VARIABILIDADE GENÉTICA E BIOLÓGICA DE *Meloidogyne exigua* E PATOGENICIDADE DE *Meloidogyne* spp. EM GENÓTIPOS DE CAFEEIRO

MARIA DE FÁTIMA SILVA MUNIZ

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Orientador

Prof. Dr. Vicente Paulo Campos

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Dra. Regina Maria Dechechi Gomes Carneiro	EMBRAPA/CENARGEN
Dr. Antônio Alves Pereira	EPAMIG
Prof. Dra. Antônia dos Reis Figueira	UFLA
Prof. Dr. Mário Sobral de Abreu	UFLA

Prof. Dr. Vicente Paulo Campos
UFLA
(Orientador)

LAVRAS
MINAS GERAIS – BRASIL

A Deus, pelo dom da vida.

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SUMÁRIO

RESUMO

MUNIZ, Maria de Fátima Silva. **Variabilidade genética e biológica de *Meloidogyne exigua* e patogenicidade de *Meloidogyne* spp. em genótipos de cafeeiro.** 2007. 99p. Tese (Doutorado em Fitopatologia) – Universidade Federal de Lavras, Lavras, MG.*

Estudos isoenzimáticos e técnicas moleculares (SCAR e RAPD-PCR) foram realizados em 15 populações de três raças de *Meloidogyne exigua*, parasitas do cafeeiro no Brasil, Bolívia e Costa Rica e em uma população obtida de seringueira no Brasil. Esses estudos revelaram quatro fenótipos de esterase (E1, E2, E2a e E3) e três de malato-desidrogenase (N1, N1a e N2). Os *primers* SCAR em condição multiplex-PCR permitiram a identificação de todas as populações de *M. exigua*. Análises filogenéticas mostraram alto polimorfismo intra-específico (25,9-59,6%) para todas as populações estudadas. Entretanto, todas agruparam-se com 100% de bootstrap. Além disso, a caracterização de uma população de *M. exigua* obtida de seringueira revelou algumas diferenças, comparadas àquelas populações descritas no cafeeiro, principalmente na morfologia do estilete de macho e fêmea. Todavia, apresentou similaridades em várias características morfológicas e morfométricas, fenótipo E1 de esterase (Rm 1,5), em dados citológicos (número de cromossomos) e moleculares (SCAR e RAPD-PCR). Em outro estudo, 10 populações de *Meloidogyne* spp. foram inoculadas em sete genótipos de cafeeiro em casa de vegetação. As cultivares Obatã IAC 1669-20, Sarchimor IAC 4361 e Tupi Amarelo IAC 5111 exibiram suscetibilidade às quatro populações brasileiras de *M. exigua*. Entretanto, cv. Tupi Vermelho IAC 1669-33 mostrou-se resistente (FR = 0,7) a uma população de *M. exigua* proveniente de Lavras, MG, Brasil. A população de *M. exigua* oriunda de Bom Jesus de Itabapoana, RJ, Brasil, foi altamente virulenta (FR = 165,7) à cv. IAPAR 59, portadora do gene de resistência Mex-1 e ao genótipo H 419-5-4-5-2 (FR = 396,2). A população de *Meloidogyne* sp. do cafeeiro, Garça, SP, Brasil, reproduziu-se em baixos níveis (FR = 0,1-3,9) sobre todos os genótipos. Todas as cultivares foram suscetíveis a *M. incognita* e *M. paranaensis*. A reprodução de *M. mayaguensis* obtida de goiabeira, PR, Brasil, foi baixa (FR = 0,0-1,6), em todos os genótipos testados. Entretanto, a mesma espécie obtida do cafeeiro na Costa Rica apresentou valores de FR que variaram de 0,8 a 12,4. Os resultados deste trabalho mostraram, pela primeira vez, a quebra de resistência da cultivar IAPAR 59, resultante do cruzamento *C. arabica* cv. Villa Sarchi x Híbrido do Timor por uma população de *M. exigua* obtida em campo.

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*Orientador: Prof. Dr. Vicente Paulo Campos – UFLA e Dra. Regina Maria Dechechi Gomes Carneiro – Embrapa/Cenargen (co-orientadora)

ABSTRACT

MUNIZ, Maria de Fátima Silva. **Genetic and biological variability of *Meloidogyne exigua* and pathogenicity of *Meloidogyne* spp. on coffee genotypes.** 2007. 99p. Thesis (Doctorate Program in Phytopathology) – Federal University of Lavras, Lavras, MG.*

Isozyme profiles and molecular techniques (SCAR and RAPD-PCR) were studied on fifteen populations of three races of *Meloidogyne exigua,* collected from coffee-producing areas in Brazil, Bolívia and Costa Rica and one population from rubber tree plantations in Brazil**.** This study revealed four esterase phenotypes (E1, E2, E2a, E3) and three malate dehydrogenase phenotypes (N1, N1a, N2). Multiplex PCR using SCAR primers allowed the identification of all *M. exigua* populations. Phylogenetic analyses showed high intraspecific polymorphism (25.9–59.6%) for all *M. exigua* studied. However, all populations clustered together with 100% of bootstrap. Furthermore, the characterization of one population of *M. exigua* from rubber tree revealed some differences compared with those described on coffee, particularly in the female and male stylet morphology. Nevertheless, the population from rubber tree was closely related with *M. exigua* from coffee, considering several morphological and morphometric features, esterase band E1 (Rm=1.5), cytological (chromosome number) and molecular approaches (SCAR and RAPD-PCR). In an other study, ten populations of *Meloidogyne* spp. were inoculated on seven coffee genotypes under greenhouse conditions. The cultivars Obatã IAC 1669-20, Sarchimor IAC 4361 and Tupi Amarelo IAC 5111 exhibited susceptibility to the four Brazilian *M. exigua* populations tested. However, cv. Tupi Vermelho IAC 1669-33 revealed resistance (RF= 0.7) to *M. exigua* population from Lavras, MG, Brazil. The *M. exigua* population from Bom Jesus de Itabapoana, RJ, Brazil was highly virulent (RF= 165.7) on cv. IAPAR 59, bearing resistance gene Mex-1 and on genotype H 419-5-4-5-2 (RF= 396.2). *Meloidogyne* sp. population from coffee, Garça, SP, Brazil, reproduced at low rates (RF = 0.1-3.9) on all genotypes. All cultivars were susceptible to *M. incognita* and *M. paranaensis*. *M. mayaguensis* from guava PR, Brazil, multiplied at low rates (RF= 0.0-1.6) in all coffee genotypes. However, the same species from coffee, Costa Rica showed RF value that ranged from 0.8 to 12.4. Our results showed the first naturally resistance–breaking field populations of *M. exigua* on the cultivar IAPAR 59 derived from crossing *C. arabica* cv. Villa Sarchi x Timor Hybrid.

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*Adviser: Prof. Dr. Vicente Paulo Campos – UFLA and Dra. Regina Maria Dechechi Gomes Carneiro – Embrapa/Cenargen (co-adviser)

INTRODUÇÃO GERAL

O gênero *Meloidogyne* compreende mais de 80 espécies descritas (Karssen & Van Hoenselaar, 1998), dentre as quais 17 já foram relatadas parasitando o cafeeiro (*Coffea arabica* L.) no mundo (Campos & Villain, 2005). Recentemente, *M. izalcoensis* Carneiro, Almeida Gomes & Hernandez foi relatada em El Salvador (Carneiro et al., 2005). No Brasil, *M. exigua* Göldi, *M. incognita* (Kofoide & White) Chitwood e *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida são consideradas as espécies mais importantes (Campos & Villain, 2005).

Em condição de campo no Noroeste do estado do Rio de Janeiro, Barbosa et al. (2004) estimaram perdas de produtividade de até 45% em cafeeiros afetados por *M. exigua*. Essa espécie foi a primeira reconhecidamente importante economicamente para a agricultura brasileira (Göldi, 1987). *Meloidogyne exigua* está amplamente disseminada em áreas produtoras de café no Brasil (Campos & Villain, 2005) tendo sido relatada nos estados de São Paulo (Lordello & Zamith, 1958), Espírito Santo (Chebabi, 1968), Bahia (Lordello, 1971; Souza et al., 1997), Ceará (Ponte & Senna-Silva, 1972), Minas Gerais (Campos & Melles, 1987), Rio de Janeiro (Barbosa et al., 2004) e Paraná (Portz et al., 2006). Além do cafeeiro, *M. exigua* é também um importante patógeno da seringueira (*Hevea brasiliensis* Muell. Arg.) no estado do Mato Grosso (Santos et al., 1992; Bernardo et al., 2003).

Meloidogyne incognita foi assinalada em São Paulo (Lordello & Mello-Filho, 1970), Espírito Santo (Lordello & Hashizume, 1971; Lima et al., 2007), Paraná (Lordello & Lordello, 1972), Ceará (Ponte & Castro, 1975), Minas Gerais (Campos & Melles, 1987) e Bahia (Souza et al., 2000). Recentemente, uma nova espécie do gênero *Meloidogyne* foi descrita em cafeeiros no estado do Paraná e denominada *M. paranaensis* (Carneiro et al., 1996b). Posteriormente,

foi também detectada em Minas Gerais (Castro et al., 2003; Castro & Campos, 2004). De acordo com Campos & Villain (2005), essa espécie é tão destrutiva para o cafeeiro quanto *M. incognita*, principalmente nos estados do Paraná e São Paulo.

Meloidogyne mayaguensis Rammah & Hirschmann é um fitopatógeno economicamente importante em goiabeira (*Psidium guajava* L.), no Brasil (Carneiro et al., 2001; Torres et al., 2004; 2005; Almeida et al., 2006; Silva et al., 2006; Asmus et al., 2007). Em casa de vegetação, uma população de *M. mayaguensis* proveniente de goiabeira infectou o cafeeiro cv. Mundo Novo (Carneiro, 2003). Entretanto, não existem estudos sobre sua patogenicidade em outros genótipos de cafeeiro no Brasil.

A utilização de nematicidas, rotação de culturas, destruição de plantas atacadas, plantio em local isento dos fitonematóides, uso de mudas sadias, de porta-enxerto resistente e o emprego de cultivares resistentes são táticas indicadas para o controle da meloidoginose no cafeeiro (Campos & Villain, 2005). Segundo Moura (1997), o uso de cultivares resistentes é a mais eficiente, a mais prática e a mais econômica de todas.

Em 1987, o Instituto Agronômico de Campinas (IAC) liberou o porta-enxerto Apoatã IAC 2258 de *C. canephora* Pierre ex Froehner, resistente aos nematóides *M. exigua*, *M. incognita* e *M. paranaensis* (Fazuoli et al., 2007). Estudos realizados por Gonçalves & Pereira (1998), Bertrand et al. (1997), Bertrand et al. (2001) e Silva et al. (2007) mostraram que muitas linhagens derivadas do híbrido interespecífico entre *C. arabica* e *C. canephora* (Híbrido de Timor) apresentaram resistência a *M. exigua* similar às observadas em *C. canephora*. Recentemente, foi identificado o gene Mex-1, obtido a partir de *C. canephora*, que confere resistência a *M. exigua* em *C. arabica* (Noir et al., 2003).

A extensiva variação morfológica entre as espécies, o grande número de espécies descritas dentro do gênero *Meloidogyne* e a alta variabilidade intra-especifica dificultam a identificação e a caracterização dos nematóides das galhas (Hartman & Sasser, 1985). A identificação dessas espécies foi inicialmente baseada em características morfológicas (configuração perineal) e hospedeiros diferenciais (Eisenback et al., 1981). Entretanto, devido à grande variação e subjetividade dessas características, outros critérios taxonômicos que empregam marcadores enzimáticos (esterases) e moleculares (regiões amplificadas de seqüência caracterizada - SCAR em multiplex – PCR, DNA-satélite) foram propostos por vários pesquisadores (Cenis, 1993; Carneiro et al., 1996a, 2000, 2004; Zijlstra, 2000; Zijlstra et al., 2000; Fourie et al., 2001; Randig et al., 2002 a, b).

Vários estudos têm mostrado que o padrão de esterase permite distinguir as principais espécies de *Meloidogyne* e é, particularmente, útil para sua identificação (Dalmasso & Bergé, 1978; Esbenshade & Triantaphyllou, 1985; Kunieda de Alonso et al., 1995; Carneiro et al., 1996a; 2000).

Embora a caracterização das principais espécies de *Meloidogyne* tenha sido alcançada por meio de eletroforese de proteínas ou isoenzimas, essa técnica não pode distinguir raças de uma mesma espécie (Janati et al., 1982). Assim sendo, aumentou o número de estudos baseados em análise de DNA, a partir de 1985, quando a tentativa inicial para distinguir alguns gêneros de nematóides de vida livre e zooparasitas teve sucesso. Também nesse período, várias das mais importantes espécies de *Meloidogyne* foram caracterizadas por técnicas que utilizaram o RFLP - polimorfismo de comprimento dos fragmentos de restrição (Ferraz & Brown, 2002). Mas, foi somente com a técnica de amplificação de DNA por PCR (reação em cadeia da polimerase) que uma melhor discriminação interespecífica pode ser obtida e que métodos de diagnóstico foram propostos, tais como a amplificação de regiões de DNA mitocondrial ou ribossômico

(Powers & Harris, 1993; Petersen et al., 1997). O método de RAPD (DNA polimórfico amplificado ao acaso) (Williams et al., 1990) tem sido utilizado para muitos estudos, por ser sensível, rápido e relativamente simples, além de não requerer informações sobre a seqüência nucleotídica do DNA genômico.

Carneiro et al. (2004) avaliaram 18 populações de *Meloidogyne* spp. do cafeeiro provenientes do Brasil, América Central e Havaí e, por meio dos marcadores RAPD, os autores detectaram baixos níveis de polimorfismo intra-específico em *M. exigua* (8,6%), *M. incognita* (11,2%) e *M. paranaensis* (20,3%). Entretanto, Randig et al. (2002a), estudando duas populações de *M. exigua* do cafeeiro e seringueira, mostraram uma variabilidade genética da ordem de 67,5 %, o que é extremamente alto para nematóides da mesma espécie.

Recentemente, a conversão dos marcadores de RAPD em SCAR foi designado por Paran & Michelmore (1993) para definir marcadores RAPD cuja seqüência interna tenha sido determinada, permitindo compor conjuntos de *primers* mais longos, ricos em guanina e citosina e de seqüência específica. Esses marcadores já possibilitaram a identificação de *M. incognita*, *M. javanica* e *M. arenaria* (Zijlstra et al., 2000), *M. chitwoodi*, *M. fallax* e *M. hapla*, (Zijlstra, 2000). SCAR-PCR é mais sensível que outras técnicas moleculares e possibilita a detecção de espécies presentes em mistura de populações em proporção inferior a 1% (Fourie et al., 2001). Randig et al. (2002a) estabeleceram marcadores SCAR-PCR para as três principais espécies do nematóide das galhas ocorrentes em cafeeiro no Brasil: *M. exigua*, *M. incognita* e *M. paranaensis*.

Dessa forma, este trabalho foi realizado com os seguintes objetivos: a) examinar a variabilidade genética entre populações de *M. exigua* coletadas em diferentes áreas produtoras de café e seringueira no Brasil; b) verificar qualquer associação possível entre marcadores enzimáticos/molecular e raças em *M. exigua*; c) validar a especificidade de marcadores SCAR, previamente descritos, para diferentes populações de *M. exigua*; d) estudar, por meio de caracteres

morfológicos, citogenéticos, enzimáticos e moleculares, uma população de *M. exigua* proveniente da seringueira e e) avaliar a resistência de genótipos de cafeeiro a diferentes populações de *Meloidogyne* spp.

REFERÊNCIAS BIBLIOGRÁFICAS

ALMEIDA, E.J.; SOARES, P. L. M.; SANTOS, J. M.; MARTINS, A. B. G. Ocorrência de *Meloidogyne mayaguensis* na cultura da goiaba (*Psidium guajava*) no Estado de São Paulo. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006. p. 85.

ASMUS, G. L.; VICENTINI, E. M.; CARNEIRO, R. M. D. G. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado de Mato Grosso do Sul. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 61.

BARBOSA, D. H. S. G.; VIEIRA, H. D.; SOUZA, R. M.; VIANA, A. P.; SILVA, C. P. Field estimates of coffee yield losses and damage threshold by *Meloidogyne exigua*. **Nematologia Brasileira,** Brasília, v. 28, n. 1, p. 49-54, jun. 2004.

