators of environmental disturbances, soil quality and sustainability.

SESSION 12 MARINE NEMATODES

EFFECT OF SALINITY ON THE CAPACITY INFECTIVE PARASITIC NEMATODE *ROMANOMERMIS IYENGARI* (NEMATODA: MERMITHIDAE) IN MOSQUITO LARVAE OF *CULEX QUINQUEFASCIATUS* (Efecto de la salinidad en la capacidad infectiva del nematodo parásito *Romanomermis iyengari* (nematoda:mermithidae) en larvas de mosquitos *Culex quinquefasciatus*).

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Los nematodos parásitos del género *Romanomermis* (Mermithidae) son una alternativa efectiva, específica y sostenible para el control biológico de mosquitos. Sin embargo, el potencial de control biológico de estos nematodos, es afectado por su sensibilidad a condiciones ambientales extremas como la temperatura, salinidad y pH de los cuerpos de agua donde se aplican, afectando su viabilidad y capacidad parasítica, reduciendo su máximo potencial. El objetivo de la investigación fue determinar el efecto que causan diferentes concentraciones de NaCl sobre la capacidad infectiva del nematodo *R. iyengari*. Se evaluaron

diez concentraciones de salinidad en un rango 0 - 2000 mg L⁻¹ con cuatro repeticiones y un testigo (agua desionizada). El nematodo *R. iyengari* presentó parasitismo en todas las concentraciones de salinidad evaluadas. Sin embargo, los niveles de parasitismo disminuyeron con respecto a las concentraciones de salinidad. En altas concentraciones de salinidad se registraron menores niveles de parasitismo y viceversa. Las altas concentraciones de NaCl causaron importante reducción de la capacidad infectiva del nematodo, observándose en una reducción drástica en las concentraciones de salinidad mas altas, en las que se registraron 3.1, 3.1 y 1.25 porcentajes de parasitismo con 1600, 1800 y 2000 mg L⁻¹. Concluyendo que la salinidad es un factor de estrés en los Juveniles infectivos del nematodo *R. iyengari* por el aumento la presión osmótica y la subsecuente perdida de agua en los organismos, disminuyendo su capacidad infectiva y potencial de control biológico de larvas de mosquitos.

TEMPORAL VARIATION OF *MEXICONEMA CICHLASOMAE* (NEMATODA: DANICONEMATIDAE) IN THE MAYAN CICHLID FISH *CICHLASOMA UROPHTHALMUS* AND ITS INTERMEDIATE HOST *ARGULUS YUCATANUS* FROM A TROPICAL COASTAL LAGOON (Variación temporal de *Mexiconema cichlasomae* (Nematoda: Daniconematidae) en el ciclido maya *Cichlasoma urophthalmus* y en su hospedero intermediario *Argulus yucatanus* en una laguna costera tropical).

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The aim of the present investigation was to determine whether temporal variation in environmental factors such as rainfall or temperature influence long-term fluctuations in the prevalence and mean abundance of the nematode *Mexiconema cichlasomae* in the cichlid fish *Cichlasoma urophthalmus* and its crustacean intermediate host, *Argulus yucatanus*. The study was undertaken in a tropical coastal lagoon in the Yucatan Peninsula (south-eastern Mexico) over an eight year period. Variations in rainfall and monthly infection levels for both hosts were analyzed using time series and cross-correlations to detect possible recurrent patterns. Infections of *M. cichlasomae* in *A. yucatanus* showed annual peaks, while in *C. urophthalmus* peaks were bi-annual. The latter appear to be related to the accumulation of several generations of this nematode in *C. urophthalmus*. Rainfall and temperature appear to be key factors in influencing temporal variation in the infection of *M. cichlasomae* over periods longer than a year.

POSTER SESSIONS

POSTER COMPETITION

AGGRESSIVENESS AND GENETIC VARIABILITY OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* POPULATIONS IN COTTON (*GOSSYPIUM* SPP.) (Agresividad y variabilidad genética de poblaciones del nematodo agallador (*Meloidogyne incognita*) en algodón (*Gossypium* spp.).

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The root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 is widely distributed and recognized as a major pathogen of cotton crop (*Gossypium* spp.) in Brazil. The objectives of this study were to assess the genetic variability and variation in aggressiveness among populations of *M. incognita* in cotton. Five isolates of *M. incognita*, esterases (Est I1 Rm 1.0 and Est I2 Rm 1.05 and 1.1) and one isolate of *Meloidogyne enterolobii* (an outgroup) were used in the analyses. DNA amplifications were done using 22 RAPD, 9 ISSR and 13 AFLP primers and cluster analyses were done using UPGMA. Our results showed that only 2.7% of the fragments were polymorphic. Despite the existence of two races (race 3 and 4) and two esterase phenotypes (I1 and I2), a low genetic variability among isolates was observed and this might be due to their mitotic parthenogenetic mode of reproduction. The aggressiveness of isolates to different cotton genotypes was also studied. The experiment was conducted under greenhouse conditions in which eight cotton cultivars were inoculated with 10.000 eggs per plant with each isolate. Four months post inoculation, nematode reproduction factor (RF) was determined. None of the populations was pathogenic to the cotton resistant genotypes M-315, TX -25, Semi Áspero Huanuco, Wild Mexican Jack Jones and CIR1348. Two populations of *M. incognita* from the states of Mato Grosso and Bahia, Brazil, were highly aggressive to the moderate resistant accessions La-887 and Clevewilt-6.

AGRESIVIDAD Y CAPACIDAD REPRODUCTIVA DE UN AISLADO DE *MELOIDOGYNE JAVANICA* EN TRES PORTAINJERTOS DE FRUTALES DE CAROZO (Aggressiveness and reproductive fitness of one isolate of *Meloidogyne javanica* on tree fruit rootstocks).

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Los frutales de carozo constituyen una de las principales actividades agrícolas de la provincia de Mendoza, Argentina. Entre los nematodos fitoparásitos que afectan estos cultivos en la región, *Meloidogyne* spp. provoca los mayores daños económicos, generando, en algunos casos, hasta el 80% de pérdidas en montes frutales de 2 a 3 años de edad. El objetivo del presente estudio fue evaluar la agresividad y capacidad reproductiva de un aislado local de *M. javanica* en tres portainjertos (Mariana 2624, Hansen 2168 y GxN 3). Se utilizó tomate cv. Platense como testigo. El ensayo se llevó a cabo bajo condiciones de invernáculo,

en macetas de 2L de capacidad con suelo esterilizado. Las plantas fueron inoculadas al trasplante con 1000 huevos y/o juveniles de segundo estadio (J2) del nematodo. Se realizaron cuatro repeticiones por portainjerto. A los 145 días se descalzaron las plantas, se contabilizó el número de agallas y de huevos en cada sistema radical. Se tomaron 100 gr del sustrato de cada maceta y se lo procesó mediante la técnica de flotación-centrifugación. En los diferentes portainjertos se estimó el índice de agallamiento (como indicador de agresividad) y el factor de reproducción (FR=población final/población inicial). Se encontraron diferencias significativas en los valores de ambos índices que sugieren que el portainjerto GxN 3 es más susceptible a este aislado de *M. javanica* que Mariana 2624 y Hansen 2168.

NEMATODE SUPPRESSION IN SOILS DRENCHED WITH SUPERNATANT FROM ANAEROBIC FERMENTATION CHAMBERS (Supresión de nematodos en suelos inundados con el sobrenadante de cámaras anaerobias de fermentación).

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There is increasing effort to develop cropping systems utilizing soil amendments, flooding, and tarping to control nematodes. The decrease in nematode populations under such anaerobic conditions is attributed to the fermentative metabolites produced by soil bacterium. To produce these metabolites outside the soil environment, anaerobic fermentation chambers were prepared with bioglycerin from biodiesel production and urea with soil and water in 121 liter barrels. After fermenting for three weeks, supernatant from these chambers was tested for herbicidal and nematicidal properties in greenhouse pots, microplots, and polyethylene covered vegetable beds. Soils drenched with supernatant from anaerobic chambers had decreased root knot nematode (*Meloidogyne incognita*) and reniform nematode (*Rotylenchulus reniformis*) populations, and increased beneficial nematode and fungi populations. Positive growth response and increased yield occurred in 'Marketmore 76' cucumber (*Cucumis sativus*), 'Tiny Tim' tomato (*Solanum lycopersicum*), and 'Conqueror III' squash (*Cucumis pepo*).

PARASITISMO DEL HONGO NEMATÓFAGO *POCHONIA CHLAMYDOSPORIA* EN HUEVOS DE *MELOIDOGYNE INCOGNITA* EN MEDIO LÍQUIDO ADICIONADO CON EXTRACTOS VEGETALES EN CRUDO (Parasitism of the nematophagous fungus *Pochonia chlamydosporia* on *Meloidogyne incognita* eggs in a liquid medium supplemented with crude plant extracts).

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Se realizó un ensayo in vitro para conocer el efecto de extractos vegetales acuosos en crudo con propiedades nematicidas o nematostáticas con los aislamientos Pc341 y Pc10 del hongo nematófago *Pochonia chlamydosporia* en el porcentaje de parasitismo en huevos de *Meloidogyne incognita*. Los extractos en crudo se obtuvieron a partir de las especies *Argemone mexicana*, *Chenopodium album*, *Datura stramonium*, *Nerium oleander* y *Raphanus raphanistrum* colectadas en Morelos, México y una muestra de *Crotalaria* sp. (proveniente de Kenya). Los huevos del nematodo se obtuvieron a partir de masas de huevos colectadas de las raíces de plantas de tomate (*Solanum esculentum* var. Tiny Tim) de 6 semanas de edad. Una suspensión de huevos del nematodo (100 ml⁻¹), conidias (5.5x10³ ml⁻¹) de *P. chlamydosporia* y 1 ml del extracto vegetal a una concentración del 25% se adicionaron a un medio líquido con extracto de levadura. El diseño experimental para los tratamientos fue en bloques al azar. Los tubos y su contenido

se incubaron a 26°C y se mantuvieron en agitación constante a 150 rpm por dos días. A las 24 y 48 horas, se tomaron alícuotas de los tratamientos registrando el número de los huevos parasitados o no parasitados a partir de una muestra de 100 huevos para estimar el porcentaje de parasitismo. Los datos se analizaron con ANOVA, la comparación de medias de los tratamientos, mostró diferencias significativas a las 24 y 48 horas (LSD (0.05)= 3.742), en el porcentaje de parasitismo ejercido por los dos aislamientos del hongo en los huevos del nematodo, el cual aumentó con el tiempo de exposición de los huevos al hongo y extracto vegetal. Hubo además diferencias significativas entre tratamientos (LSD (0.05)= 4.205): en todos los tratamientos con extracto vegetal, el porcentaje de parasitismo fue disminuido siendo *Crotalaria* sp. el tratamiento que tuvo el menor porcentaje de parasitismo y *N. oleander* el mayor. En el aislamiento Pc10 se disminuyó más el porcentaje de parasitismo a las 24 y 48 horas por efecto del extracto vegetal en comparación con el aislamiento Pc341.

THE ARABIDOPSIS ICK2/KRP2 GENE HAS A CRITICAL FUNCTION ON CELL CYCLE CONTROL DURING ROOT-KNOT NEMATODE FEEDING SITE DEVELOPMENT (El gen *Arabidopsis* ICK2/KRP2 posee una función crítica en el control del ciclo celular durante el desarrollo del sitio de alimentación de nematodos agalladores).

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Plant-parasitic nematodes of the genera *Meloidogyne* trigger the formation of giant cells that undergo recurring acytokinetic mitosis and endocycles. Expression analyses of key cell cycle genes showed their early induction in the nematode feeding site (NFS). Intense DNA synthesis and enlarged nuclei suggest that giant cells undergo endoreduplication cycles. How precisely nematodes manipulate the cell cycle and cellular processes that includes prevention of cytokinesis remains to be understood. A systematic comparison of the temporal and spatial expression pattern of core cell cycle genes between uninfected roots and in galls of *Arabidopsis thaliana* resulted in the identification of a collection of genes up- or downregulated in NFS. The disruption of many *Arabidopsis* cell cycle regulators has been shown to affect both mitotic and endoreduplication cycles. Among them, negative regulators are candidates to control the cell cycle in NFS. Previous work has shown that *ICK2/KRP2*, a member of the cyclin-dependent kinase/kip-related proteins (ICK/KRP), regulate mitosis-to-endocycle transition in plant cells, and is expressed in endoreduplicating cells. The *ICK2/KRP2* gene showed to be expressed during gall development. In order to study the relevance of this cell cycle inhibitor for NFS ontogeny, mutant lines over-expressing and knocked-out were tested to determine their effect on NFS development. Herein we present the data related with the *ICK2/KRP2* gene. Our data provide a potential role of this gene in cell cycle control during the NFS formation and development.

PLANT PARASITIC NEMATODES COMMUNITIES ASSOCIATED IN THE RHIZOSPHERE OF PEACH TREES IN AN ORCHARD OF ZACUALPAN, MORELOS, MEXICO (Comunidades de nematodos fitoparásitos asociados en la rizosfera de árboles de Durazno en un huerto del municipio de Zacualpan, Morelos, México).

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The death of peach tree (DP) is a complex disease of great economic importance that affects the production of peaches in the state of Morelos, Mexico. The main causal agent of this disease is the bacteria *Pseudomonas syringae* pv. *syringae* and between the biotic factors predisposing to this disease are the species of *Mesocriconema xenoplax* and *Criconeema mutabile*. Other populations of plant parasitic nematodes (PPN) could be associated in the rhizosphere of peach trees but there is no information about their damage. The aim of this work was to make a nematological survey in an orchard located in the municipality of Zacualpan of Morelos. A directed sampling was done in patches of trees that showed apparent symptoms of DP. Four sub-samples of soil, per trees (approximately 300 g per sub sample) were taken, within the drip line to a depth of 15 and 30 cm of the soil surface and then were mixed as one soil representative of the tree. Nematodes were extracted from soil samples by flotation-centrifugation method. The samples were fixed in formaldehyde and mounted for observation of morphometric characteristics. Extraction of cysts nematodes were done by Fenwick funnel technique. The identification of plant parasitic nematodes was done by major morphological features, using identification keys. *Mesocriconema xenoplax*, *Helicotylenchus* spp, and cysts of *Cactodera* spp were found in the rhizosphere of peach orchard. This the first report of *Cactodera* in Peach trees.