BERNARDO, E. R. A.; SANTOS, J. M.; SILVA, R. A.; CASSETARI NETO, D.; SANTOS, S. S.; DELMADI, L.; ROCHA, V. F. Levantamento de *Meloidogyne exigua* na cultura da seringueira em São José do Rio Claro, MT, Brasil. **Ciência Rural**, Santa Maria, v. 33, n. 1, p. 157-159, jan./fev. 2003.

BERTRAND, B.; AGUILAR, G.; BOMPARD, E.; RAFINON, A.; ANTHONY, F. Comportement agronomique et résistance aux principaux déprédateurs des lignées de Sarchimor et Catimor au Costa Rica. **Plantations, Recherche, Développement**, Montpellier, v. 4, n. 5, p. 312-318, Sept./Oct. 1997.

BERTRAND, B.; ANTHONY, F.; LASHERMES, P. Breeding for resistance to *Meloidogyne exigua* in *Coffea arabica* by introgression of resistance genes of *Coffea canephora*. **Plant Pathology,** Avenel, v. 50, n. 5, p. 637-643, Oct. 2001.

CAMPOS, V. P.; MELLES, C. C. A. Ocorrência e distribuição de espécies de *Meloidogyne* em cafezais dos Campos das Vertentes e do Sul de Minas. **Nematologia Brasileira,** Piracicaba, v. 11, p. 233-241, mar. 1987.

CAMPOS, V. P.; VILLAIN, L. Nematode parasites of coffee and cocoa. In: LUC, M.; SIKORA, R. A.; BRIDGE, J. (Ed.). **Plant parasitic nematodes in subtropical and tropical agriculture.** 2.ed. Wallingford, UK: CAB International, 2005. p. 529-579.

CARNEIRO, R. M. D. G. Uma visão mundial sobre a ocorrência e patogenicidade de *Meloidogyne mayaguensis* em goiabeira e outras culturas. **Nematologia Brasileira,** Brasília, v. 27, n. 2, p. 229-230, dez. 2003.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; CARNEIRO, R. G. Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp. **Fundamental and Applied Nematology**, Paris, v. 19, n. 6, p. 555-560, Nov. 1996a.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; QUÉNÉHERVÉ, P. Enzyme phenotypes of *Meloidogyne* spp. populations. **Nematology,** Leiden, v. 2, n. 6, p. 645-654, 2000.

CARNEIRO, R. M. D. G., CARNEIRO, R. G.; ABRANTES, I. M. O.; SANTOS, M. S. N. A.; ALMEIDA, M. R. A. *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. **Journal of Nematology**, Hanover, v. 28, n. 2, p. 177-189, June 1996b.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; GOMES, A. C. M. M.; HERNANDEZ, A. *Meloidogyne izalcoensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitising coffee in El Salvador. **Nematology,** Leiden, v. 7, n. 6, p. 819-832, Dec. 2005.

CARNEIRO R. M. D. G.; MOREIRA, W.A.; ALMEIDA, M. R. A.; GOMES A. C. M. M. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. **Nematologia Brasileira,** Brasília, v. 25, n. 2, p. 223-228, dez. 2001.

CARNEIRO, R. M. D. G.; TIGANO, M. S.; RANDIG, O.; ALMEIDA, M. R. A.; SARAH, J. L. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida:Meloidogynidae) on coffee from Brazil, Central America and Hawaii. **Nematology,** Leiden, v. 6, n. 2, p. 287-298, Mar. 2004.

CASTRO, J. M. C.; CAMPOS, V.P. Ocorrência de *Meloidogyne paranaensis* em cafeeiros na região Sul de Minas Gerais. **Fitopatologia Brasileira,** Brasília, v. 29, p. 89, 2004. Suplemento.

CASTRO, J. M. C.; CAMPOS, V. P.; NAVES, R. L. Ocorrência de *Meloidogyne paranaensis* em cafeeiros na região do Alto Paranaíba em Minas Gerais. **Fitopatologia Brasileira,** Brasília**,** v. 28, n. 5, p. 565, set./out 2003.

CENIS, J. L. Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). **Phytopathology,** St. Paul, v. 83, n. 1, p. 76-78, Jan. 1993.

CHEBABI, A. Ocorrência de nematóides em cafezais do estado do Espírito Santo. **Revista de Agricultura,** Piracicaba, v. 43, p. 140, 1968.

DALMASSO, A.; BERGÉ, J. B. Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: aplication to the taxonomy of *Meloidogyne*. **Journal of Nematology,** Hyattsville, v.10, n. 4, p. 323-332, Oct. 1978.

EISENBACK, J. D.; HIRSCHMANN, H.; SASSER, J. N.; TRIANTAPHYLLOU, A. C. **A guide to the four most common species of root-knot nematodes (*Meloidogyne* species) with a pictorial key**. Raleigh, NC: North Carolina State University, 1981. 48 p.

ESBENSHADE, P. R.; TRIANTAPHYLLOU, A. C. Use of enzyme phenotypes for identification of *Meloidogyne* species. **Journal of Nematology,** Lawrence, v. 17, n 1, p. 6-20, Jan. 1985.

FAZUOLI, L. C.; SILVAROLLA, M. B.; SALVA, T. J. G.; GUERREIRO FILHO, O.; MEDINA FILHO, H. P.; GONÇALVES, W. Cultivares de café arábica do IAC: um patrimônio da cafeicultura brasileira. **O Agronômico,** Campinas, v. 59, n. 1, p. 12-15, 2007.

FERRAZ, L. C. C. B.; BROWN, D. J. F. Taxonomy and classification. In: _____. **An introduction to nematodes: plant nematology**. Sofia, Moscow: Pensoft, 2002. p.184-207.

FOURIE, H.; ZIJLSTRA, C.; McDONALD, A. H. Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. **Nematology,** Leiden, v. 3, n. 7, p. 675-680, Dec. 2001.

GÖLDI, E. A. **Relatório sobre a moléstia do cafeeiro na Província do Rio de Janeiro, 1887.** Recife: UFRPE. Fadurpe, 1998. 121p. (Reeditado por Romero Marinho Moura).

GONÇALVES, W.; PEREIRA, A. A. Resistência do cafeeiro a nematóides IV – Reação de cafeeiros derivados do Híbrido de Timor a *Meloidogyne exigua*. **Nematologia Brasileira,** Brasília, v. 22, n. 1, p. 39-50, jun.1998.

HARTMAN, K. M.; SASSER, J. N. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University, 1985. v. 2, p. 69-77.

JANATI, A.; BERGÉ, J. B.; TRIANTAPHYLLOU, A. C.; DALMASSO, A. Nouvelles données sur l'utilisation dês isoestérases pour l'identification des *Meloidogyne*. **Revue de Nématologie**, Bondy, v. 5, n.1, p. 147-154, 1982.

KARSSEN, G.; VAN HOENSELAAR, T. Revision of the genus *Meloidogyne* Göldi, 1892 (Nematoda: Heteroderidae) in Europe. **Nematologica,** Leiden, v. 44, n. 6, p. 713-788, Dec. 1998.

KUNIEDA DE ALONSO, S.; ALFENAS, A. C; SANTOS, J. M.; FERRAZ, S. Análise de isoenzimas para identificação de espécies de *Meloidogyne*. **Fitopatologia Brasileira,** Brasília, v. 20, n. 1, p. 20-23, mar. 1995.

LIMA, I. M.; MARTINS, M. V. V.; SERRANO, L. A. L.; CARNEIRO, R. M. D. G. Ocorrência de *Meloidogyne mayaguensis* em goiabeira 'Paluma' no Estado do Espírito Santo. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 96.

LORDELLO, L. G. E. Bahia: nematóides atacam o cafeeiro. **Revista de Agricultura,** Piracicaba, v. 46, n. 4, p. 175, 1971.

LORDELLO, L. G. E.; HASHIZUME, H. Suscetibilidade da variedade Kouillou de *Coffea canephora* a um nematóide. **Revista de Agricultura,** Piracicaba, v. 46, n. 4, p. 157-158, 1971.

LORDELLO, L. G. E.; LORDELLO, R. R. A. *Meloidogyne incognita* ataca o cafeeiro no Paraná. **O Solo,** Piracicaba, v. 64, n. 2, p. 27, nov. 1972.

LORDELLO, L. G. E.; MELLO FILHO, A. T. Mais um nematóide ataca o cafeeiro. **Revista de Agricultura,** Piracicaba, v. 45, n. 2/3, p. 102, 1970.

LORDELLO, L. G. E.; ZAMITH, A. P. L. Nematódeos atacando cafeeiro no Estado de São Paulo. **Revista de Agricultura**, Piracicaba, v. 33, n. 1, p. 59-62, 1958.

MOURA, R. M. O gênero *Meloidogyne* e a meloidoginose. Parte II. **Revisão Anual de Patologia de Plantas,** Passo Fundo, v. 5, p. 281-315, 1997.

NOIR, S.; ANTHONY, F; BERTRAND, B.; COMBES, M.C.; LASHERMES, P. Identification of a major gene (Mex-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. **Plant Pathology**, Avenel, v.52, n.1, p. 97-103, Feb. 2003.

PARAN, I.; MICHELMORE, R.W. Development of reliable PCR – based markers linked to downy mildew resistance genes in lettuce. **Theoretical and Applied Genetics,** Berlin, v. 85, n. 8, p. 985-993, Fev. 1993.

PETERSEN, D. J.; ZIJLSTRA, C.; WISHART, J.; BLOK, V.; VRAIN, T. C. Specific probes efficiently distinguish root-knot nematode species using signature sequences in the ribossomal intergenic spacer. **Fundamental and Applied Nematology,** Paris, v. 20, n. 6, p. 619-626, Nov. 1997.

PONTE, J. J.; CASTRO, E. F. Lista adicional de plantas hospedeiras de nematóides das galhas, *Meloidogyne* spp., no Estado do Ceará (Brasil), referente a 1969/74. **Fitossanidade,** Fortaleza, v. 1, n. 2, p. 29-30, 1975.

PONTE, J. J.; SENA-SILVA, M. S. *Meloidogyne exigua* atacando cafeeiros no Ceará. **Pesquisa Agropecuária Brasileira,** Fortaleza, v. 7, p. 93-94, 1972.

PORTZ, R. L.; STANGARLIN, J. R.; FRANZENER, G.; BALBI-PEÑA, M. I.; FURLANETTO, C. *Meloidogyne* spp. associadas à cafeicultura em municípios do oeste do Paraná. **Nematologia Brasileira,** Brasília, v. 30, n. 1, p. 23-27, abr. 2006.

POWERS, T. O.; HARRIS, T. S. A polymerase chain reaction method for identification of five major *Meloidogyne* species. **Journal of Nematology,** Lakeland, v.25, n. 1, p.1-6, Mar. 1993.

RANDIG, O.; BONGIOVANNI, M.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. **Genome,** Ottawa, v. 45, n. 5, p. 862-870, Oct. 2002a.

RANDIG, O.; BONGIOVANNI, M., CARNEIRO, R.M.D.G.; SARAH, J.L. & CASTAGNONE-SERENO, P. A species–specific satellite DNA family in the genome of the coffee root-knot nematode *Meloidogyne exigua*: application to molecular diagnostics of the parasite. **Molecular Plant Pathology**, Bristol, v. 3, n. 6, p. 431-437, Nov. 2002b.

SANTOS, J. M.; MATTOS, C.; BARRÉ, L.; FERRAZ, S. *Meloidogyne exigua*, sério patógeno da seringueira nas plantações E. Michelin, em Rondonópolis, MT. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 16., 1992, Lavras. **Anais...** Lavras: Sociedade Brasileira de Nematologia, 1992. p. 75.

SILVA, G. S.; ATAÍDE SOBRINHO, C.; PEREIRA, A. L.; SANTOS, J. M. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado do Piauí. **Nematologia Brasileira,** Brasília, v. 30, n. 3, p. 307-309, dez. 2006.

SILVA, R. V.; OLIVEIRA, R. D. L.; PEREIRA, A. A.; SÊNI, D. J. Respostas de genótipos de *Coffea* spp. a diferentes populações de *Meloidogyne exigua*. **Fitopatologia Brasileira,** Brasília, v. 32, n. 3, p. 205-212, maio/jun. 2007.

SOUZA, S. E.; SANTOS, J. M.; MATOS, R. V.; RAMOS, J. A.; SANTOS, F. S.; FERRAZ, R. C. N.; CARVALHO, G. S.; OLIVEIRA, C. A. Levantamento preliminar de *Meloidogyne* em cafeeiros do Estado da Bahia Planalto de Vitória da Conquista e Chapada Diamantina. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL,1., 2000, Poços de Caldas. **Anais...** Poços de Caldas: EMBRAPA, 2000. p. 167-170.

SOUZA, S. E.; SOUZA, L. H.; MAIA, J. S.; LIMA, E.; CASTRO, J.; SANTOS, F. G.; SANTOS, F. S.; SILVA, R. V. Ocorrência de nematóide das galhas em cafeeiros no município de Barra do Choça, Bahia. **Nematologia Brasileira,** Brasília, v. 21, n. 1, p. 24, dez. 1997. Resumo.

TORRES, G. R. C.; COVELLO, V. N.; SALES JÚNIOR, R.; PEDROSA, E. M. R.; MOURA, R. M. *Meloidogyne mayaguensis* em *Psidium guajava* no Rio Grande do Norte. **Fitopatologia Brasileira,** Brasília, v. 29, n. 5, p. 570, set./out. 2004.

TORRES, G. R. C.; SALES JÚNIOR, R.; REHN, V. N. C.; PEDROSA, E. M. R.; MOURA, R.M. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado do Ceará. **Nematologia Brasileira,** Brasília, v. 29, n. 1, p. 105-107, jun 2005.

WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research,** Oxford, v. 18, n. 22, p. 6531-6535, Nov. 1990.

ZIJLSTRA, C. Identification of *Meloidogyne chitwood*, *M. fallax* and *M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. **European Journal of Plant Pathology**, Dordrecht, v. 106, n. 3, p. 283-290, Mar. 2000.

ZIJLSTRA, C.; DONKERS-VENNE, D. T. H. M.; FARGETTE, M. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. **Nematology,** Leiden, v. 2, n. 8, p. 847-853, Nov. 2000.

ARTIGO 1

Diversity of *Meloidogyne exigua* (Tylenchida: Meloidogynidae) populations from coffee and rubber tree

(Preparado de acordo com as normas da revista "Nematology")

Maria de Fátima S. MUNIZ [1,*], Vicente Paulo CAMPOS [2], Philippe CASTAGNONE-SERENO[3], José Mauro da Cunha e CASTRO[4], Maria Ritta A. ALMEIDA[5] and Regina M.D.G. CARNEIRO [5]

[1]UFAL – Centro de Ciências Agrárias, 57100-000 Rio Largo, AL, Brazil

[2]UFLA – Departamento de Fitopatologia, C.P. 3037, 37200-000 Lavras, MG, Brazil

[3]INRA, UMR1064 IPMSV, BP167, F-06903 Sophia Antipolis, France

[4]EMBRAPA - Semi-Árido, C.P. 23, 56302-970 Petrolina, PE, Brazil

[5]EMBRAPA – Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-970 Brasília, DF, Brazil

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[*]Corresponding author, e-mail: mf.muniz@uol.com.br

Summary - Isozymes (Esterase and malate dehydrogenase), SCAR and RAPD-PCR were studied in 15 populations of three races of *Meloidogyne exigua,* collected in coffee-producing areas in Brazil, Bolivia and Costa Rica and one population from rubber tree plantations in Brazil**.** This study revealed four esterase phenotypes (E1, E2, E2a, E3) and three malate desydrogenase phenotypes (N1, N1a, N2) for *M. exigua* populations. The most common multienzyme phenotype was E2N1. The enzymatic phenotypes do not separate *M. exigua* races. Sixteen populations of *M. exigua* were tested in Multiplex PCR using SCAR primers ex-D15F/R that allowed the identification of all *M. exigua* populations. Phylogenetic analyses showed high intraspecific polymorphism (25.9–59.6%) for all *M. exigua* studied. However, all populations clustered together with 100% of bootstrap showing the consistency of species identification. In general, no correlation was found between enzymatic profile, race and genetic polymorphism of the studied populations.

Keywords – *Coffea arabica*, *Hevea brasiliensis*, isozymes, electrophoresis, molecular markers, SCAR, RAPD, root-knot nematode.

Root-knot nematodes (*Meloidogyne* spp.) are major agricultural pests of a wide range of crops. However, some less common species, such as *M. exigua* Göldi, 1887, are very host-specific (Shurtleff & Averre, 2000). The genus comprises more than 80 described species that are distributed worldwide (Karsen & Van Hoenselaar, 1998). Seventeen of them have been detected on coffee (*Coffea arabica* L.). In Brazil, *M. exigua*, *M. incognita* (Kofoide & White, 1919) Chitwood, 1949 and *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996 are considered the main species of root-knot that attack coffee plantations (Campos & Villain, 2005). Amongst the most damaging species, *M. exigua* constitutes a serious agronomic constraint because of its wide distribution in Latin America. It is the dominant species in Brazil (Campos & Villain, 2005) and Costa Rica (Flores & López, 1989), where it causes general weakening of coffee trees, associated with a yield loss estimated at 10-15% in

Costa Rica (Bertrand et al., 1997) and 45% in Brazil (Barbosa et al., 2004). According to Campos & Villain (2005) *M. exigua* causes typical rounded galls mostly on new roots formed. The galls are initially white to yellowish brown and turn dark brown as the root becomes older. Egg masses are produced in the cortex. In addition, a different population of *M. exigua* that parasitizes only rubber tree (*Hevea brasiliensis* Muell. Arg.) is a very important pathogen in Rondonópolis and São José do Rio Claro, Mato Grosso State, Brazil (Santos et al., 1992; Bernardo et al., 2003).

Eight populations of *M. exigua* from coffee in Brazil appeared to be very similar to each other morphologically (Lima & Ferraz, 1985). Only a few of variants within this species have been reported (Eisenback & Triantaphyllou, 1991). Nevertheless, physiological variability was observed and three races of *M. exigua* have been described: race 1 (pepper and coffee), race 2 (tomato, pepper and coffee) and race 3 (rubber tree) (Carneiro & Almeida, 2000).

The comparison of esterase patterns (Est) shows great consistency in the separation of the major *Meloidogyne* species (Dalmasso & Bergé, 1978; Esbenshade & Triantaphyllou, 1985a; Kunieda de Alonso et al., 1995; Carneiro et al., 1996; 2000). The isozyme malate dehydrogenase is helpful in the identification, when the esterases show similar patterns such as *M. naasi* Franklin, 1965 and *M. exigua* (Esbenshade & Triantaphyllou, 1985a). Three different esterase phenotypes of *M. exigua* have been reported: E1a, E1b and E2 (Carneiro et al., 2000; 2005; Oliveira et al., 2005). *Meloidogyne exigua* can be identified by isoenzyme electrophoresis, using a large number of macerated females, because the esterase activity for this species is very low (Carneiro et al., 1996; 2000).

Considering the difficulty in characterizing *M. exigua* using esterase phenotypes, molecular markers (Sequence Characterized Amplified Region – SCAR), in multiplex PCR and satellite DNA were developed to identify *M.*

exigua populations (Randig et al., 2002a, b). These two techniques are the most interesting for use in routine analyses. Recently, a study of 54 populations of *Meloidogyne* spp. in coffee fields was carried out in São Paulo and Minas Gerais States using six SCAR primers together in a single reaction. The multiplex PCR allowed the unambiguous differentiation of the three main *Meloidogyne* species from coffee (including *M. exigua*), alone or in a mixture, and its potential for application in routine diagnostic procedure has been confirmed (Carneiro et al., 2005).

Polymerase chain reaction (PCR) based methods are relatively fast and very reliable, besides their independency of the nematode life cycle stages (Zijlstra, 2000). The random amplified polymorphic DNA (RAPD) technique can reveal considerable polymorphism, even between closely related species (Williams et al., 1990), and it is most used for intra or interspecific variability studies (Randig et al., 2002a; Carneiro et al., 2004). These papers also showed that two races of *M. exigua* (races 1 and 3), from coffee and rubber trees, respectively) exhibited higher levels (67.5%) of genetic variability (Randig et al., 2002a) and race 1 and 2 from coffee only 8.6% of polymorphic fragments (Carneiro et al., 2004).

The objectives of the present study were: *i)* to examine the genetic variability and relationships among the *M. exigua* populations encountered in different regions from Brazil; *ii)* to look for any possible association between enzymatic/molecular markers and host races in *M. exigua*; and *iii)* to validate the specificity of the previously described SCAR markers on a wide variety of *M. exigua* populations.

Materials and methods

NEMATODE POPULATIONS

Twenty-one populations of *Meloidogyne* spp. were examined. Thirteen of them were collected in coffee fields from the Brazilian States of Minas Gerais, São Paulo and Rio de Janeiro, and another from rubber tree (Rondonópolis, Mato Grosso State, Brazil). Two populations from other countries (populations 11 and 12 from Costa Rica and Bolivia, respectively) were included, which belong to the nematode collection of Embrapa Recursos Genéticos e Biotecnologia. Five populations from coffee (Brazil and Costa Rica) which had already been identified through esterase and malate dehydrogenase phenotypes as *M. incognita*, *M. paranaensis*, *M. javanica* (Treub, 1885) Chitwood, 1949 and *M. mayaguensis* Rammah & Hirschmann, 1988) were used as a reference for DNA analysis. All populations studied were maintained on tomato (*Lycopersicum esculentum* Santa Cruz cv. Kada) or coffee cv. Catuaí plants in the greenhouses. The population from rubber tree was propagated on its respective host.

ISOZYME ANALYSES: ESTERASE AND MALATE DEHYDROGENASE

Isozyme characterizations were conducted for esterase (Est) and malate dehydrogenase (Mdh) using polyacrylamide gel electrophoresis according to the technique described by Carneiro & Almeida (2001). *Meloidogyne exigua* females were dissected from coffee, tomato or rubber tree roots using a stereomicroscope and macerated (thirty females for esterase studies and fifteen for Mdh in 3 µl of extraction buffer containing sucrose-Triton (Esbenshade & Triantaphyllou, 1985b) or Tris/HCl (Trudgill & Carpenter, 1971), for Est or

Mdh, respectively. Protein extracts of five females of pure populations of *M. hapla* Chitwood, 1949, and *M. javanica* were used as reference phenotypes in each gel. After electrophoresis, the gels were stained for specific enzymes according to methods previously published (Carneiro & Almeida, 2001). The values of the rate of migration (Rm) of the various bands in the gels were measured. Enzyme phenotypes were designated by a suggestive letter for the species and an indicative number for the bands according to Esbenshade & Triantaphyllou (1985a, 1990).

DIFFERENTIAL HOST TEST

Considering previous works (Carneiro & Almeida, 2000; Oliveira et al., 2005), the differential host test was performed with the following host plants: coffee cv. Catuaí Vermelho IAC 144, tomato Santa Cruz cv. Kada and rubber tree clone GD1, following the methodology described by Hartman & Sasser (1985).

EGG EXTRACTION AND DNA PREPARATION

The extraction of eggs to be used in PCR-SCAR-RAPD was performed according to the protocol described by Carneiro et al. (2004). Total genomic DNA was extracted from 200 to 300µl of nematode eggs of each population that had been stored at –80ºC before use, as described by Randig et al. (2002a). Eggs were crushed in liquid nitrogen with a mortar and pestle. DNA was extracted from the resulting fine powder and purified by phenol-chloroform extraction. Samples of DNA were precipitated by mixing with absolute ethanol. The pellet was collected, rinsed with 70% (v/v) ethanol, dried at room temperature and then stored at – 20 ºC in sterile milli-Q water. The quantity of DNA was estimated in

a 1% agarose gel compared to the total DNA on five concentrations of lambda DNA. DNA samples were then diluted in sterile Milli-Q water to obtain the DNA concentration of 6 ng/µl. The aliquots were stored at –20 ºC for further analyses.

MULTIPLEX SCAR–PCR-ANALYSIS

The analysis was performed using four sets of SCAR primers (Table 1) produced by Invitrogen Life Technologies in a single PCR reaction, according to Randig et al. (2002a) and Zijlstra et al. (2000). The multiplex PCR was performed in a final volume of 25 µl containing 6 ng of the total genomic DNA, in addition to 4 µM of primer, dATP, dCTP, dGTP, and dTTP (Fermentas Life Sciences) each at 200 µM final concentration, 1x Taq incubation buffer, and 1 unit of Taq polymerase (Phoneutria Biotecnologia & Serviços, MG, Brazil). Control reaction with all reagents except template DNA was included in the test. Each reaction mixture was overlaid with mineral oil to prevent evaporation. Amplification was performed on a PTC-100 MJ Research thermal cycler (MJ Research Inc., Watertown, Mass, USA), according to the following amplification conditions: 10 min at 94ºC; 30 cycles of 30 s at 94ºC, 45 s at 62ºC, and 1 min at 72ºC; and a final incubation of 8 min at 72ºC. Amplification products were separated by electrophoresis in 1.3% w/v agarose gels in TBE buffer (90 mM Tris base, 89 mM boric acid, and 2 mM EDTA, pH 8.3). The 1 kb Plus DNA Ladder was used as the molecular weight standard. Gels were stained with ethidium bromide (0.2 µg/mL) and the DNA fragments were photographed under ultraviolet light.

Table 1. Characteristics of the SCAR markers developed for *Meloidogyne exigua*, *M. incognita*, *M. paranaensis* and *M. javanica* according to Randig et al. (2002a) and Zijlstra et al. (2000).

Species	SCAR Primer	SCAR primer sequence (5´→ 3´)	Size of the SCAR (bp)
M. exigua	EX-D15-F	CAT CCG TGC TGT AGC TGC GAG	562
	EX-D15-R	CTC CGT GGG AAG AAA GAC TG	
M. incognita	INC-K14-F	GGG ATG TGT AAA TGC TCC TG	399
	INC-K14-R	CCC GCT ACA CCC TCA ACT TC	
M. paranaensis	PAR-C09-F	GCC CGA CTC CAT TTG ACG GA	208
	PAR-C09-R	CCG TCC AGA TCC ATC GAA GTC	
M. javanica	JAV A01-F	CAG GCC CTT CAG TGG AAC TAT AC	670
	JAV A01-R	GCC CGA CTC CAT TTG ACG GA	

bp – base pairs

PCR-RAPD-ANALYSIS

Thirty-three random 10-mer primers, obtained from Invitrogen Life Technologies or Operon Technologies Inc., were used in RAPD trials, and with these, two replicates of the reactions were made. RAPD-PCR was performed in a final volume of 25 µl containing 6 ng of total genomic DNA, in addition to 8 µM of primer, dATP, dCTP, dGTP, and dTTP each at 200 µM final concentration, 1x Taq incubation buffer, and 1 unit of Taq polymerase

(Phoneutria Biotecnologia & Serviços Ltda). To confirm that the observed bands were amplified from the genomic DNA, but not primer artefacts, the genomic DNA was then omitted from control reactions for each primer. The cycling program was 5 min at 94ºC; 40 cycles of 30 s at 94 ºC, 45 s at 36ºC, and 2 min at 70ºC; and a final incubation of 10 min at 70ºC. Amplification products were separated by electrophoresis as described earlier.

PHYLOGENETIC ANALYSES

DNA bands were scored as 1 (presence) or 0 (absence) directly from the gels. For each population, two independent PCR reactions were electrophoresed in the same gel; only DNA fragments consistently present or absent in these replicated samples were recorded and considered as binary characters. DNA fingerprints from each population were converted to a 0-1 matrix, and two phylogenetic analyses were performed using PAUP 4.0 (Swofford, 1998). For the parsimony analysis, characters were run unordered with no weighting, and the heuristic search algorithm was used to find the most parsimonious tree. Characters that were phylogenetically uninformative were deleted, i.e., invariant bands among all populations, and bands either present or absent in one single population only (Li & Graur, 1991).The distance-based neighbor-joining algorithm (Saitou & Nei, 1987) was used on the same data set, using the mean-character difference option of PAUP to compute distances. For both analyses, 1000 bootstrap replicates were performed to test the support of nodes for the most parsimonious tree (Felsenstein, 1985) and a consensus dendrogram was computed.

Results

ISOZYME CHARACTERIZATION

Four phenotypes for esterase (Est) activity were recognized among sixteen *Meloidogyne* populations: fifteen of them from coffee fields and one population from rubber tree (Table 2). We detected the phenotype E1 with a single band (Rm 1.5), the phenotype E2 with two bands (Rm 1.5, 1.9) and a new phenotype designated E3 showing one strong band (Rm 1.5) and two weak bands (Rm 1.7, 1.8) (Figure 1). In addition to, the *M. exigua* population from rubber tree gave different enzymatic profile, with two bands phenotype (E2a - Rm 1.5, 1.6). All phenotypes confirmed the diagnosis of *M. exigua* because of the presence of the main band (Rm 1.5) (Figure 1).

Three distinct malate dehydrogenase phenotypes were observed. The N1 phenotype (Rm 1.0) was detected in almost all populations of *M. exigua* from coffee and one from rubber tree. Two undescribed phenotypes (N1a, Rm 1.3; N2 Rm 1.0, 1.3) were found in four *M. exigua* populations obtained from coffee fields (Table 2; Figure 2).

Table 2. Geographical origin, host preference, enzyme phenotypes and identification of *Meloidogyne* spp.

Geographical origin and population codes	Number of populations	Reproduction on		Enzyme phenotypes		Species/Race
		Coffee	Tomato	Est	Mdh	
Varginha, MG, Brazil (7)	1	+	+	E1	N1	*M. exigua* Race 2
São Sebastião do Paraíso, MG, Brazil (4, 16, 17)	3	+	+	E2, E3, E3	N1, N1, N2	*M.exigua* Race 2
Lavras, MG, Brazil (13, 14)	2	+, +	+, -	E1, E2	N1	*M.exigua* Race 1, 2
São Francisco do Glória, MG, Brazil (10)	1	+	+	E1	N1a	*M. exigua* Race 2
São Sebastião da Vargem Alegre, MG, Brazil (9)	1	+	+	E3	N1	*M. exigua* Race 2
Canaã , MG, Brazil (6)	1	+	-	E2	N2	*M .exigua* Race 1
Itirapuã, SP, Brazil (15)	1	+	+	E2	N1	*M. exigua* Race 2

Table 2. Continued.

Geographical origin and population codes	Number of populations	Reproduction on		Enzyme phenotypes		Species/Race
		Coffee	Tomato	Est	Mdh	
Campinas, SP, Brazil (23)	1	+	-	E2	N1	*M. exigua* Race 1
Varre-Sai, RJ, Brazil (18[1], 19)	2	+	+, -	E2	N1, N1a	*M. exigua* Race 1, 2
Rondonópolis, MT, Brazil (24) [2]	1	-	-	E2a	N1	*M. exigua* Race 3
Bolívia (12)	1	+	+	E1	N1	*M. exigua* Race 2
Costa Rica (11)	1	+	+	E2	N1	*M. exigua* Race 2
Avilândia, SP, Brazil (25)	1	+	+	I1	N1	*M. incognita*
Londrina, PR, Brazil (26)	1	+	+	I2	N1	*M. incognita*
Londrina, PR, Brazil (27)	1	+	+	P1	N1	*M. paranaensis*
Petrolina, PE, Brazil (30) [3]	1	-	+	J3	N1	*M. javanica*
Costa Rica (32)	1	+	+	M2	N1	*M. mayaguensis*
Total	21					

[1]The population 18 is virulent to the Mex-1 coffee resistant gene (Barbosa et al., 2007); [2]Population collected from rubber tree; [3]Population collected from tomato. The remaining populations were collected from coffee plantations. Est= esterase, Mdh= malate dehydrogenase.