POPULATION DETECTION OF MELOIDOGYNE SPECIES WITH FAME ANALYSIS (Detección poblacional de especies de *Meloidogyne* con análisis FAME).

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Continuing work to develop FAME analysis as a means for nematode diagnostics has provided promising results using both clean nematodes and infected plant tissue, but the limits of detection and identification have yet to be determined. A series of dilutions of eggs, juveniles, and mature females was used to determine the required number of nematodes for an accurate identification. Twelve replications of samples containing a range of 1–1000 eggs or juveniles and 1–10 mature females were extracted and analyzed using the Instant FAMETM and Rapid analysis methods. Statistical comparisons using stepwise and canonical analysis coupled with the Sherlock Analysis Software® were used to evaluate each dilution for reproducibility and accuracy of FAME profiles for identification. Nematodes were detectable at all dilutions, but identifiable profiles were consistently produced using 2 mature females, 5 juveniles, and 25 eggs. Fatty acids indicating the presence of nematodes included 18:1 ω7c and 20:1 ω7c and were detected at all dilution levels. Proportions of 12:0 2OH, 16:0 and 18:0 in conjunction with the indicator fatty acids were required for accurate identification using Sherlock Analysis Software®. These results can be used in further research to improve the sensitivity of FAME analysis and provide an indicator for population densities within soil and root samples.

POPULATION DEVELOPMENT OF ROTYLENCHULUS RENIFORMIS IN A FIELD OVER A NINE YEAR PERIOD (Desarrollo poblacional de *Rotylenchulus reniformis* en un campo durante un periodo de nueve años).

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The population development of *Rotylenchulus reniformis* population was monitored in a nine year period

in a predominantly Commerce silt loam field in Tensas Parish, Northeast Louisiana. To characterize the nematode population, 77 georeferenced samples were collected in a 31.8 ha area in 2003 and 2012. Twelve soil cores to a depth of 15.2-20.3 cm were collected within a 4.6 m area around each sample site. Nematodes were extracted from the samples by elutriation and centrifugation and were identified by an inverted microscope. Soil samples were analyzed for texture based on a pipette methodology. Each sampling site was characterized for electrical conductivity and elevation by the Veris 3100 soil mapping implement ranging from 8.7 to 122.9 mS/m for the 0-1 m soil depth and elevation from 21.4 to 22.8 m. In this field, the clay and sand content ranged from 6.6 to 45.7% and 3.0 to 63.9%, respectively. Initially, the nematode was found in only two locations within the field at very low levels (80-360 vermiform stages per 500 cm³ of soil). Nine years after the first sampling, the population of the nematode had both spread and increased significantly throughout the field (62 locations and 40-164,800 vermiform stages per 500 cm³ of soil). Population densities and spread of *R. reniformis* were not correlated to clay, sand, or silt content, electrical conductivity, elevation, or soil zones. Highest populations of *R. reniformis* were found when clay content was less than 20%. Reproduction of *R. reniformis* that has been reported as greater in more fine-textured soils did not follow this pattern in this study.

SESSION 1 NEW APPROACHES

A NEW ESTERASE PHENOTYPE FOUND IN *MELOIDOGYNE INCOGNITA*: A CASE STUDY (Un nuevo fenotipo de esterasa encontrado en *Meloidogyne incognita*: Un caso de estudio).

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Root systems of ornamental plants, namely *Callistemon viminalis*, *Gardenia* sp., *Plumbago auriculata*, and *Plectranthus scutellarioides* were found infected by a root-knot nematode in a home garden, Alachua County, FL. Infected plants exhibited typical root-knot disease symptoms both above and below ground. Nematode species identification was performed using morphology of selected characters of the second-stage juveniles (J2) and perineal patterns of females, and biochemical (esterase [EST] and malate dehydrogenase [Mdh]) and molecular (mtDNA, ITS and SCAR) analyses of individual females. Body, stylet and tail length of the J2 matched those of *M. incognita*. The perineal patterns were similar to those of *M. incognita*, but there was considerable variability among them. The isozyme profiles showed two bands for EST activity, with Rm = 24.5; 36.7, phenotype S1-M1, which has not been reported for *M. incognita*; and a single band of Mdh activity (N1). The latter is common for *M. incognita*, but has low diagnostic value because of similarity with other *Meloidogyne* spp. The unique EST profile was similar for all specimens tested for this population. Single egg mass isolates were established and reared on tomato 'Rutgers', and used for further investigations. Both EST and Mdh phenotypes proved to be stable. PCR amplification of mtDNA using (C2F3/1108 primer set) and ITS (F194/5368 primer set) produced fragments of ca 1.7 kb and ca. 640 bp, respectively. These are similar for both *M. incognita* and *M. javanica*. Further analysis was carried out using the *M. incognita* SCAR MI-F/MI-R primer set. A single DNA fragment of ca. 1 kb size was obtained with this SCAR primer, which has only been reported for *M. incognita*. In summary, the importance of using a combination of different methods for root-knot nematode identification is confirmed.

IDENTIFICATION OF *MELOIDOGYNE INCOGNITA* IN TWO FIG PLANTATION (*FICUS CARICA* L.) IN COSTA RICA (Identificación de *Meloidogyne incognita* en dos plantaciones de Higos en Costa Rica).

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The genus *Meloidogyne* commonly known as root-knot nematode is an important agricultural pest that causes significant crop damage and its effects are associated with loss production and its commercial value. The fig plantations have increased in recent years in Costa Rica. Small farmers have shown great interest in this crop as a commercial alternative. Current production is around 7000 kg per year, still insufficient to supply the local market. In March 2012, was conducted a soil and root sampling in some fig farms in Cartago province, root samples shows an evident *Meloidogyne* damage, small to large galls were found in root systems of two fig plantations (*Ficus carica* L.). Females, egg-masses and second-stage juveniles of *Meloidogyne* sp. were extracted from the galled roots. Females were examined for perineal patterns and second-stage juveniles were analyzed by PCR-RFLP by amplifying intergenic mitochondrial region flanked by the cytochrome oxidase II gene and the large subunit of the ribosomal RNA gene. Morphological and molecular analyzes identified the nematode population as *M. incognita*. The sizes of the PCR products obtained were of 1.7 kb. When PCR products were treated with restriction enzymes they generated four fragments of sizes 800, 480, 200 and 150 bp with *AluI* and fragments of 1300 and 400 bp with *HinfI*. These results concur with those from other studies conducted in the USA, Korea, and Japan. The use of mitochondrial DNA is an efficient and fast method for molecular identification of *Meloidogyne* species and involves fragments that change rapidly flanked by conserved regions. Production areas should be monitored in order to prevent the spread and occurrence of this pathogen in other producing areas.

SESSION 2 MAJOR NEMATODES

ANATOMICAL CHANGES CAUSED BY *MELOIDOGYNE ENTEROLOBII* ON TOMATO PLANTS (Alteraciones anatómicas causadas por *Meloidogyne enterolobii* en plantas de tomate).