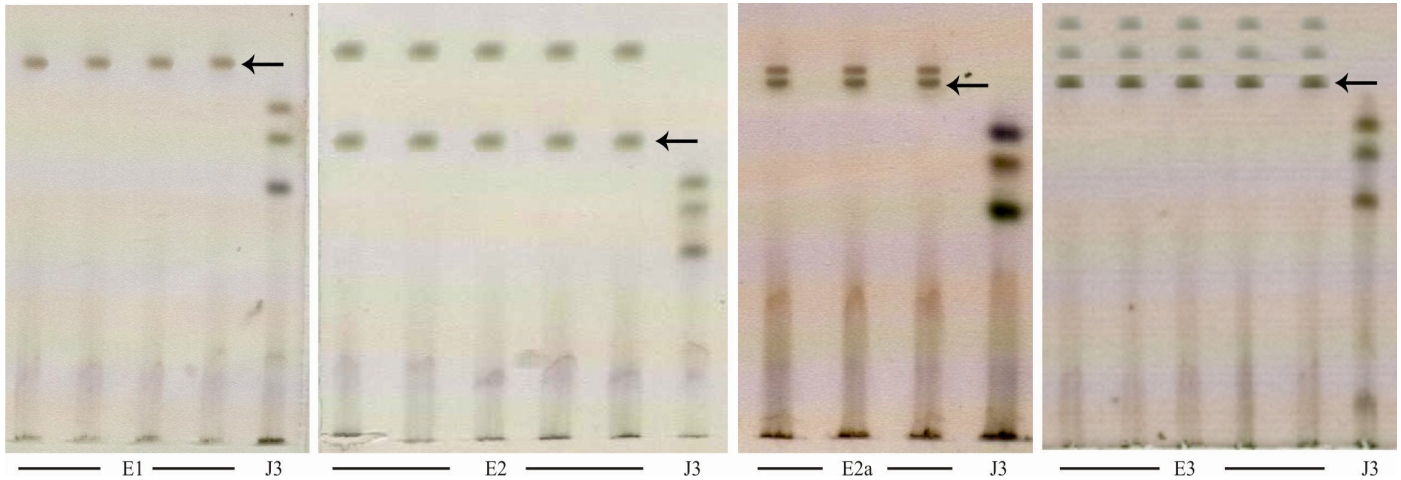

Fig. 1. Esterase phenotypes of *Meloidogyne exigua*: E1, E2, E3 (from coffee); E2a (from rubber tree) compared to reference phenotype J3 for *M. javanica*. The main band (Rm 1.5) is marked by an arrow.

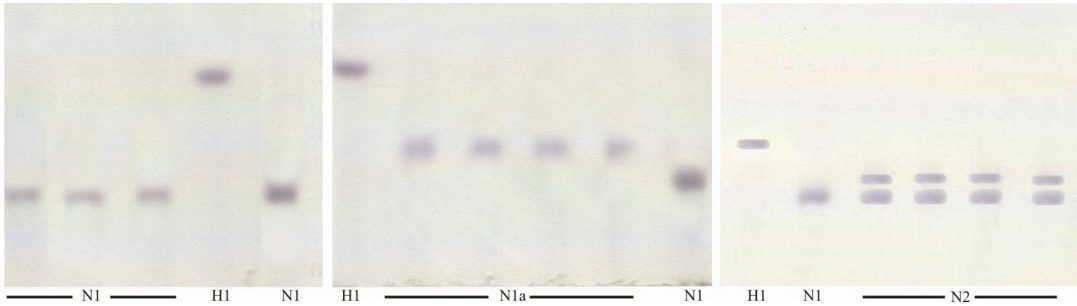

Fig. 2. Malate dehydrogenase phenotypes of *Meloidogyne exigua*: N1, N1a, N2 compared to phenotypes N1 and H1 for *M. javanica* and *M. hapla* used as standard, respectively.

DIFFERENTIAL HOST TEST

In the greenhouse conditions, four *M. exigua* populations reproduced on coffee plants but not on tomato which were them characterized as race 1 (Table 2). In contrast, eleven *M. exigua* populations reproduced on coffee and tomato roots (race 2) and one population reproduced only in rubber tree (race 3).

SCAR ANALYSIS

The populations of *Meloidogyne* spp. were investigated using SCAR primers (Table 1) in Multiplex-PCR. These reactions always resulted in species-specific fragments of 562 bp for 16 *M. exigua* populations, 399 bp for *M. incognita*, 208 bp for *M. paranaensis* and 670 bp for *M. javanica*. No band could be observed when DNA from one population of *M. mayaguensis* was used as a template (Figure 3). The specificity of the SCAR primers was confirmed for different populations (enzymatic phenotypes and races) of *M. exigua* from coffee, including the population from rubber tree.

RAPD ANALYSES

All primers produced good amplification products. An example is shown in Figure 4. As can be observed, PCR with primer B13 produced identical patterns for all populations of *M. exigua* and could be considered diagnostic. On the other hand, the primer G6 contained species-specific bands but showed a lot of intraspecific variation. The majority of the random primers tested in this study produced common bands that discriminated *M. exigua* populations from each other.

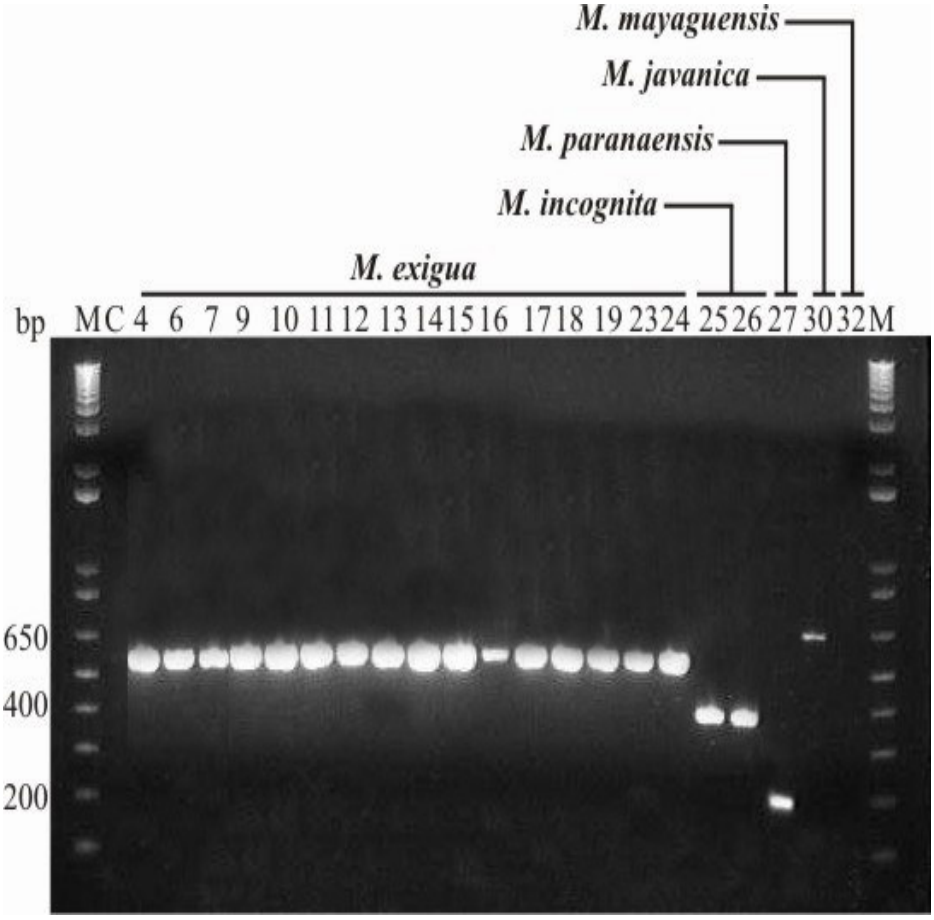

Fig. 3. Typical amplification products of *Meloidogyne* spp. using SCAR primers in multiplex-PCR. M: 1 kb size marker DNA in base pairs (bp); C: A negative control without DNA. Population codes are given in Table 2.

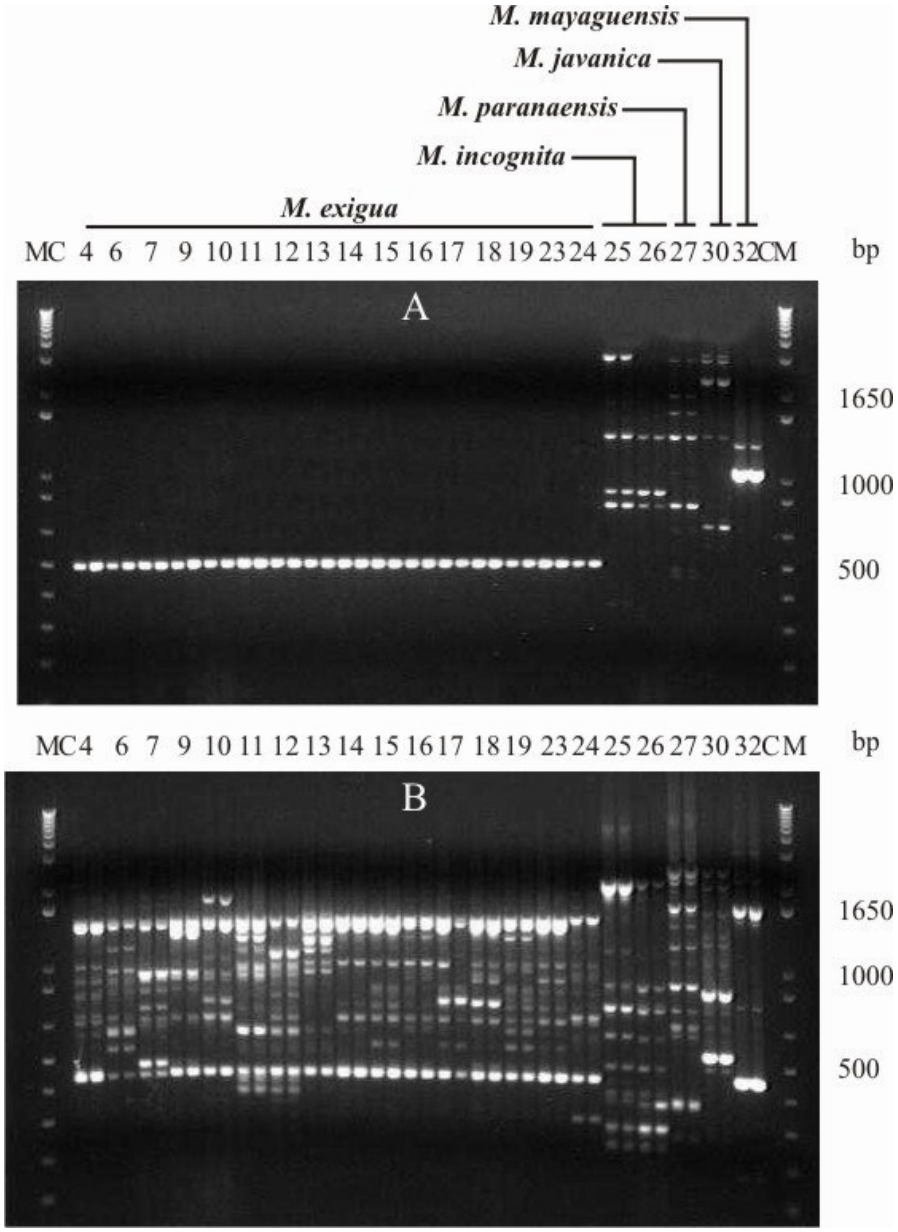

Fig. 4. RAPD patterns for 21 *Meloidogyne* spp. populations generated with primers B13 (A) and G6 (B). For each population, two duplicate amplifications were loaded side by side on the gel. M: 1 kb size marker DNA in bp; C: A negative control without DNA. Population codes are given in Table 2.

With the 33 random primers used, the number of amplified fragments ranged from 1 to 17/population and varied in size from, approximately, 150 to 4000 bp. The global results of the RAPD analysis are provided in Table 3. On the overall trial, each primer produced from 12 to 37 polymorphic bands. From overall, 815 fragments were amplified and considered as RAPD markers, 814 of them were polymorphic, and one monomorphic. In general, the *M. exigua* populations were not grouped according to their geographical distribution, enzymatic profile or race. For *M. exigua* populations from coffee, the effects of the geographical origin, isozyme esterase and race, ranged from 25.9-46.4%, 28.9-59.6%, and 28.3-56.1% of rate of RAPD polymorphisms, respectively. Considering the virulence, the population 18 (virulent, according to Barbosa et al., 2007) was compared with populations 11, 13, 14 and 23 (not virulent) (Noir et al., 2003; Muniz et al., 2007). In this case, 33.6-54.8% of RAPD bands were polymorphic. Moreover, comparing the population from rubber tree with those from coffee, 43.1-57.8 % of RAPD bands were polymorphic.

All *M. exigua* populations from coffee and rubber tree were clustered together with a high confidence level (bootstrap value of 100%) in the two resulting dendrograms (Figures 5 and 6), but the population from rubber tree was distinctly separated from the others. In the dendrograms, *M. incognita*, *M. paranaensis*, *M. javanica* and *M. mayaguensis* were analysed as outgroups. The two *M. incognita* populations clustered together with a bootstrap support of 100% in the analyses. *Meloidogyne paranaensis* and *M. javanica* clustered together with 87% and 90% in the parsimony and the neighbor-joining methods, respectively. By contrast, *M. mayaguensis* was the most genetically distinct from the other species.

Table 3. Oligonucleotide primers used for RAPD analysis and number of amplified fragments scored for *Meloidogyne* spp.

RAPD primer	RAPD primer sequence (5´→ 3´)	% GC	Minimum/ population	Maximum/ population	Polymorphic	Total
A04	AAT CGG GCT G	60	1	7	22	22
AB04	GGA ACG GGT T	60	3	11	31	31
OPAB-06	GTG GCT TGG A	60	3	8	23	23
OPB-11	GTA GAC CCG T	60	3	9	31	31
OPB-12	CCT TGACGC A	60	1	5	12	12
OPB-13	TTC CCC CGC T	70	1	10	19	19
C07	GTC CCG ACG A	70	2	11	27	27
C09	CTC ACC GTC C	70	4	13	31	31
C16	CAC ACT CCA G	60	2	11	25	25
OPC-18	TGA GTG GGT G	60	1	6	17	17
OPF-06	GGG AAT TCG G	60	1	8	22	22
G03	GAG CCC TCC A	70	3	9	21	21
G05	CTG AGA CGG A	60	2	11	29	29
G06	GTG CCT AAC C	60	3	12	37	37
G13	CTC TCC GCC A	70	2	6	15	15
OPJ-19	GGA CAC CAC T	60	4	17	27	27
K01	CAT TCG AGC C	60	1	5	26	26
K04	CCG CCC AAA C	70	4	9	30	30
K06	CAC CTT TCC C	60	3	10	19	19
K07	AGC GAG CAA G	60	1	10	23	23
OPK-13	GGT TGT ACC C	60	1	6	19	19
K16	GAG CGT CGA A	60	1	7	23	23
OPK-17	CCC AGC TGT G	70	3	12	25	25
K19	CAC AGG CGG A	70	1	9	29	29
K20	GTG TCG CGA G	70	3	6	31	31
L08	AGC AGG TGG A	60	2	8	17	17
M20	AGG TCT TGG G	60	4	12	26	26
OPN-07	CAG CCC AGA G	70	1	9	27	27
P01	GTA GCA CTC C	60	1	9	22	22
P02	TCG GCA CGC A	70	1	10	28	28
P05	CCC CGG TAA C	70	1	11	29	29
R07	ACT GGC CTG A	60	1	7	27	27
R08	CCC GTT GCC T	70	2	10	24	25
Total			-	-	814	815

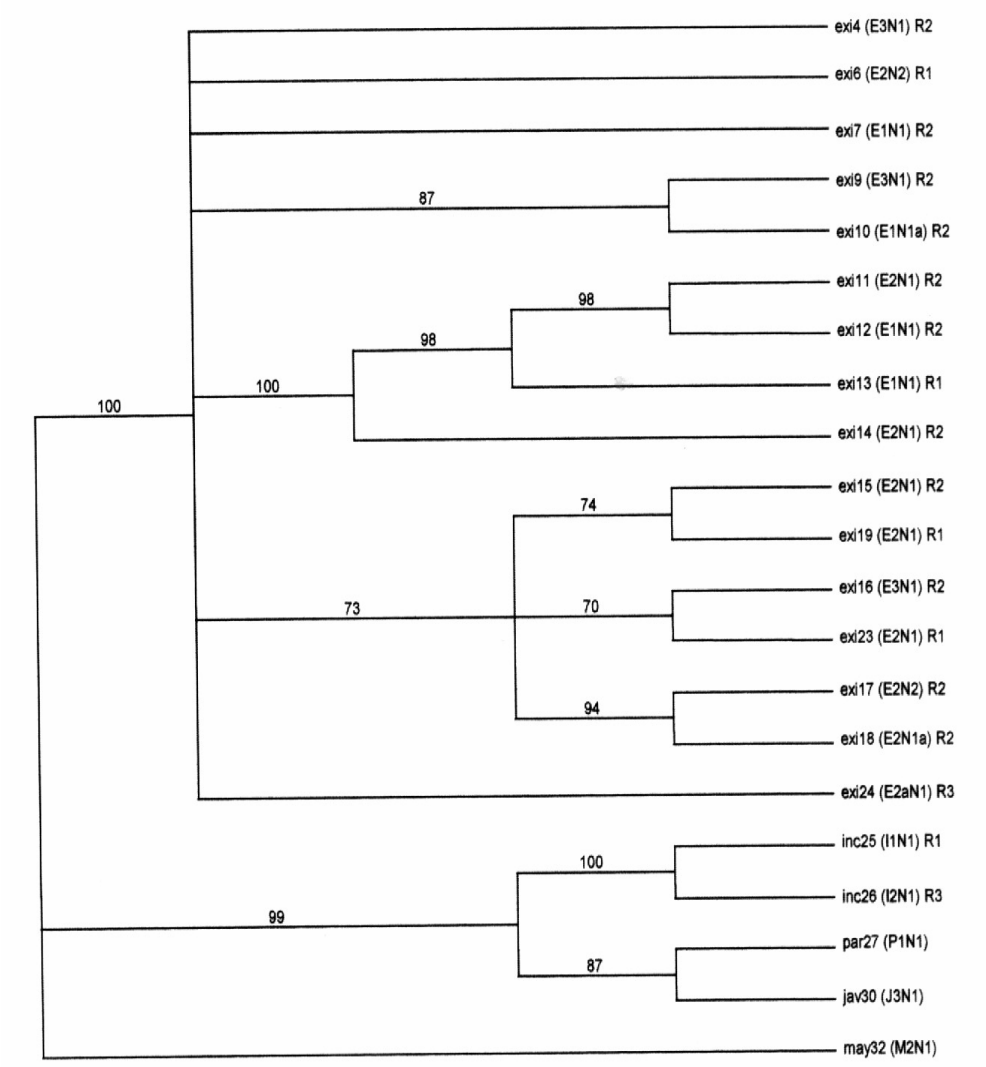

Fig. 5. Consensus dendrograms of relationship of *Meloidogyne* spp. populations (*M. exigua*: exi, *M. incognita*: inc, *M. paranaensis*: par, *M. javanica*: jav, *M. mayaguensis*: may; R1 – race 1, R2 – race 2, R3 – race 3). Parsimony-based tree. Bootstrap percentages based on 1000 replicates are given on each node. The populations 25 to 32 were considered as outgroup. Population codes are given in Table 2.