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Meloidogyne enterolobii has been causing great concern for Sao Paulo State horticultural. In tomato, the resistance to *M. incognita*, *M. javanica* and *M. arenaria*, conferred by Mi gene, does not include *M. enterolobii* species. This study aimed to verify the anatomical changes caused by *M. enterolobii* in resistant tomatoes roots. The experimental design was completely randomized, with three tomato hybrids (Cordillera, Ellen and Sanni) and one root-knot nematode (*M. enterolobii*) with three replications. Plants were inoculated with 1,000 J2 of *M. enterolobii* and evaluated 30 day after inoculation. 1.0 cm long infected segments were fixed in modified Karnovsky solution and later sectioned in microtome with razor manual rotating steel type C. The 5 mm thick sections

were stained with toluidine blue 0.05% in acetate buffer, pH 4.7 for 5 minutes; slides were mounted in synthetic resin. It was observed, under light microscope, more than one feeding sites per section on vascular cylinder of 'Cordillera' and 'Ellen'. They were formed by nurse cells characterized by thick cell walls, dense and granular cytoplasm, multinucleated and with several small vacuoles. The vascular tissues are compressed and showed disorganized, with hypertrophy of cortical parenchyma cells. While in 'Sanni' there were little changes, with small nurse cells presented only in some evaluated section. This hybrid can be considered a promising for *M. enterolobii* infested areas.

INFLUENCIA DE LOS NIVELES TECNOLÓGICOS EN LA DINÁMICA POBLACIONAL DE LOS NEMATODOS EN EL CULTIVO DE BANANO. TEAPA, TABASCO. MÉXICO (Influence of technological levels in the dynamics population of nematodes in banana crop. Teapa, Tabasco. Mexico).

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Diferentes niveles tecnológicos y condiciones climatológicas fueron evaluados en la zona bananera de Teapa, Tabasco (México) en la dinámica poblacional de los nematodos *Radopholus similis*, *Helicotylenchus multicinctus* y *Meloidogyne incognita*, entre otras especies, en el período 2011-2012. Para ello se evaluaron mensualmente, siempre del mismo lote, tres muestras de raíces compuestas de tres plantas tomadas de cada finca. Los resultados mostraron que las variables evaluadas se comportan de forma diferente a través de los meses, donde las temperaturas superiores a los 34 °C son las que más influyen directamente, en forma negativa, sobre la longitud y peso de raíces y en la dinámica poblacional de *R. similis*. Mientras que la precipitación afectó el comportamiento de *H. multicinctus*. *Meloidogyne incognita* fue el único nematodo que no tiene correlación, de forma directa, con alguna variable climatológica; pero depende de la cantidad de raíces sanas. Los niveles tecnológicos no incidieron estadísticamente en las poblaciones de los nematodos, pero si marcaron diferencias en las cantidades de raíces sanas y podridas; variables muy importantes en la producción de banano.

POSITIONING NEMATOLOGY RESEARCH AND DEVELOPMENT THROUGH NIESA (NEMATOLOGY INITIATIVE FOR EASTERN AND SOUTHERN AFRICA) (Posicionando la investigación y desarrollo en nematología a través de NIESA (Iniciativa de Nematología para el Este y Sureste de África)).

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Since its inception in 2005, the Nematology Initiative for Eastern and Southern Africa (NIESA)

is a project that has been sponsored by the Gatsby Charitable Foundation (UK) to build capacity in the discipline of nematology, and to develop a network of expertise in Eastern and Southern Africa, originally with technical support from a UK consortium – CABI Bioscience, Rothamsted Research and the University of Reading. At present, NIESA is composed of a cadre of qualified nematologists from Kenya, Malawi, Tanzania, Uganda and Zimbabwe. The project has also started to move from its capacity building phase to sharing and transferring NIESA nematologists' expertise to ascertain the extent to which plant-parasitic nematodes act as a constraint to local and regional crop production and to create awareness among farmers and local communities about the importance of nematodes. To achieve this, NIESA, as a group, will continue carrying out scientific research and training of farmers and phytosanitation staff for the practical benefit for local communities, crop health and food security. Networking, joint research fund application, cross-learning and peer support among practising nematologists in Africa can facilitate an active and interactive support to overcome the lack of a critical mass of nematologists in any one country. It can also link the network to information services available through partner scientists, formal research and training collaborations to improve understanding and raise the profile of nematology within Africa. Further details of the NIESA partners and activities can be found at the web site www.africannematology.org

PROSPECCIÓN FITONEMATOLÓGICA EN VIÑEDOS DEL VALLE DE GUADALUPE, B.C. MÉXICO (Survey of plant parasitic nematodes in vineyards in the Valle de Guadalupe, B.C. Mexico).

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Se realizó una prospección de nematodos fitoparásitos en viñedos (*Vitis vinifera* L.) del Valle de Guadalupe (654.04 ha) en 25 variedades de vid en 14 ranchos. De las muestras de suelo y raíces (época de brotación), se identificaron los géneros de nematodos y las tres especies de nematodos de mayor frecuencia; se midió porcentaje de agallamiento y porcentaje de arcilla en el suelo. Los datos se analizaron mediante el modelo lineal generalizado mixto y estadística descriptiva, la relación entre los géneros de nematodos se calculó a través del valor de importancia. Se detectaron once géneros de nematodos fitoparásitos, cuatro se consideran altamente patogénicos al cultivo de vid: *Meloidogyne*, *Tylenchulus*, *Pratylenchus* y *Trichodorus*. Los géneros predominantes fueron *Meloidogyne* y *Aphelenchus*. Las especies *Meloidogyne arenaria*, *M. incognita* y *M. javanica* ocasionaron daños a las raíces con porcentaje de agallamiento mayores al 80%. La variedad de vid Gamay fue la más susceptible. Estudios preliminares sobre el combate de estos nematodos en la región mostraron diferentes grados de control con respecto a los testigos sin tratar.

RELEVAMIENTO DE NEMATODOS FITÓFAGOS RELACIONADOS A CULTIVOS DE PAPA ANDINA DEL MORENO PROVINCIA JUJUY, ARGENTINA (Survey of phytophagous nematodes related to andean potato crops of the Moreno province Jujuy, Argentina).

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Los objetivos del presente trabajo fueron identificar y determinar la riqueza y abundancia de los géneros de nematodos fitófagos de la localidad de El Moreno provincia de Jujuy. Se evaluaron 20 muestras de suelo correspondientes a dos parcelas de 20 m x 35 m cada una, tomadas en abril de 2011 correspondientes a siete variedades de *Solanum tuberosum* subsp. *andigenum*. Para su análisis se utilizó la técnica de flotación – centrifugación, en el laboratorio de Zoología General de la Facultad de Ciencias Agrarias. La abundancia relativa de *Nacobbus aberrans* se presentó con valores altos para la totalidad de las variedades de papa, “azul” (5 muestras): *N. aberrans* 86% *Tylenchorhynchus* 11% y *Meloidogyne* 3%; “collareja” (5 muestras): *N. aberrans* 82%, *Criconematidae?* sp. 16.4% y *Tylenchorhynchus* 1.6%; “blanca larga” (2 muestras): 100% *N. aberrans*; “tuni” (1 muestra): *N. aberrans* 98.3% y 1.7% *Meloidogyne*; “airampilla” (1 muestra): 100% *N. aberrans*; “charera morada” (1 muestra): 100% *N. aberrans*. La mayor riqueza genérica se observó en la variedad “ojo de señorita” (5 muestras) con r=4: *N. aberrans* 81.6%, *Tylenchorhynchus* 10.6%, *Helicotylenchus* 6.3% y 1.5% *Criconematidae?* sp.