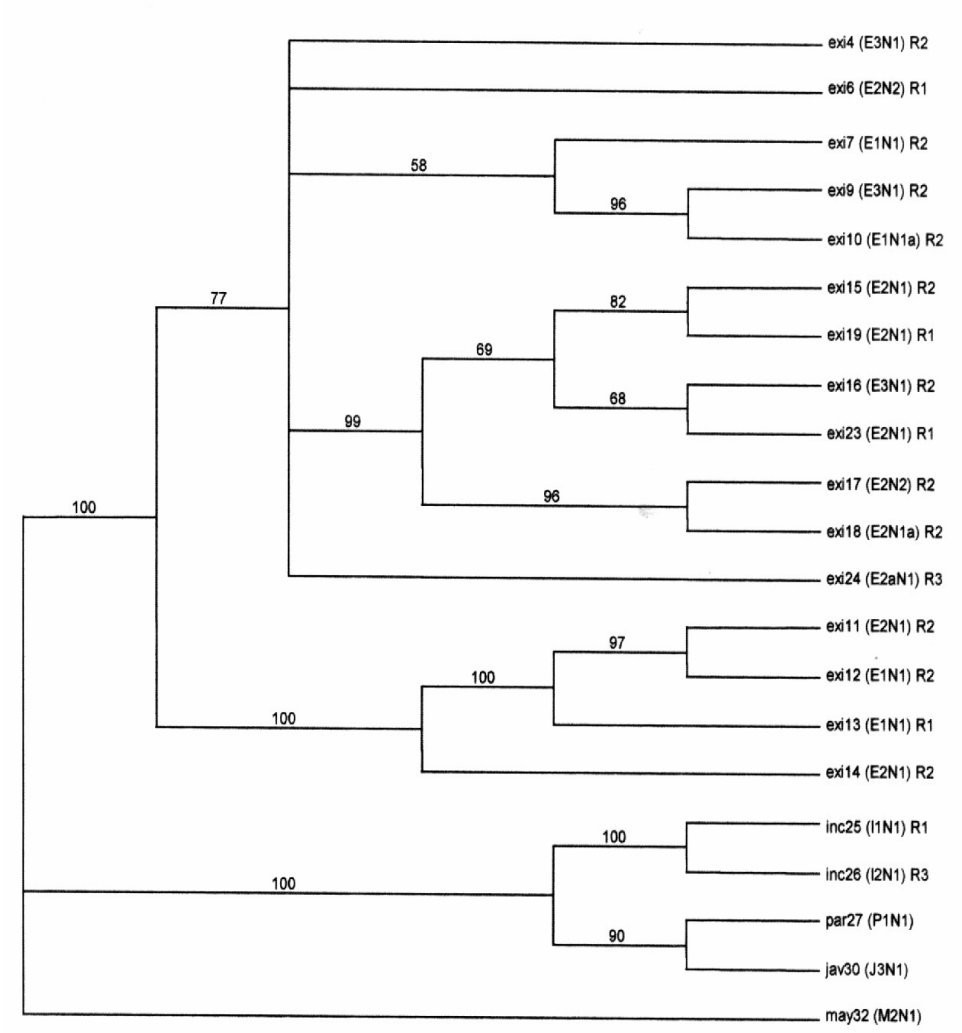

Fig. 6. Consensus dendrograms of relationship of *Meloidogyne* spp. populations (*M. exigua*: exi, *M. incognita*: inc, *M. paranaensis*: par, *M. javanica*: jav, *M. mayaguensis*: may; R1 – race 1, R2 – race 2, R3 – race 3). Neighbor-joining tree. Bootstrap percentages based on 1000 replicates are given on each node. The populations 25 to 32 were considered as outgroup. Population codes are given in Table 2.

The dendrograms generated through RAPD data showed one homogeneous cluster within *M. exigua* populations (11, 12, 13 and 14) with 100% bootstrap support in the analyses (Figures 5 and 6). The *M. exigua* populations from Costa Rica (sample 11, E2N1 race 2) and Bolivia (sample 12, E1N1 race 2) were clustered together (97% and 98% of bootstrap in the neighbor-joining and the parsimony methods, respectively).This cluster was close to the populations from Lavras, Minas Gerais State, Brazil (samples 13 and 14; E1N1 race 1 and E2N1 race 2, respectively).

In addition, the populations from São Sebastião da Vargem Alegre (sample 9, E3N1 race 2) and São Francisco do Glória (sample 10, E1N1a race 2), both from Minas Gerais State, clustered together with 87% and 96% of bootstrap in the analyses. Populations from Itirapuã, São Paulo State (E2N1 race 2) and Varre-Sai, Rio de Janeiro State (E2N1 race 1) (samples 15 and 19, respectively) were clustered together with 74% and 82% of bootstrap in the analyses, and this cluster was close to the populations from São Sebastião do Paraíso, Minas Gerais State (sample 16, E3N1 race 2) and Campinas, São Paulo State (sample 23, E2N1 race 1) and also to the populations from São Sebastião do Paraíso, Minas Gerais State (sample 17, E2N2 race 2) and Varre-Sai, Rio de Janeiro State, Brazil (sample 18, E2N1a race 2). Conversely, the populations from São Sebastião do Paraíso (sample 4, E3N1 race 2), Canaã (sample 6, E2N2 race 1) and Varginha (sample 7, E1N1 race 2), obtained from coffee, and from rubber tree (sample 24, E2aN1 race 3) appear in different branches.

Discussion

Four populations of *M. exigua* collected in Brazil reproduced only on coffee (race 1) and eleven on coffee and tomato (race 2). The ability of *M. exigua* from coffee to reproduce on tomato has already been noticed (Carneiro & Almeida, 2000; Carneiro et al., 2004; Hernandez et al., 2004). This result confirms the physiological variability of this species and the prevalence of *M. exigua* race 2 in Brazil which agrees with the observations made by Oliveira et al. (2005).

The analyses of esterase enzyme showed four phenotypes (E1, E2, and E3 from coffee and E2a for rubber tree). *Meloidogyne exigua* populations that showed the Est phenotype E2 were the most widespread in coffee areas of Brazil, according to Oliveira et al. (2005) and Carneiro et al. (2005). These results agree with the data obtained in the present paper. The occurrence of more than one phenotype for the same enzyme is known in others *Meloidogyne* species (Esbenshade & Triantaphyllou, 1985b; Santos & Triantaphyllou, 1992; Carneiro et al., 1996, 2000). In fact, the esterase band E1 (Rm 1.5) seems to be the most species- specific band present in all *M. exigua* populations. The lack of resolution of other *M. exigua* bands was apparently related to the low esterase activity of those bands (Carneiro et al., 1996).

The *M. exigua* population from rubber tree, studied previously by Carneiro et al. (2000), exhibited the E1a phenotype. In this study the band E1 is the same, but a secondary band appeared in the position Rm: 1.6. The N1 phenotype for Mdh was found in 57 *M. exigua* populations from Minas Gerais State, Brazil (Oliveira et al., 2005) and also from Honduras and Costa Rica populations (Hernandez et al., 2004). In this study, three different phenotypes were observed. Consequently, the isozyme profiles of Mdh detected intraspecific variability among populations of *M. exigua*.

Analyzing the two enzymatic profiles (Est and Mdh) together, seven different phenotypes were detected and showed high diversity among *M. exigua* populations. The occurrence of similar variability was detected only for *M. arenaria*: A3N1, A2N1, A2N3, A1N1 (Cofcewicz et al., 2004, 2005)

Randig et al. (2002a, 2004) and Carneiro et al. (2005) tested the diagnostic potential of the SCAR markers for a few *M. exigua* populations collected from Minas Gerais State, Brazil. The present study included 14 populations of *M. exigua* originating from different States of Brazil (different races and enzymatic phenotypes) and also two populations from Bolivia and Costa Rica and, consequently, provided the validation of SCAR-PCR markers for different *M. exigua* populations. For the SCAR markers, a simple visual evaluation of the gels is enough to give a rapid and easy assessment of a great number of samples. The SCAR-PCR technique used in this study proved to be very sensitive and a reliable tool for routine work in laboratories interested in identification of root-knot nematode populations.

RAPD has been shown valuable in discriminating *Meloidogyne* species (Cenis, 1993; Castagnone-Sereno et al., 1994; Handoo et al., 2004) and to study intraspecific variability (Blok et al., 1997, 2002; Randig et al. 2002a; Carneiro et al., 2004; Cofcewicz et al., 2004, 2005).

Our results showed that *M. exigua* populations from coffee and rubber tree showed high diversity (43.1 - 57.8% of polymorphic amplified fragments). These results agree with Randig et al. (2002a). These authors observed abundant polymorphism (67.5% of polymorphic amplified fragments) between one *M. exigua* population from coffee and another from rubber tree, the same used in the present study. However, both populations clustered together with 100% of bootstrap in the parsimony and the neighbor-joining analyses. On the other hand, studies made on light microscopy and scanning electron microscopy showed that the *M. exigua* population from rubber tree presented similarities with coffee

population with regard to female perineal patterns and head region of males (Santos, 1997). According to Carneiro et al. (2000) the population from rubber tree did not affect coffee or tomato plants in experiments carried out in greenhouse conditions. This population presented a new esterase phenotype not previously detected.

Carneiro et al. (2004) observed low levels (8.6%) of intraspecific polymorphism in race 1 and race 2 of two *M. exigua* (Est E1) populations from coffee. Conversely, for the 15 populations from this host (Est E1, E2, E3) used in this study, the number of RAPD bands scored as polymorphic ranged from 25.9 – 59.6%. More variability is expected in field populations of *M. exigua*, which reproduces by facultative meiotic parthenogenesis (n = 18) compared to mitotic parthenogenesis (Triantaphyllou, 1985).

Meiotic parthenogenesis in *Meloidogyne* is facultative, in that a single population can reproduce by cross-fertilization when males are present or by meiotic parthenogenesis when males are absent (Triantaphyllou, 1985). According to Cook & Evans (1987) the sexual reproduction permits recombination between homologous chromosomes and so permits some reassortment of genes. This reassortment may activate previously masked genes.

Two populations of *M. exigua* (population 18 included in this study) from Varre Sai and another from Bom Jesus de Itabapoana, both from Rio de Janeiro State were of particular concerns because they can reproduce on coffee cv. Iapar 59 with the Mex-1 resistance gene and on genotype H 419-5-4-5-2 which probably has the same gene (Barbosa et al., 2007, Muniz et al., 2007). Enzymatic phenotypes or RAPD markers were not able to differentiate the virulent populations of *M. exigua*. In addition, RAPD markers did not show consistency in the separation of races 1 and 2. This result is in agreement with previous investigations that showed *M. arenaria* and *M. incognita* populations did not cluster according to host race designation (Cenis, 1993; Baum et al.,

1994). This finding suggested that host races do not form monophyletic groups or did not originated from a common ancestor, i.e. the notion of races was not sustained by a genetic determinism.

This study demonstrated that RAPD-PCR is a powerful methodology for the detection of genetic variability among populations of *M. exigua*. The clustering derived from RAPDs was fully consistent with that obtained from enzymatic and SCAR-PCR analyses. In general, no relationships were found between enzyme profiles, races and genetic polymorphism. The high genetic variability among *M. exigua* revealed in this study is a factor that should be systematically considered in breeding programs for durable resistance of coffee genotypes in fields infested by root-knot nematodes.

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References

BARBOSA, D. H. S. G.; VIEIRA, H. D.; SOUZA, R. M.; VIANA, A. P.; SILVA, C. P. Field estimates of coffee yield losses and damage threshold by *Meloidogyne exigua*. **Nematologia Brasileira,** Brasília, v. 28, n. 1, p. 49-54, jun. 2004.

BARBOSA, D. H. S. G.; VIEIRA, H. D.; SOUZA, R. M.; DIAS, P. P.; VIANA, A. P. Desenvolvimento vegetativo e reação de genótipos de *Coffea* spp. a uma população de *Meloidogyne exigua* virulenta a cultivares resistentes. **Nematologia Brasileira,** Piracicaba, v. 31, n. 1, p. 1-6, abr. 2007.

BAUM, T. J.; GRESSHOFF, P. M.; LEWIS, S. A.; DEAN, R. A. Characterization and phylogenetic analysis of four root-knot nematode species using DNA amplification fingerprint and automated polyacrylamide gel electrophoresis. **Molecular Plant-Microbe Interactions,** St. Paul, v. 7, n. 1, p. 39-47, Jan./Feb. 1994.

BERNARDO, E. R. A.; SANTOS, J. M.; SILVA, R. A.; CASSETARI NETO, D.; SANTOS, S. S.; DELMADI, L.; ROCHA, V. F. Levantamento de *Meloidogyne exigua* na cultura da seringueira em São José do Rio Claro, MT, Brasil. **Ciência Rural**, Santa Maria, v. 33, n. 1, p. 157-159, jan./fev. 2003.

BERTRAND, B.; AGUILAR, G.; BOMPARD, E.; RAFINON, A.; ANTHONY, F. Comportement agronomique et résistance aux principaux déprédateurs des lignées de Sarchimor et Catimor au Costa Rica. **Plantations, recherche, développement**, Montpellier, v. 4, n. 5, p. 312-318, Sept./Oct. 1997.

BLOK, V. C.; PHILLIPS, M. S.; MCNICOL, J. W.; FARGETTE, M. Genetic variation in tropical *Meloidogyne* spp. as shown by RAPDs. **Fundamental and Applied Nematology,** Paris, v. 20, n. 2, p. 127-133, Mar.1997.

BLOK, V. C.; WISHART, J.; FARGETTE, M.; BERTHIER, K.; PHILLIPS, M. S. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. **Nematology,** Leiden, v. 4, n. 7, p. 773-781, Oct. 2002.

CAMPOS, V. P.; VILLAIN, L. Nematode parasites of coffee and cocoa. In: LUC, M.; SIKORA, R. A.; BRIDGE, J. (Ed.). **Plant parasitic nematodes in subtropical and tropical agriculture.** 2.ed. Wallingford, UK: CAB International, 2005. p. 529-579.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A. Caracterização isoenzimática e variabilidade intraespecífica dos nematóides de galhas do cafeeiro no Brasil. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 1., 2000, Poços de Caldas. **Anais...** Poços de Caldas: EMBRAPA, 2000. p. 280-282.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. **Nematologia Brasileira,** Brasília, v. 25, n. 1, p. 35-44, jun. 2001.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; CARNEIRO, R. G. Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp. **Fundamental and Applied Nematology**, Paris, v. 19, n. 6, p. 555-560, Nov. 1996.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; QUÉNÉHERVÉ, P. Enzyme phenotypes of *Meloidogyne* spp. populations. **Nematology,** Leiden, v. 2, n. 6, p. 645-654, Sept. 2000.

CARNEIRO, R. M. D. G.; RANDIG, O.; ALMEIDA, M. R. A.; GONÇALVES, W. Identificação e caracterização de espécies de *Meloidogyne* em cafeeiros nos Estados de São Paulo e Minas Gerais através dos fenótipos de esterase e SCAR-Multiplex PCR. **Nematologia Brasileira,** Brasília, v. 29, n. 2, p. 233-241, dez. 2005.

CARNEIRO, R. M. D. G.; TIGANO, M. S.; RANDIG, O.; ALMEIDA, M. R. A.; SARAH, J. L. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. **Nematology***,* Leiden, v. 6, n. 2, p. 287-298, Mar. 2004.

CASTAGNONE-SERENO, P.; VANLERBERGHE-MASUTTI, F.; LEROY, F. Genetic polymorphism between and within *Meloidogyne* species detected with RAPD markers. **Genome,** Ottawa, v. 37, n. 6, p. 904-909, Dec. 1994.

CENIS, J. L. Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). **Phytopathology,** St. Paul, v. 83, n. 1, p.76-78, Jan. 1993.

COFCEWICZ, E. T.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P.; QUÉNÉHERVÉ, P. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitising *Musa* in Brazil. **Nematology**, Leiden, v. 6, n. 1, p. 85-95, May 2004.

COFCEWICZ, E. T.; CARNEIRO, R. M. D. G.; RANDIG, O.; CHABRIER, C.; QUÉNÉHERVÉ, P. Diversity of *Meloidogyne* spp. on *Musa* in Martinique, Guadeloupe, and French Guiana. **Journal of Nematology,** Hanover, v. 37, n. 3, p. 313-322, Sept. 2005.

COOK, R.; EVANS, K. Resistance and tolerance. In: BROWN, R. H.; KERRY, B. R. (Ed.). **Principles and practice of nematode control in crops**. New York: Academic, 1987. p. 179-231.

DALMASSO, A.; BERGÉ, J. B. Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: aplication to the taxonomy of *Meloidogyne*. **Journal of Nematology,** Hyattsville, v. 10, n. 4, p. 323-332, Oct. 1978.

EISENBACK, J. D.; TRIANTAPHYLLOU, H. H. Root-knot nematode: *Meloidogyne* species and races. In: NICKLE, W. R. (Ed.). **Manual of agricultural nematology**. New York: M. Dekker, 1991. p.191-274.

ESBENSHADE, P. R.; TRIANTAPHYLLOU, A. C. Use of enzyme phenotypes for identification of *Meloidogyne* species. **Journal of Nematology,** Lawrence, v. 17, n 1, p. 6-20, Jan. 1985a.

ESBENSHADE, P. R.; TRIANTAPHYLLOU, A. C. Electrophoretic methods for the study of root-knot nematode enzymes. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University Graphics, 1985b. v. 2, p. 115-123.

ESBENSHADE, P. R.; TRIANTAPHYLLOU, A. C. Isozyme phenotypes for the identification of *Meloidogyne* species. **Journal of Nematology,** Lawrence, v. 22, n. 1, p. 10-15, Jan. 1990.

FELSENSTEIN, J. Confidence limits on phylogenies: an approach using the bootstrap. **Evolution,** Lawrence, v. 39, n. 4, p.783-791, July 1985.

FLORES, L.; LÓPEZ, R. Caracterización morfológica del nematodo nodulador del cafeto *Meloidogyne exigua* (Nemata: Heteroderidae). I. Hembras y huevos. **Turrialba,** San José, v.39, n. 3, p.352-360, July/Sept. 1989.

HANDOO, Z. A.; NYCZEPIR, A. P.; ESMENJAUD, D.; VAN DER BEEK, J. G.; CASTAGNONE-SERENO, P.; CARTA, L. K.; SKANTAR, A. M.; HIGGINS, J. A. Morphological, molecular, and differential-host characterization of *Meloidogyne floridensis* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode parasitizing peach in Florida. **Journal of Nematology,** Hanover, v. 36, n. 1, p. 20-35, Mar. 2004.

HARTMAN, K. M.; SASSER, J. N. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University Graphics, 1985. v. 2, p. 69-77.

HERNANDEZ, A.; FARGETTE, M.; SARAH, J. L. Characterisation of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. **Nematology,** Leiden, v. 6, n. 2, p. 193-204, Mar. 2004.

KARSSEN, G.; VAN HOENSELAAR, T. Revision of the genus *Meloidogyne* Göldi, 1892 (Nematoda: Heteroderidae) in Europe. **Nematologica,** Leiden, v. 44, n.6, p. 713-788, Dec. 1998.

KUNIEDA DE ALONSO, S.; ALFENAS, A. C; SANTOS, J. M.; FERRAZ, S. Análise de isoenzimas para identificação de espécies de *Meloidogyne*. **Fitopatologia Brasileira,** Brasília, v. 20, n. 1, p. 20-23, mar. 1995.

LI, W.; GRAUR, D. **Fundamentals of molecular evolution**. Sunderland, MA: Sinauer Associates, 1991. 284 p.

LIMA, R. D.; FERRAZ, S. Análise comparativa das variações morfométricas entre diferentes populações de *Meloidogyne exigua*. **Revista Ceres,** Viçosa, v. 32, n. 183, p. 362-373, set./out. 1985.

MUNIZ, M. F. S.; CAMPOS, V. P.; MOITA, A. W.; GONÇALVES, W.; CARNEIRO, R. M. D. G. Reação de genótipos de cafeeiro a populações de *Meloidogyne exigua*: detecção de virulência natural ao gene Mex-1. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 84-85.

NOIR, S.; ANTHONY, F; BERTRAND, B.; COMBES, M.C.; LASHERMES, P. Identification of a major gene (Mex-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. **Plant Pathology**, Avenel, v. 52, n. 1, p. 97-103, Feb. 2003.

OLIVEIRA, D. S.; OLIVEIRA, R. D. L.; FREITAS, L. G.; SILVA, R.V. Variability of *Meloidogyne exigua* on coffee in the Zona da Mata of Minas Gerais State, Brazil. **Journal of Nematology,** Hanover, v. 37, n. 3, p. 323-327, Sept. 2005.

RANDIG, O.; BONGIOVANNI, M.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. **Genome,** Ottawa, v. 45, n. 5, p. 862-870, Oct. 2002a.

RANDIG, O.; BONGIOVANNI, M., CARNEIRO, R.M.D.G.; SARAH, J.L.; CASTAGNONE-SERENO, P. A species–specific satellite DNA family in the genome of the coffee root-knot nematode *Meloidogyne exigua*: application to molecular diagnostics of the parasite. **Molecular Plant Pathology**, Bristol, v. 3, n. 6, p. 431-437, Nov. 2002b.

RANDIG, O.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P. Identificação das principais espécies de *Meloidogyne* parasitas do cafeeiro no Brasil com marcadores SCAR-café em multiplex PCR. **Nematologia Brasileira,** Brasília, v. 28, n. 1, p. 1-10, jun. 2004.

SAITOU, N.; NEI, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. **Molecular Biology and Evolution,** Chicago, v. 4, n. 4, p. 406-425, July 1987.

SANTOS, J. M. **Estudos das principais espécies de *Meloidogyne* Goeldi que infectam o cafeeiro no Brasil com descrição de *Meloidogyne goeldii* sp.n**. 1997. 153p. Tese (Doutorado em Proteção de Plantas) - Universidade Estadual Paulista, Botucatu, SP.

SANTOS, J. M.; MATTOS, C.; BARRÉ, L.; FERRAZ, S. *Meloidogyne exigua*, sério patógeno da seringueira nas plantações E. Michelin, em Rondonópolis, MT. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 16., 1992, Lavras. **Anais...** Lavras: Sociedade Brasileira de Nematologia, 1992. p. 75.

SANTOS, J. M.; TRIANTAPHYLLOU, H. H. Determinação dos fenótipos isoenzimáticos e estudos comparativos da morfologia de 88 populações de *Meloidogyne* spp. parasitas do cafeeiro. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 16., 1992, Lavras. **Anais...** Lavras: Sociedade Brasileira de Nematologia, 1992. p. 42.

SHURTLEFF, M. C.; AVERRE, C. H. **Diagnosing plant diseases caused by nematodes**. St. Paul, Minnesota: APS, 2000. 187 p.

SWOFFORD, D. L. **PAUP:** phylogenetic analyses using parsimony (and other methods). Version 4. Sunderland, MA: Sinauer Associates, 1998. 257 p.

TRIANTAPHYLLOU, A. C. Cytogenetics, cytotaxonomy and phylogeny of root-knot nematodes. In: SASSER, J. N.; CARTER, C. C. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University Graphics, 1985. v. 1, p. 113-126.

TRUDGILL, D. L.; CARPENTER, J. M. Disk electrophoresis of proteins of *Heterodera* species and pathotypes of *Heterodera rostochiensis*. **Annals of Applied Biology,** London, v. 69, n. 1, p. 35-41, 1971.

WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI, J. A.; TINGEY, S. V. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. **Nucleic Acids Research,** Oxford, v. 18, n. 22, p. 6531-6535, Nov.1990.

ZIJLSTRA, C. Identification of *Meloidogyne chitwood*, *M. fallax* and *M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. **European Journal of Plant Pathology**, Dordrecht, v. 106, n. 3, p. 283-290, Mar. 2000.

ZIJLSTRA, C.; DONKERS-VENNE, D. T. H. M.; FARGETTE, M. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. **Nematology,** Leiden, v. 2, n. 8, p. 847-853, Nov. 2000.

ARTIGO 2

Additional information on atypical population of *Meloidogyne exigua* Göldi, 1887 (Tylenchida: Meloidogynidae), parasitizing rubber tree in Brazil

(Preparado de acordo com as normas da revista "Nematology")

Maria de Fátima S. MUNIZ [1*], Vicente Paulo CAMPOS [2], Maria Ritta A. ALMEIDA[3], Ana Cristina M.M. GOMES[3], Marcilene F. dos SANTOS[3], Fabiane de Castro MOTA[3]
and Regina M.D.G. CARNEIRO[3]

[1]UFAL – Centro de Ciências Agrárias, 57100-000 Rio Largo, AL, Brazil
[2]UFLA – Departamento de Fitopatologia, C.P. 3037, 37200-000 Lavras, MG, Brazil
[3]EMBRAPA/CENARGEN, C.P. 02372, 70849-970 Brasília, DF, Brazil

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[*]Corresponding author, e-mail: mf.muniz@uol.com.br

Summary – Females, males and second-stage juveniles of one unusual population of *Meloidogyne exigua* race 3 that parasitized only rubber tree were examined by light and scanning electron microscopy. Previous molecular characterization using SCAR marker showed consistency with other *M. exigua* populations from coffee. However, RAPD analysis showed high polymorphism (43.1 – 57.8 %) but with 100% of bootstrap among populations from coffee and rubber tree. The morphology of this atypical population was very close to *M. exigua* from coffee for the most important characters: perineal pattern and male head region. However, some differences occurred in some morphological and morphometric features, particularly in the female and male stylet morphology: shape of the cone, shaft and knobs. In addition, this population showed the main *M. exigua* esterase band (Rm:1.5) and one additional band (Rm: 1.6) never detected on coffee populations. Cytogenetic study confirmed the occurrence of meiotic parthenogenesis with haploid chromosome number (n = 18).

Keywords – *Hevea brasiliensis*, root-knot nematode, morphology, taxonomy, cytogenetics

Meloidogyne exigua Göldi, 1887 is a serious plant pathogen on coffee, *Coffea arabica* L. in Brazil and other countries (Campos & Villain, 2005). An atypical population of *M. exigua* was found on rubber tree (*Hevea brasiliensis* Muell. Arg.) in Mato Grosso State, Brazil (Santos et al., 1992; Fonseca et al., 2003; Bernardo et al., 2003). According to Santos (1997) this species was also found on *H. brasiliensis* in Pará and Amazonas States, Brazil. *M. exigua* on rubber tree forms small galls, rounded or oval, up to 5 mm in diameter, mainly on root tip. Necroses were also observed on the galled roots; egg masses were not deposited on the root surface. Histological studies of one gall section revealed up to 12 feeding sites with mature females and males (J4). The females were in the pericycle tissues with the anterior body region inside the central cylinder where the giant cells were formed. The xylem around the giant cells was drastically altered, affecting water and nutrient absorption (Santos et al. 1992; Santos 1997).

In Brazil, there is no information available on the actual yield losses caused by *M. exigua* on rubber tree. However, according to Santos et al. (1992), this species in conjunction with the secondary invading fungus *Botryodiplodia theobromae* Pat. (= *Lasiodiplodia theobrome* (Pat.) Griff. & Maubl.) was responsible for a general decline of the trees, leading to the death of approximately 3% of a rubber tree plantation in Mato Grosso State (MT, Brazil), during 1979 and 1986.

High intraspecific variability of *M. exigua* from coffee and rubber tree was observed by molecular approaches, using RAPD markers (Randig et al., 2002). In this study the two populations presented a polymorphism of 67.5%. Unlike *M. exigua* populations from coffee, this population from rubber tree did not affect coffee or tomato (*Lycopersicon esculentum* Mill.) (Santos et al., 1992; Santos, 1997; Carneiro & Almeida, 2000; Silva et al., 2007). In addition, studies made by Lordello & Lordello (2004) with other hosts, including cotton (*Gossypium hirsutum* L. cv. Deltapine 16), tobacco (*Nicotiana tabacum* L. cv. NC95), tomato cv. Rutgers, pepper (*Capsicum annuum* L. cv. California Wonder), peanut (*Arachis hypogea* L. cv. Florunner), pepper (*Capsicum frutescens* L. cv. Malagueta); corn (Zea mays L. cv. AL 25), and also coffee cv. Caturra Amarelo and rubber tree clone RRIM 600 confirmed the ability of the *M. exigua* population from rubber tree to parasite only its respective host.

Santos (1997) studied some morphological characters, such as perineal pattern of females and male head structures of this atypical *M. exigua* from rubber tree. However, other important morphological and morphometrical features that allowed the identification of this population as *M. exigua* have not been studied: female and male stylet length and shape, distance of DGO to the base of stylet, shape of head cap of females and second-stage juveniles (J2). According to Hirschmann (1985) these characters are reliable features for differentiating *Meloidogyne* species**.**

Based on morphological, biological, biochemical and molecular differences with *M. exigua* from coffee, the population from rubber tree, Rondonópolis, MT, Brazil was examined and its taxonomic identity clarified.

Materials and methods

NEMATODE POPULATION

The population of *M. exigua* obtained from infected rubber tree roots from Rondonópolis, MT, Brazil was maintained on this host in a greenhouse and used for the morphological, biochemical and cytological studies. Females were handpicked from infected roots. Males and J2 were obtained by stressing the host plant according to Carneiro et al. (1998). The aerial parts were removed from an infected rubber tree plant leaving the roots in tap water, aerating them with an aquarium pump. The nematodes were collected from the water at 2 day intervals for a month.

MORPHOLOGICAL CHARACTERIZATION

Live males, females and J2 were fixed and mounted in 2% formalin and measured immediately under LM. Specimens were examined at all three stages. Perineal patterns were prepared from mature females in 45% lactic acid and mounted in glycerin (Hartman & Sasser, 1985).

For SEM of females, small pieces of galled root tissues were placed in 2% glutaraldehyde solution at cold temperature (8ºC) buffered with 0.1 M sodium cacodylate at pH 7.0. After fixation overnight, females were removed from the root tissues. Fixation of the material continued for an additional 24 h in the same solution, whereas males and J2 were similarly fixed in 2% glutaraldehyde for 24 hs. The specimens were post-fixed with 2% osmium tetroxide for 2 hr, and dehydrated with a graded series of 10, 30, 50, 70, 90, and

100% acetone for 15 min at each step. Three 15-min changes of pure acetone were applied for complete dehydration. Then the specimens were critical-point dried from liquid CO_2, mounted onto stubs covered with double-sided adhesive tape and sputter-coated with a 20 to 30-nm layer of gold, by standard procedures (Eisenback, 1985), and observed using a Zeiss DSM-962 scanning electron microscope operated at 10 KV accelerating voltage. At least 50 females and 50 J2 were examined. Because of the scarcity of males, only 10 specimens were observed.