SESSION 3 PHYSIOL PLANT DEFENSE

HISTOLOGICAL CHARACTERIZATION OF RESISTANCE TO *MELOIDOGYNE* SPP. IN CLONES OF CONILON COFFEE (Caracterización histológica de clones de café Conilon a *Meloidogyne* spp.).

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Root-knot nematodes (RKN), *Meloidogyne* spp. cause major economic impact on coffee production in Brazil. Although *Coffea canephora* is a source of resistant genes to *Meloidogyne* spp., there are no histological studies comparing mechanisms of resistance of susceptible and resistant coffee plants. Two clones of Robusta coffee plants, 14 and 22, were selected as respectively resistant and susceptible to *M. incognita* and *M. paranaensis*. Roots of both clones plus *C. arabica* (Catuaí IAC 81, a susceptible standard pattern) were fixed according to methods of Byrd (1983) and Pegard et al. (2005). The reaction of different clones to the two nematode species studied was similar. Clone 14 showed hypersensitivity reaction (HR) between the 4th and 8th days after inoculation (DAI) in the root cortex, leading to cell death, which prevented the nematode development. At 12 DAI, giant cells formed in the vascular cylinder besides normally developing J3/J4. From the 32th to 45th DAI, HR was observed along with dead cells around females and completely degenerated giant cells. During this period, some fully-grown females even developed early stages of ovaries, although they did not produce eggs. In the susceptible clones, 22 and Catuaí IAC 81, well-formed giant cells and adult females appeared between the 38th and 45th DAI, also with egg production. Plants used in the histopathological studies were replanted and evaluated to corroborate to previous results on resistance/susceptibility. Three hundred DAI, plants were evaluated and the responses obtained in previous studies were confirmed. These results provide bases for further gene expression studies, which will profoundly examine resistance to RKN at molecular the level.

SESSION 4 NOVEL SOURCES

MALEZAS PRESENTES EN EL AGROECOSISTEMA DE ESPÁRRAGO (*ASPARAGUS OFFICINALIS* L.) QUE FAVORECEN LA DISEMINACIÓN DE *MELOIDOGYNE* SPP. (Weeds present in the agroecosystem of asparagus (*Asparagus officinalis* L.) that promote the dissemination of *Meloidogyne* spp).

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El presente trabajo se realizó con el propósito de analizar el rol que juegan las malezas, presentes en los campos de producción de espárrago, en la disseminación y multiplicación del “nematodo del nódulo de la raíz” *Meloidogyne* spp. Para tal propósito se colectaron e identificaron las diferentes especies de malezas que predominan en los campos de espárrago. De estas especies se tomaron muestras de raíces que fueron procesadas mediante el método del hipoclorito de sodio al 0.5 % para determinar la presencia de huevos y juveniles. Las malezas predominantes en el agroecosistema del espárrago fueron *Cloris halofila*, *Eragrostis ciliaris*, *Eleusine indica*, *Portulaca oleracea*, *Amaranthus celosoides*, *Chenopodium ambrosoides*, *Dactylactemium aegyptum*, *Sonchus oleraceus* y *Amaranthus viride*. En las raíces de todas las especies evaluadas se encontró gran cantidad de huevos y juveniles (J2), destacando *Eleusine indica* con 1 775 500 de huevos y 5 700 juveniles (J2) en 5 gramos de raíces. Pese a la densa población de huevos y juveniles que se encontró, las raíces no presentaban los nódulos típicos producidos por *Meloidogyne* sino un frisado y ligero engrosamiento de los ápices. Estos resultados reafirman el carácter polífago de *Meloidogyne* spp. y la importancia de la eliminación de estas especies como un componente cultural en el manejo integrado de este fitopatógeno.

SCREENING OF SOYBEAN CULTIVARS FOR RESISTANCE TO *HETERODERA GLYCINES* IN NORTHWESTERN ARGENTINE (Evaluación de la resistencia de variedades de soja a *Heterodera glycines* en el Noroeste Argentino).

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The soybean cyst nematode, *Heterodera glycines*, is one of the most important plant parasitic nematode of soybean, *Glycine max*, in Northwestern Argentina. This nematode was detected in the soybean area in 1998 and some populations were classified in races 3, 5 (Hg Type 2,5,7) and 6 (Hg Type 5,7). Crop rotation and resistant cultivars are effective means of managing this pest. Numerous cultivars adapted to the region have been evaluated in greenhouse for their reaction to races 5 and 6 of soybean cyst nematode, few varieties showed resistance and moderated resistance. The objective of this research was to evaluate the response of commercial soybean cultivars to *Heterodera glycines*, race 6 (HG Type 5,7). The experiment was conducted under greenhouse conditions in a completely randomized design with seven repetitions per cultivar. Seventeen soybean varieties were evaluated and seedlings of each cultivar were inoculated with 4,770 eggs and juveniles. The evaluation was done 28 days after inoculation by counting the number of females on each root-system. Reaction of cultivars was determined base on female index. Most of the cultivars tested were classified as susceptible (female index values ranged from 70.5 to 114.8 %). Only two varieties were moderately susceptible, DM 6500 RR and TJ 2171 RR, with female index of 55.2 and 54.1 % respectively. Further information about reaction of varieties to this nematode is necessary to improve the control of this pest in the region.

SESSION 5 ENTOMOPATOGENOS

AISLAMIENTO Y SELECCIÓN DE NEMATODOS ENTOMOPATÓGENOS ASOCIADOS AL CULTIVO DE MAÍZ EN EL MUNICIPIO DE GUASAVE, SINALOA, MÉXICO (Isolation and selection of entomopathogenic nematodes associated with corn in Guasave, Sinaloa, Mexico).

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Las plagas más importantes del cultivo de maíz en Sinaloa son el “gusano cogollero” *Spodoptera frugiperda* Smith (Noctuidae: Lepidóptera), el “gusano elotero” *Helicoverpa zea* Boddie (Noctuidae: Lepidóptera) y un complejo de moscas de los estigmas de los géneros *Euxesta stigmatias* Loew, *Chaetopsis massyla* Walker y *Eumecosommyia nubila* Wiedemann. Se combaten mediante control químico, que resulta poco eficiente además de causar contaminación; por lo tanto es necesario desarrollar otras alternativas de control como el uso de Nematodos Entomopatógenos (ENP). Estos, han sido poco estudiados en esta región y pueden ser desarrollados como bioplaguicidas. El objetivo de esta investigación fue aislar y seleccionar los ENP asociados al cultivo de maíz. Durante los ciclos agrícolas 2011 y 2012 se procesaron 42 muestras de suelos de la región, se han aislado cinco poblaciones de nematodos entomopatógenos pertenecientes a la familia Rhabditidae, incluyendo el género *Heterorhabditis* y otros no identificados que a nivel laboratorio han manifestado capacidad infectiva sobre larvas de *Galleria melonella* L., sobre el gusano cogollero de maíz *S. frugiperda* Smith y en pruebas sobre pupas de la mosca de los estigmas *E. stigmatias* (Loew) también han resultado positivas. Se tienen consideradas evaluaciones de invernadero y campo para determinar la factibilidad de incorporarlos en un programa de manejo integrado de las principales plagas del maíz con énfasis en control biológico.