The stylets of *M. exigua* females and males were dissected in 45% lactic acid, fixed in 2% formalin and air dried, coated with gold, viewed, and photographed by SEM according to Eisenback (1985).

BIOCHEMICAL STUDY

Isozyme characterizations were conducted for esterase using polyacrylamide gel electrophoresis according to the technique described by Carneiro & Almeida (2001). Protein extracts of *M. javanica* (Treub, 1885) Chitwood, 1949 females were used as reference phenotype.

CYTOGENETICS

Cytogenetic studies were carried out using with a propionic-orcein staining method, according to Triantaphyllou (1985b). The females were dissected from galled root and processed for the preparation of slides, hydrolysis and fixation. Smeared females were stained with orcein, following the temporary mount for microscopic examination of the chromosomes.

Results

Meloidogyne exigua Göldi, 1887 from *Hevea brasiliensis*

(Figs 1-4)

MEASUREMENTS

The measurements of females, males and J2 are listed in Tables 1, 2, 3.

MORPHOLOGICAL CHARACTERIZATION

Female

Females and egg masses completely embedded in root tissue. Body white, variable in size, typically rounded with a defined neck, short or elongated. In SEM observations, stoma slit-like, located in ovoid prestoma, surrounded by pit-like openings, of six inner labial sensilla. Labial disc rounded, slightly raised, fused with medial and lateral lips with almost rectangular amphidial apertures located between the labial disc and lateral lips. Medial lips with indentations, larger than labial disc; lateral lips large and triangular often fused to the cephalic region. Stylet delicate, 12.5–15.0 µm long, cone slightly curved dorsally, increasing in width posteriorly; shaft cylindrical, slightly widening at the junction with the knobs; heart shaped, backward-sloping knobs. The distance of DGO to the base of the knobs is long, usually 4.5–7.5 µm. Perineal pattern in LM and SEM was typical of *M. exigua*, with low and slightly flattened dorsal arch, usually indented laterally; striae coarse and widely-spaced, broken and folded striae at sides of pattern ventral to lateral line region. Lateral fields inconspicuous; phasmids widely separated, near the anus. All other female characters were very close to the description of typical *M. exigua* populations from coffee (Lordello & Zamith, 1958; López, 1985; Flores & López, 1989a; Eisenback & Triantaphyllou, 1991).

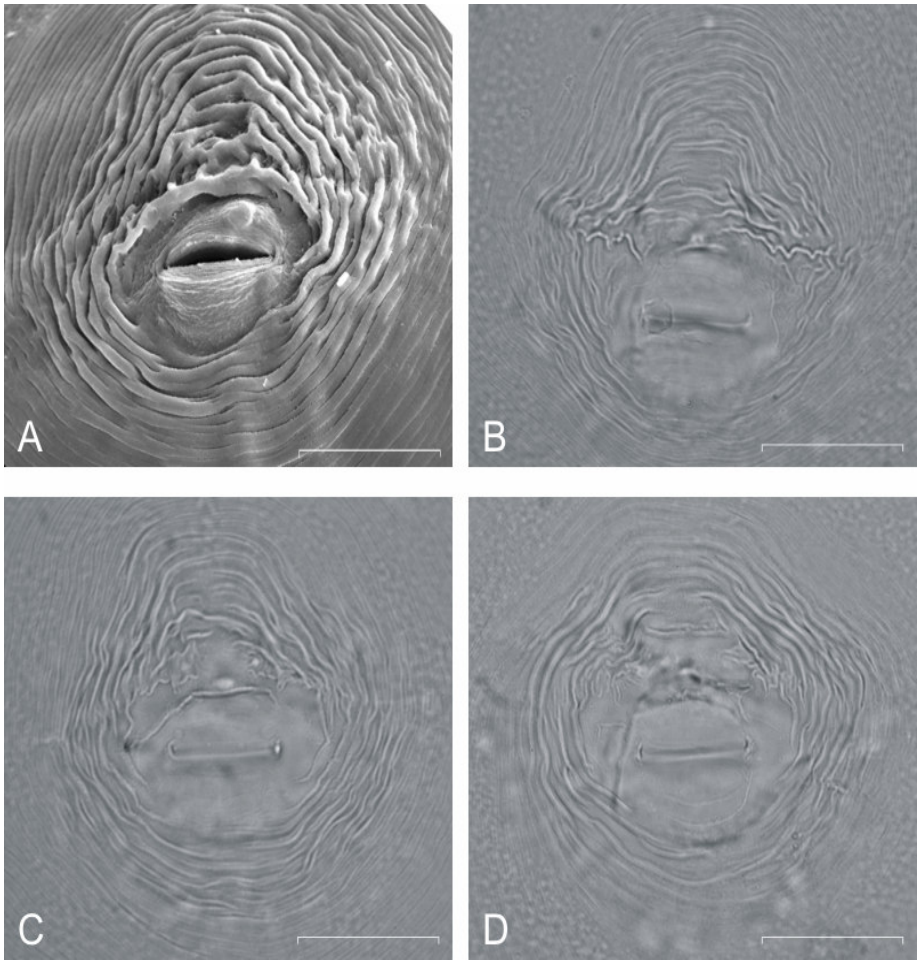

Fig.1. *Meloidogyne exigua* perineal patterns. A: Scanning electron microscopy. B-D: Light micrographs (scale bars: A-D = 25 µm).

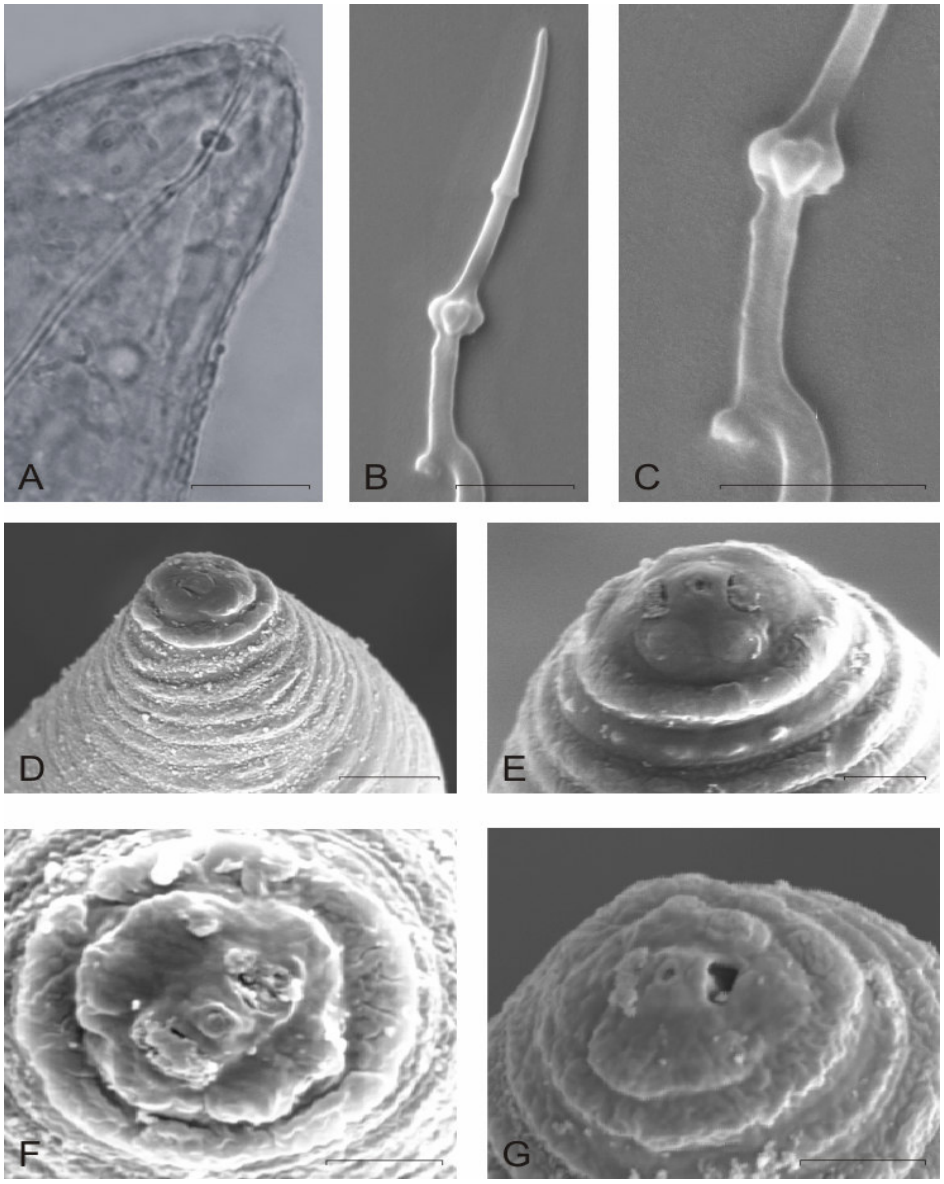

Fig.2. *Meloidogyne exigua* females. A: Light micrograph of anterior end; B-C: Scanning electron microscopy (SEM) of excised stylet; D-E: SEM of the anterior end in lateral view; F-G: SEM of the anterior end in face view (scale bars: A = 7 µm; B-D = 5 µm; E-G: 2 µm).

Male

Body vermiform not twisted, bluntly rounded posteriorly, with sclerotized head region. In SEM the labial disc and medial lips are fused and form one smooth continuous head cap. The head region is smooth and the triangular lateral lips are completely formed and separated from the medial lips; medial lips not divided medially by a shallow groove, but sometimes divided into lip pairs, wider laterally than the labial disc, sometimes with slightly indentations at junctions with labial disc. Amphidial apertures elongated, located below lateral edges of labial disc; amphids often producing exudates. The head region and body annules are in the same contour; head region without annulations. Lateral fields beginning as two incisures located at 8 annules behind the cephalic region, usually with four areolated incisures in other areas of the body. Stylets are 17.0-20.0 µm long, cone bluntly pointed, gradually increasing in diameter posteriorly, knobs rounded, and set off from the shaft; shaft straight and cylindrical and never narrows at the junction with the knobs. The distance of dorsal pharyngeal gland orifice (DGO) to knobs base exhibited narrow range of variability: 5.0-5.5 µm. All other male characters were very close to the description of typical *M. exigua* populations from coffee (Lordello & Zamith, 1958; López, 1985; Flores & López, 1989b; Eisenback & Triantaphyllou, 1991).

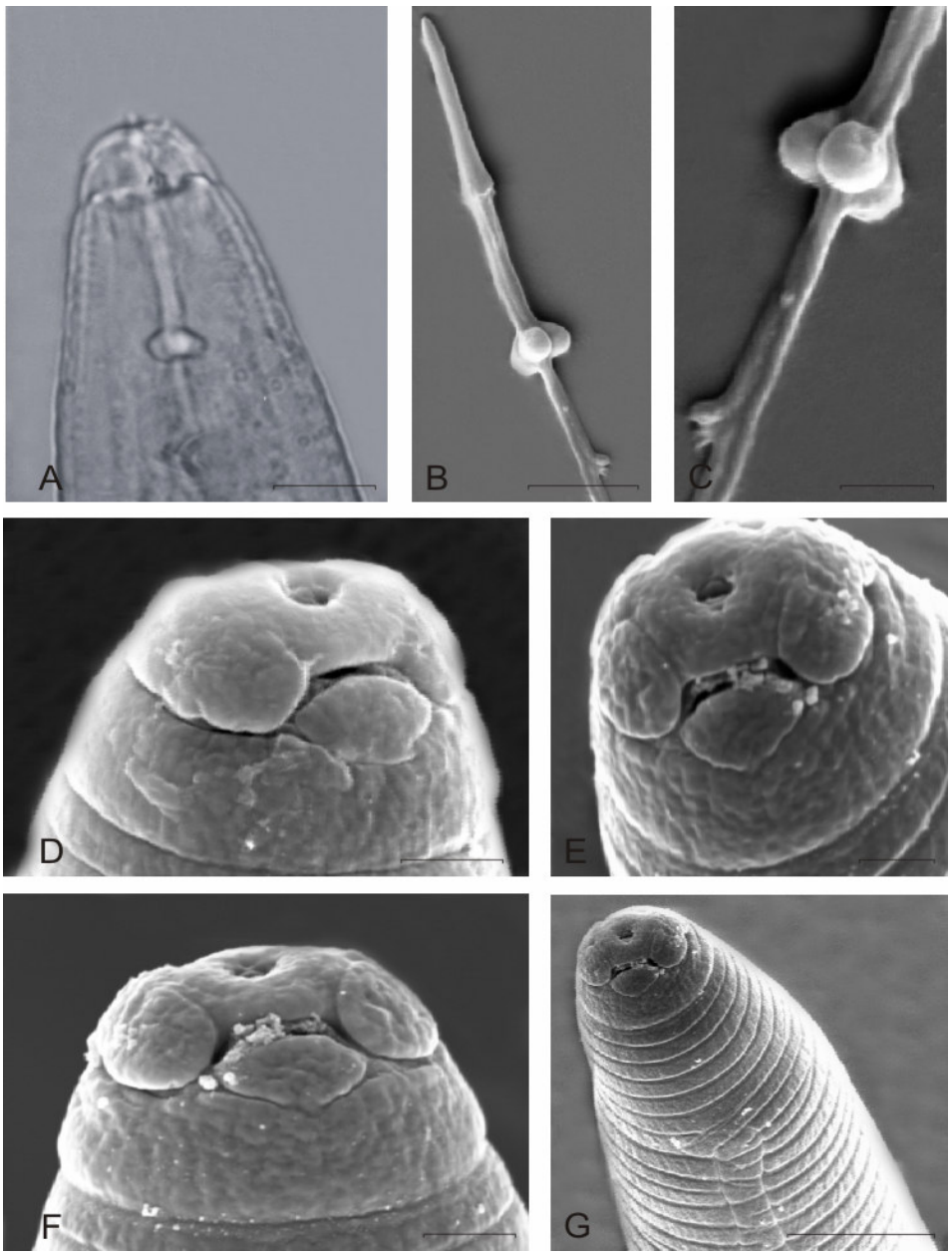

Fig.3. *Meloidogyne exigua* males. A: Light micrograph of head end. B-C: Scanning electron microscopy (SEM) of excised stylet; D, F: SEM of the anterior end in lateral view; E: SEM of the anterior end in face view; G: SEM of the anterior region (scale bars: A, G = 10 µm; B = 5 µm; C-F = 2 µm).

Second-stage juvenile (J2)

In LM, body vermiform, slender with a weak sclerotized cephalic framework; tail narrow, elongated, 45.0-49.5 µm that ends in a bluntly rounded tip; hyaline tail terminus with 10.0-18.0 µm, sometimes with constrictions in posterior region. Stylets are 11.0-12.5 µm long and the distance of DGO to the base of knobs is variable, 3.0-5.0 µm.

SEM observations (face view) confirmed that the labial disk and medial lips are fused and dumbbell-shaped. Labial disc rounded, slightly raised above medial lips; each medial lip is larger than the labial disc; lateral lips small, lower than medial lips; head region smooth and in contour with the body annules. Lateral fields with four incisures. All other J2 characters were very close to the description of typical *M. exigua* populations from coffee (Lordello & Zamith, 1958; Flores & López, 1989c; Eisenback & Triantaphyllou, 1991).