CARACTERIZACIÓN MORFOMÉTRICA Y MOLECULAR DE UN AISLAMIENTO NATIVO DE *STEINERNEMA* SP. DE LA ZONA SUR DE CHILE (Morphological and molecular characterization of a native isolate of *Steinernema* sp. from southern Chile).

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Debido al potencial de los nematodos entomopatógenos (EPN), en Chile hace desde hace una década se han incrementado los estudios sobre estos nematodos. Aislamientos de estos nematodos en bosques de robles de la localidad de Licanray, en la IX región de Chile, indicaron la factibilidad de identificar una nueva especie perteneciente al género *Steinernema*. Identificación morfológica y molecular (análisis de secuencias 28S e ITS de rDNA) fueron empleadas para los propósitos de diagnóstico e identificación. El análisis de las secuencias indicó que este aislamiento de *Steinernema*, pertenece al clado III, correspondiente al grupo *feltiae*, siendo muy cercana a *S. weiseri* y *S. feltiae*. Morfológicamente esta especie está caracterizada por un juvenil infectivo de largo medio de cuerpo de 807 μm ; largo medio de cola de 74 μm , ubicación media del poro excretor de 55 μm ; %D (media 46); %E (media 75) y %H (media 36). Ambas generaciones de machos presentan mucrón, siendo más largo en la segunda generación (11 μm) que en la primera (5 μm). La primera generación de machos posee un largo medio de 1428 μm , largo medio de espícula de 68 μm ; largo medio de gubernáculo de 46 μm y %SW de 1,7, caracterizándose además por poseer seis crestas en los campos laterales. Los antecedentes analizados sugieren la factibilidad de que el aislamiento de *Steinernema* pueda corresponder a una especie no descrita, debiendo completarse con estudios de hibridación cruzada.

INFECTIVITY OF AN ISOLATE OF STEINERNEMA SP. (STEINERNEMATIDAE) IN LARVAE OF *PSEUDOPPLUSIA INCLUDENS* (LEPIDOPTERA: NOCTUIDAE) AND *TENEBRIO MOLITOR* (COLEOPTERA: TENEBRIONIDAE) (Infectividad de un aislamiento de *Steinernema* sp. (Steinernematidae) en larvas de *Pseudoplusia includens* (Lepidoptera: Noctuidae) y *Tenebrio molitor* (Coleoptera: Tenebrionidae)).

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Pseudoplusia includens is an important defoliating species of several cultivated plants, such as cotton, tobacco, and alfalfa, whereas *Tenebrio molitor* is a stored-grain pest. Susceptibility of larvae of those insects was evaluated under laboratory conditions by applying an isolate of *Steinernema* sp. recently detected in soil from the locality of La Bolsa (Santa María department, Córdoba province, Argentina). Two doses of infective juveniles (IJ) were used: 50 and 100. Infections (n=15) were performed individually in Petri dishes (35 mm diameter) and kept at 25°C. Mortality (expressed as percentage) was checked every 24 h during 9 days post-inoculation (DPI). Once the insect was dead it was placed in a White trap to ensure IJ emergence. Larvae died after different periods, depending on the inoculum dose: a) *P. includens*: 2 to 6 DPI (at a dose of 50 IJ) and 1 to 8 DPI (100 IJ); b) *T. molitor*: 2 to 5 DPI (50 IJ) and 2 to 6 DPI (100 IJ). Mortality was 100% for *P. includens* (both doses) and 40% (50 IJ) and 60% (100 IJ) for *T. molitor*. Mortality percentage showed significant differences between species but not between doses for a single species. In all the treatments, the nematode completed its life cycle and produced new IJ. The insects, especially *P. includens*, showed susceptibility to the isolate evaluated.

PRIMER REPORTE DE POBLACIONES NATIVAS DE NEMATODOS ENTOMOPATÓGENOS EN EL VALLE DE GUASAVE, SINALOA, MÉXICO (First report of entomopathogenic nematodes in the Guasave agricultural Valley at Sinaloa, Mexico).

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Los nematodos entomopatógenos (NEP) son agentes potenciales de control biológico de numerosas plagas de insectos. En este estudio se aislaron e identificaron especies nativas de nemátodos entomopatógenos presentes en el Valle de Guasave. Se tomaron muestras al azar de suelos agrícolas y se expusieron larvas de *Galleria mellonella* Linnaeus utilizadas como cebo o trampas para aislar NEP. Las poblaciones de nematodos que se reprodujeron en las larvas, fueron sometidas a los postulados de Koch. Los especímenes se procesaron con la técnica de Seinhorst, se elaboraron preparaciones permanentes para su identificación morfológica y se fotografiaron con un microscopio de fluorescencia Leica DM6000 CS. También se caracterizaron molecularmente mediante PCR, amplificando los segmentos D2 y D3 del gen 28S del ADN Ribosomal (oligonucleótidos D2A y D3B) y LSU. Se aislaron 8 poblaciones de nematodos entomopatógenos, hasta el momento se han estudiado molecularmente dos poblaciones: Población 1 (Callejones de Tamazula) *Diplogastrellus metamasius* (98% de similitud) y población 2 (Las Playitas) *Rhabditis rainai* (100% de similitud), se han tomado fotografías para su identificación morfológica. La presencia de estos nematodos en los suelos cultivados con maíz, proporciona una oportunidad para su utilización como posibles agentes de control biológico para el manejo del gusano cogollero, principal plaga de este cultivo. Con la identificación de estas primeras dos poblaciones se están sentando las bases para desarrollar métodos compatibles con el medio ambiente en la región para el manejo de insectos plaga.

SESSION 6 NOVEL STRATEGIES

No Posters

SESSION 7 CONTROL DE NEMATODOS

ACTIVIDAD DE COMPUESTOS ORGÁNICOS VOLÁTILES DE *FUSARIUM OXYSPORUM* EN JUVENILES DE SEGUNDO ESTADIO DE *MELOIDOGYNE INCOGNITA* UTILIZANDO FRASCOS SUPELCO® (Activity of organic volatile compounds from *Fusarium oxysporum* on J2 of *Meloidogyne incognita* using Supelco flaks).

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Se emplearon frascos de SUPELCO® de 80x28 mm con tapa roscada y revestida internamente por una película de silicona, permitiendo un sellado completo. En el frasco se colocaron 15 mL de medio agar-agua. Después de la solidificación, se añadieron 6 mL de medio agar malta (AM) para permitir el cultivo de *Fusarium oxysporum* (FO), fuente productora de compuestos orgánicos volátiles (COVs). Un microtubo esterilizado de 1.5 mL fue introducido en el medio de cultivo. Dos discos de 2 mm de diámetro fueron retirados de los bordes de la colonia de FO y depositados sobre el medio AM, al lado del microtubo. Después del sellado, los tubos fueron incubados en BOD a 25°C en oscuridad durante 0, 2, 4 y 8 días. En cada periodo de incubación en los frascos cultivados con FO, con ayuda de una jeringa, fue depositado en el interior del microtubo 1 mL de una suspensión con 250 juveniles de segundo estadio (J2) de *Meloidogyne incognita* (MI). Los frascos fueron mantenidos en cámara por 48 h a 25°C. El experimento fue realizado en un diseño completamente aleatorizado con 6 repeticiones y 3 aislamientos (3, 13 y 21) de FO. En la evaluación, después de la apertura de los frascos, se contó el número de J2 móviles e inmóviles. Los COVs producidos por los tres aislamientos de FO probados causaron inmovilidad significativa a los J2 de MI comparados con el control. La mayor inmovilidad y mortalidad fue causada por los COVs del aislamiento 21 y la más baja ($P \leq 0,05$) por los COVs de los aislamientos 3 y 13. COVs del aislamiento 3 no causaron efecto nematocida en J2 de MI, demostrando que los COVs del aislamiento 21 tienen gran potencial en el desarrollo de nuevos nematocidas.

EFFECTO DEL HONGO MICORRIZICO ARBUSCULAR GLOMUS INTRARADICES EN PLANTAS DE TOMATE PARASITADAS POR *NACOBBUS ABERRANS* (Effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on tomato plants infected by *Nacobbus aberrans*).

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Nacobbus aberrans ocasiona pérdidas en diversos cultivos hortícolas, entre ellos el tomate (*Lycopersicon esculentum*). Una posible alternativa de manejo es la utilización de hongos micorrizico

arbusculares (HMA) como control biológico. El objetivo de este trabajo consistió en estudiar el efecto de *Glomus intraradices* en tomate parasitado por una población del nematodo (NEM) proveniente de Río Cuarto (Córdoba, Argentina). Se llevaron a cabo 6 tratamientos (6 réplicas) que comprendieron plantas: a) sin inocular (Control), b) inoculadas con HMA al momento del trasplante (T0), c) inoculadas con NEM T0, d) inoculadas con HMA T0+NEM T0, e) trasplantadas en T0 e inoculadas con NEM tres semanas después (T3), f) inoculadas con HMA T0+NEM T3. A los 60 días se estimó: porcentaje de micorrizas arbusculares (%MA), peso de la raíz y parte aérea, número de agallas (NA) y factor de reproducción (FR=Población final/Población inicial) de *N. aberrans*. El %MA fue mayor en presencia del nematodo (d=36%; f=44% versus b=23%). La biomasa aérea y radical se incrementó en las plantas micorrizadas sólo en T0. La aplicación de HMA en T0 redujo significativamente el NA y el FR en un 56% y 69%, respectivamente; no se observó el mismo efecto en las plantas inoculadas en T3. El empleo de *G. intraradices* al momento del trasplante produjo un aumento de la biomasa del tomate y disminuyó la cantidad de agallas inducidas por *N. aberrans* así como su multiplicación.

RHODOTORULA RUBRA ACTIVITY AND BACILLUS SP. ISOLATED FROM RHIZOSPHERE OF VITIS VINIFERA VAR CHARDONNAY, CASABLANCA VALLEY, CHILE ON MELOIDOGYNE ETHIOPICA AND TOMATO ROOTS, IN VITRO AND GREENHOUSE CONDITIONS (Actividad de *Rhodotorula rubra* y *Bacillus* sp. aislados de rizósfera de *Vitis vinifera* var Chardonnay, Valle de Casablanca, Chile, sobre *Meloidogyne ethiopica* y raíces de tomate, en condiciones in vitro e invernadero).

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Cepas de *Rhodotorula rubra* y *Bacillus* sp aisladas de la rizósfera de vid (var. Chardonnay) de aproximadamente 20 años de establecidas, en Casablanca Valley, Chile se seleccionaron esperando que hubiera alta patogenicidad debido a la coevolución entre *Meloidogyne ethiopica* y dichos microorganismos. Los microorganismos seleccionados fueron reproducidos en medios de cultivo y sobre huevos de *M. ethiopica*, con la idea de seleccionar los aislamientos con mayor potencial de control biológico. Los individuos capaces de crecer sobre huevos y juveniles fueron reproducidos en medios líquidos altamente nutritivos, y luego de obtener concentraciones de 10⁸ unidades formadoras de colonias, se probaron en condiciones de invernadero. Ambos microorganismos mostraron actividad nematófaga, pero también parasitaron a las plantas de tomate en condiciones de invernadero. Tanto *R. rubra* como *Bacillus* sp. fueron capaces de atacar a las raíces de las plantas de tomate generando daños funcionales relevantes a nivel de xilema y floema. Esta sería la primera cita que da cuenta de la capacidad de *Rhodotorula rubra* de nutrirse sobre nemátodos y dañar tejido xilemático de raíces de tomate.

COMPORTAMIENTO SOBRE LA POBLACIÓN DE NEMATODOS EN EL CULTIVO DE MAÍZ (*ZEAMAYS*) INOCULADO CON HONGOS MICORRÍZICOS (Behavioural effects on nematodes population in maize crop (*Zea mays*) inoculated with mycorrhizal fungi).

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El daño que causan los nematodos al maíz pasan desapercibidos pero pueden evidenciarse mejor si factores

abióticos afectan el desarrollo de las plantas. Los nematodos asociados al maíz en el altiplano de México son: *Aphelenchus avenae*, *Helicotylenchus erythrinae*, *Heterodera punctata* (*Puntodera chalconensis*), *Pratylenchus penetrans*, *Tylenchorhynchus acti*, *Pratylenchus nannus* y *Trichodorus* sp. Por otro lado, de las asociaciones biológicas de carácter simbiótico que se encuentran en la naturaleza, la micorrización ha cobrado gran importancia en las dos últimas décadas en los que se han establecido ensayos de inoculación de micorrizas en distintos cultivos con fines de protección. Se ha reportado que la presencia de un hongo formador de micorrizas eficaz reduce en alguna forma la invasión y reproducción de nematodos en Maíz. El objetivo general de este trabajo fue evaluar el efecto de los hongos micorrízicos inoculados a semillas y plantas de maíz sobre el comportamiento de las comunidades de nematodos. Se trabajó con suelo infestado naturalmente por nematodos fitoparásitos y se utilizó una variedad de maíz criollo local. Se aplicó un producto comercial conteniendo no menos de 2×10^4 unidades formadoras de colonias (UFC) de *Glomus* sp. por cada kilogramo de dicho producto bajo un diseño experimental completamente al azar. Los resultados del análisis de varianza mostraron diferencias significativas en todas las variables evaluadas, excepto área foliar inicial y población inicial de quistes. El tratamiento donde se tuvo el mejor desarrollo del cultivo fue el de inoculación de micorrizas en semilla (Tukey, 0.05%) seguido de la inoculación de micorrizas en plántula y el peor fue el testigo (sin inoculación de micorrizas). También se encontró que el cultivo de maíz al asociarse con hongos micorrízicos, del género *Glomus* sp., tuvo 50% más de desarrollo y crecimiento, en promedio. En altura de plantas de maíz con NMS presentó un 30% más de crecimiento con respecto a NsM, en el diámetro del tallo 34%, área foliar final 54%, peso fresco de planta 61%, peso de raíz 43% y volumen de raíz 73%. Se concluye que es mejor inocular la micorriza en semilla y trasplantarla mínimo 15 días después de la inoculación.

SESSION 8 SOIL HEALTH

EFFECT OF NUTRITIONAL CONDITIONS ON GENE EXPRESSION OF THE NEMATODE PARASITIC FUNGUS *POCHONIA CHLAMYDOSPORIA* (Efecto de las condiciones nutricionales en la expresión de genes del hongo parásito de nematodos *Pochonia chlamydosporia*).

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Pochonia chlamydosporia is a biocontrol agent of nematodes exploiting a wide range of trophic niches in the rhizosphere, including saprophytism, parasitism and endophytism. *In vitro* assays were performed on some genes involved in different metabolic pathways under varying nutritional conditions. Aim of this work was to produce data on the biochemical signals affecting the fungus behaviour in soil. The gene transcripts of monooxygenase, phospholipase D, phytase, and bzip transcription factor were quantified by qPCR of *P. chlamydosporia* mycelium grown in presence of *Meloidogyne incognita* (RKN) eggs and in the interaction with healthy and RKN-parasitized tomato plants. Fungus grown in minimal nutrient artificial medium was considered as control. Results showed that, after 8 hours incubation, RKN eggs stimulated transcription of all genes analyzed. At this time the bzip transcription factor, phytase, phospholipase D and monooxygenase transcripts were 3, 14, 900 and 67-fold higher than those of control, respectively. Healthy plants alone induced, after 4 hours incubation, a phytase transcription 6-fold higher than control. Changes in transcripts amounts were not significant when the fungus was incubated in presence of parasitized plants. The transcripts increases observed under the nutritional conditions tested suggest a clear and immediate gene activation response of *P. chlamydosporia*, confirming its parasitic behaviour. Data suggest a possible role of these genes in the events occurring when the fungus is in contact with eggs. However, roots also affected the activation or repression of the *P. chlamydosporia* genes, thus introducing a further complexity level in the tri-trophic host-parasite interactions.

SESSION 9 NEMATODES ECOLOGY

NEMATODOS FITOPARÁSITOS EN ÁREAS CULTIVADAS Y NO CULTIVADAS DE LA REGIÓN AGRÍCOLA DE CABORCA, SONORA, MÉXICO (A survey of plant parasitic nematodes on cultivated and non-cultivated soils in Caborca, Sonora, Mexico).

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La agricultura es la actividad económica más importante en el Municipio de Caborca, Sonora, México. Esta actividad se desarrolla en una superficie aproximada de 107,000 has y 1,005 pozos profundos que la irrigan destacándose principalmente los cultivos de espárrago, uva, trigo y hortalizas. Se realizaron muestreos para la detección de nematodos fitoparásitos en 21 campos agrícolas y ocho cultivos diferentes en la Región Agrícola de Caborca, Sonora, México, con el objetivo de determinar su presencia, distribución y asociación con cultivos y malezas. Los cultivos muestreados fueron vid, espárrago, olivo, alfalfa, pepino, melón, nogal y algodón. Diez muestras compuestas de suelo, fueron tomadas al azar en cada sitio de muestreo durante el periodo de abril a noviembre del 2011 y de marzo a junio del 2012. Estas muestras incluyeron tanto áreas cultivadas como áreas no cultivadas. Las muestras se tomaron a 30 cm de profundidad y se transportaron en una hielera al laboratorio de nematología de la Universidad de Sonora Unidad Norte Caborca, donde se procesaron por los métodos de embudo de Baerman y tamizado. La identificación se realizó usando las características morfométricas de los especímenes, un microscopio compuesto Olympus BX51 e Image pro plus software. En cada sitio de muestreo se tomó aproximadamente 1kg de suelo para determinar sus propiedades físicas y químicas (pH, materia orgánica y textura). Adicionalmente se registro la flora característica por sitio de muestreo. Se identificaron 16 especies de nematodos fitoparásitos. Entre las especies más comunes se encontraron, *Meloidogyne* spp., *Xiphinema* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Ditylenchus* spp., *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., y *Longidorus* spp.

UPDATING DATABASES ON PLANT-PARASITIC NEMATODES REPORTED IN BRAZIL (Atualização de bases de dados sobre nematodos fitoparásitos reportados em Brasil).

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Embrapa Genetic Resources and Biotechnology, since 1977 performs international exchange of plant germplasm to meet the needs of the Brazilian Agricultural Research System, which aggregates several research institutions responsible to develop new varieties of various crops. To ensure the sanity of materials that are brought into the country, Embrapa's Laboratory of Plant Quarantine (LQV) conducts the phytosanitary analyzes required for such materials. To support nematological analyzes and other activities that are performed in the Nematology Unit of LQV the following databases were developed: Brazilian Bibliography of Nematodes, and Geographic Distribution of Nematodes in Brazil. In order to modernize the system comprising these two databases, as well

as to update the data, a search is being conducted in all national and international publications involving nematodes in Brazil. They are being checked journals, books, conference abstracts and other forms of scientific communication. From each publication are extracted and registered in the database information as: authors, the scientific name of the nematode, host plant, subject to which refers the publication, geographic information (state in Brazil where it occurs), if available in the publication, and respective bibliographic reference. The updated databases will be available for consultation via the Internet, and it will be possible report output on the registered data.

SESSION 10 NACOBBUS

HISTOPATOLOGÍA DE RAÍCES DE CULTIVARES DE PIMIENTO (*CAPSICUM ANNUM*) INFECTADOS POR *NACOBBUS ABERRANS* (Histopathology of roots of pepper (*Capsicum annum*) cultivars infected with *Nacobbus aberrans*).

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El objetivo del trabajo fue caracterizar la asociación entre una población de *Nacobbus aberrans* proveniente de Río Cuarto (Córdoba, Argentina) con tres cultivares de pimiento utilizados en esa zona (INTA Fyuco, Híbrido 35-615 y Yatasto), para los cuales se desconoce su respuesta frente al ataque de este nematodo fitófago. Como testigo susceptible, se utilizó California wonder. Raíces (con y sin agallas) fueron procesadas (deshidratación-inclusión-coloración), obteniéndose cortes para microscopía óptica. En agallas de todos los cultivares se observaron hembras del nematodo e hiperplasia celular en el cilindro central. Los sincitios, adyacentes a los tejidos de conducción, ocuparon hasta el 80% de la superficie del cilindro vascular en el testigo (máximo desarrollo con relación a los sincitios de los otros cultivares). Esto ocasionaba reducción e interrupción de tejidos, alterando principalmente la posición de los elementos conductores del xilema. Las células sincitiales tenían citoplasma denso con numerosas vacuolas y núcleos hipertróficos. En INTA Fyuco y Yatasto, se encontraron además estadios juveniles en los estratos corticales más superficiales de las agallas, posiblemente resultado de recientes infecciones. El análisis histopatológico puso en evidencia la susceptibilidad a *N. aberrans* de los cultivares evaluados.

SESSION 11 NEMATODES AS INDICATORS

No Posters

SESSION 12 MARINE NEMATODES

No Posters