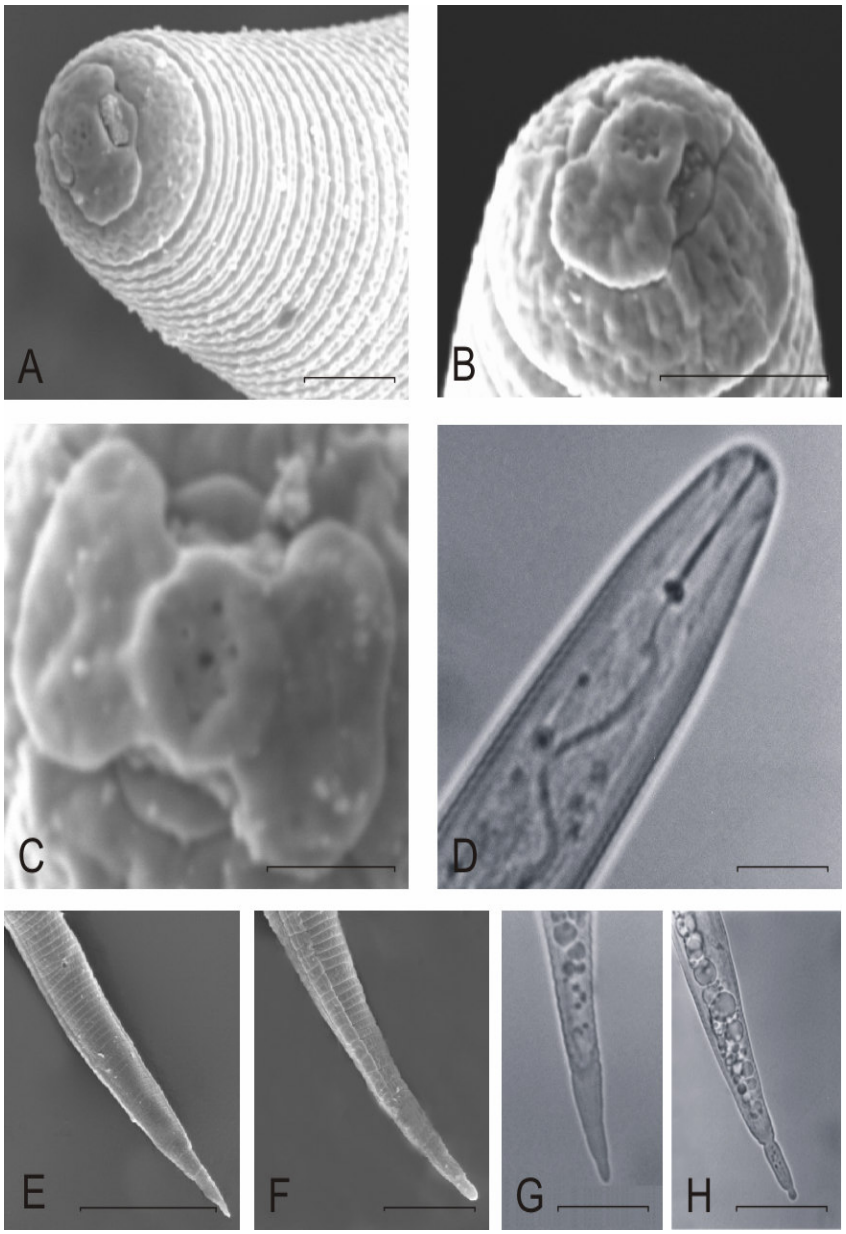

Fig.4. *Meloidogyne exigua* second-stage juveniles. A: Scanning electron microscopy (SEM) of the anterior region; B: SEM of the anterior region in lateral view; C: SEM of the anterior region in face view. D: Light micrograph (LM) of the anterior region ; E-F: SEM of tails; G-H: LM of tails (scale bars: A-B = 2 µm; C = 1 µm; D, G, H = 14 µm; E = 20 µm; F = 10 µm).

Table 1. Measurements of 16 females of *Meloidogyne exigua* from rubber tree. All measurements are in the form: mean ± standard deviation (range) and coefficient of variation.

Character	Lordello & Zamith (1958)	Lima & Ferraz (1985)	Flores & López (1989a)	Present study
Linear (µm)				
Body length	387.5 – 496.0	498.7 (345.0 – 620.0)	417.4	540.8 ± 83.15 (356 – 664) 15.4
Body width	279.0 – 372.0	337.5 (220.0 – 455.0)	242.7	310.5 ± 20.28 (280 – 344) 6.5
Neck length	-	118.2 (60.0 – 195.0)	-	144.5 ± 49.94 (72 – 240) 34.6
Stylet length	10.7	12.9 (8.2 – 14.8)	12.2	13.6 ± 0.69 (12.5 – 15.0) 5.1
Stylet knob height	-	2.2 (1.5 – 3.0)	2.0	1.8 ± 0.23 (1.5 – 2.3) 12.8
Stylet knob width	-	3.5 (2.4 – 4.5)	2.7	2.8 ± 0.18 (2.5 – 3.0) 6.5
DGO from base of stylet	4.6 - 7.7	4.6 (3.8 – 6.5)	4.7	5.4 ± 0.76 (4.5 – 7.5) 14.0
Length of the metacorpus	30.6 - 33.6	35.2 (30.0 – 38.5)	35.4	45.6 ± 6.86 (36.0 – 60.0) 15.0
Width of the metacorpus	24.5 - 26.0	27.5 925.2 – 30.0)	28.5	45.5 ±6.27 (31.0-55.0) 13.8
Median bulb valve length	-	-	10.9	12.0 ± 0.55 (11.0- 13.0) 4.6
Median bulb valve width	-	-	9.7	9.3 ± 0.57 (8.5 – 10.0) 6.1
Center of median bulb from anterior end	-	-	69.0	72.2 ± 10.42 (47.0 – 88.0) 14.4
Excretory pore from anterior end	-	19.2 (10.0 – 59.5)	27.6	37.6 ± 9.37 (20.0 – 56.0) 25.0
Vulval slit length	-	20.5 (15.0 – 26.5)	18.9	19.5 ± 1.64 (16.3 – 22.5) 8.4
Vulval slit to anus distance	-	15.3 (10.0 – 23.2)	15.2	13.1 ± 1.51 (10.0 – 15.0) 11.5
Interphasmidial distance	-	22.6 (11.5 – 31.0)	21.0	17.3 ± 3.66 (12.5 – 25.0) 21.2
Ratios				
a = total body length/maximum body width	-	1.5 (1.1 – 2.0)	1.8	1.70 ± 0.30 (1.2 – 2.2) 17.0

Table 2. Measurements of 10 males of *Meloidogyne exigua* from rubber tree. All measurements are in the form: mean ± standard deviation (range) and coefficient of variation.

Character	Lordello & Zamith (1958)	Lima & Ferraz (1985)	Flores & López (1989b)	Present study
Linear (µm)				
Body length	832.3 – 1092.4	1128.2 (660.0 – 1450)	1014.7	1068.4 ± 77.86 (990.0 – 1240.0) 7.3
Maximum body width	26.0 – 46.0	34.2 (20.0 – 48.0)	37.9	36.5 ± 3.77 (32.0 – 42.0) 10.3
Body width from base of stylet	-	18.1 (12.5 – 23.4)	-	18.4 ± 1.41 (16.0- 20.5) 7.7
Body width from excretory pore	-	29.8 (19.0 – 37.5)	-	30.9 ± 3.82 (24.0 – 36.0) 12.4
Stylet length	18.4 – 19.9	18.3 (13.9 – 22.5)	18.3	18.7 ± 0.91 (17.0 – 20.0) 4.9
Stylet knob height	3.0	3.1 (2.2 – 4.5)	2.4	2.9 ± 0.24 (2.5 – 3.0) 8.5
Stylet knob width	4.0 – 6.1	4.6 (3.5 – 6.0)	4.0	3.1 ± 0.61 (2.5 – 4.0) 19.8
Center of metacorpus valve from anterior end	3.0	-	71.9	73.3 ± 5.20 (67.0 – 84.0) 7.1
DGO from base of stylet	-	4.6 (3.0 – 6.5)	4.3	5.2 ± 0.26 (5.0 – 5.5) 5.0
Excretory pore from anterior end	-	128.6 (76.0 – 155.0)	114.0	114.7 ± 30.20 (55.0 – 146.0) 26.3
Tail length	6.1 – 10.0	9.2 (5.2 – 14.0)	10.5	10.5 ± 1.70 (9.0 – 11.0) 16.2
Spicules length	20.0 – 26.0	28.2 (22.5 – 37.0)	24.8	28.7 ± 2.18 (25.0– 32.0) 7.6
Gubernaculum length	7.7	-	7.0	8.0 ± 1.25 (6.5 – 10.0) 15.6
Ratios				
a = total body length/maximum body width	23.8 – 32.0	32.9 (21.9 – 48.8)	26.9	29.9 ± 3.84 (24.8– 35.0) 12.8
c = total body length/length of the tail	95.8 – 110.0	127.0 (58.7 – 248.1)	98.1	103.8 ± 15.81 (93.1 – 124.0) 15.2

Table 3. Measurements of 16 second-stage juveniles (J2) of *Meloidogyne exigua*. All measurements are in the form: mean ± standard deviation (range) and coefficient of variation.

Character	Lordello & Zamith (1958)	Lima & Ferraz (1985)	Flores & Lópes (1989c)	Present study
Linear (µm)				
Body length	333.5 – 358.0	375.9 (330.0 – 450.0)	366.9	375.0 ± 26.52 (330.0 – 435.0) 7.1
Maximum body width	13.7 – 15.3	16.6 (13.0 – 22.0)	14.8	14.9 ± 1.01 (13.5 – 17.5) 6.8
Stylet length	9.2	11.7 (9.5 – 14.0)	9.8	11.6 ± 0.38 (11.0 – 12.5) 3.2
Anterior end from base of stylet	-	13.9 (11.5 – 15.0)	13.4	12.9± 0.34 (12.0 – 13.5) 2.7
DGO from base of stylete	-	3.8 (2.6 – 5.0)	3.5	3.9 ± 0.50 (3.0 – 5.0)12.7
Center of median bulb from anterior end	-	56.9 (45.0 – 69.2)	50.8	48.9 ± 2.23 (44.0 – 53.0) 4.6
Excretory pore from anterior end	-	74.9 (62.0 – 92.0)	74.6	72.5 ± 4.83 (66.0 – 79) 6.7
Tail length	44.4 – 46.0	49.5 (38.0 – 62.5)	46.6	46.9 ± 1.56 (45.0 – 49.5) 3.3
Body width from anus	7.7 – 9.2	11.8 (8.8 – 16.0)	9.3	9.7 ± 0.48 (9.0 – 10.5) 4.9
Hyaline tail length	-	-	13.9	12.9 ± 1.92 (10.0 – 18.0) 14.9
Ratios				
a = total body length/maximum body width	22.2 – 26.0	22.1 (17.6 – 30.6)	25	25.2 ± 2.07 (21.4 – 29.3) 8.2
c = total body length/length of the tail	7.3 – 7.8	8.1 (5.8 – 10.7)	7.9	8.0 ± 0.69 (6.8-9.1) 8.7
c'= tail length/body width at the anus	-	4.2 (3.2 – 5.2)	5.0	4.9 ± 0.32 (4.4 – 5.5) 6.7

BIOCHEMICAL STUDY

The esterase phenotype E2a with two bands (Rm 1.5, 1.6) was observed in the *M. exigua* population from rubber tree (Muniz et al., 2008).

CYTOGENETICS

The reproduction of *M. exigua* from rubber tree is by meiotic parthenogenesis with a haploid chromosome number (n = 18). This result agrees with Triantaphyllou (1985a).

DIAGNOSIS AND RELATIONSHIPS

M. exigua from rubber tree is characterized as similar to *M. exigua* from coffee, since its female face view and perineal partens are similar (Lopez, 1985; Eisenback & Triantaphyllou, 1991); female stylet length (12.5-15.0, 13.6 µm) in the range described for *M. exigua* from coffee (12.0–14.0 µm) (Eisenback & Triantaphyllou, 1991); distance from the DGO to stylet base 4.5-7.5, 5.5 µm *vs* 4.6-7.7 µm (Lordello & Zamith, 1958). Males with head region shape similar to *M. exigua* from coffee with lateral lips completely formed (Eisenback & Triantaphyllou, 1991); stylet length 17.0-20.0, 18.7 µm *vs* 18.4-19.9 µm (Lordello & Zamith, 1958). Second-stage juveniles' characteristics appeared to be very similar to those of *M. exigua* from coffee (Lordello & Zamith, 1958; Eisenback & Triantaphyllou, 1991). Biochemical analysis showed the same main esterase band (Rm=1.5), characteristic of *M. exigua* from coffee (Carneiro & Almeida 2000; Oliveira et al., 2005). One secondary band (Rm=1.6) was observed in the population from rubber tree, but other secondary bands were also observed in some populations from coffee (Muniz et al., 2008).

Molecular analysis using SCAR markers of the population from rubber tree resulted in a fragment of 562 bp, specifically amplified in *M. exigua* from coffee (Randig et al., 2002, Muniz et al., 2008). Moreover, using RAPD pattern, we detected high polymorphism (43.1-57.8 %) among populations from coffee and the population from rubber tree (Muniz et al., 2008). Phylogenetic analyses showed that this population clustered together with 15 *M. exigua* populations from coffee with very high bootstrap support of 100%, but formed in distinct branch (Muniz et al., 2008). Furthermore, our cytological study also indicated the occurrence of meiotic parthenogenesis (n=18), the type of reproduction that occurs in *M. exigua* from coffee (Triantaphyllou, 1985a).

We can differentiate populations of *M. exigua* from rubber tree and from coffee by a relatively longer body, neck and metacarpus (Table 1). The female stylet is very different in our SEM images, compared with the female stylet morphology of *M. exigua* on coffee, illustrated by Eisenback & Triantaphyllou (1991): the cone was slightly curved dorsally (*vs* straight) and slightly widened at the junction of shaft (*vs* clearly widened); the shaft was straight, widened at the junction with the knobs (*vs* narrow at the junction with the knobs), knobs were heart shaped and backward-sloping (vs knobs pear shaped and set off from the shaft).

Males of *M. exigua* from rubber tree differ from *M. exigua* from coffee in having longer distance from DGO to the base of stylet 5.2 (5.0-5.5 µm) *vs* 4.6 (3.0-6.5) (Lima & Ferraz, 1985). According to López (1985), in SEM, the lateral lips of populations from coffee are incompletely to completely formed. In the population from rubber tree, all specimens showed the lateral lips completely formed. In addition, in SEM images illustrated by Eisenback & Triantaphyllou (1991) the medial lips are often divided medially by a shallow groove, whereas in our SEM images these depressions in the cuticle were not observed. The shape of male stylet presented some differences in populations from rubber tree

and coffee: the cone slightly widened at the junction of shaft (*vs* clearly widened); the shaft was cylindrical and straight at the junction with the knobs (*vs* narrow at the junction with the knobs); knobs are round shaped (*vs* pear shaped) (Eisenback & Triantaphyllou, 1991). In addition, the population from rubber tree was physiologically distinct (race 3) parasitizing only *H. brasiliensis* and not coffee, tomato, or pepper like the populations from coffee (Carneiro & Almeida, 2000; Lordello & Lordello, 2004).

Discussion

The present study was comparable to previous LM and/or SEM characterization of females, males and J2 of *M. exigua* populations from coffee. In general, morphological and morphometric features of the population from rubber tree agree with descriptions and measurements from some populations from coffee (Lordello & Zamith, 1958; Lima & Ferraz, 1985; López, 1985; Flores & López, 1989a; 1989b; 1989c; Eisenback & Triantaphyllou, 1991).

Variations within *M. exigua* have not been widely reported. According to Lima & Ferraz (1985) distinct populations of *M. exigua* from coffee showed a low morphological variability. Likewise, López (1985) reported variants of males of *M. exigua* populations on the basis of minor differences in the head morphology in SEM. On the other hand, enzymatic variability was reported by Oliveira et al. (2005) within 57 populations on coffee. Thirteen populations (22.8%) showed the typical one-band (E1) esterase phenotype, whereas the remaining (77.2%) presented one additional band phenotype (E2). Similar results were observed by Muniz et al. (2008), which detected a new phenotype E3 for *M. exigua* from coffee. Randig et al. (2002), studying two populations of *M. exigua* from coffee and rubber tree (PCR-RAPD) detected a high polymorphism of 67.5%, but these two populations clustered together with

100% of bootstrap. Similar molecular variability was observed on 16 populations of *M. exigua* from coffee (25.9-59.6 %) and from rubber tree (43.1 – 57.8 %), but all *M. exigua* populations clustered together with 100% of bootstrap support (Muniz et al., 2008). High variability is expected in populations of *M. exigua*, which reproduces by facultative meiotic parthenogenesis (n=18) compared to mitotic parthenogenesis (Triantaphyllou, 1985a). In addition, studies with ribosomal DNA using sequence data of the D2/D3 expansion segments showed that *M. exigua* populations from coffee and from rubber tree clustered together forming one monophyletic group (Tenente et al., 2004).

Coffee is the typical host of *M. exigua* and very few crops are known for this species. In Brazil, *Grevilea robusta* A. Cunn. (Santos, 1988); watermelon (*Citrullus vulgaris* Schrad) (Moraes et al., 1972); onion (*Allium cepa* L.) (Moraes et al., 1972; Oliveira et al., 2005); pepper (Lordello, 1964; Oliveira et al., 2005); tomato (Oliveira et al., 2005); common bean (*Phaseolus vulgaris* L.) and cacao (*Theobromae cacao* L.) (Oliveira et al., 2005); soybeans [*Glycine max* (L.) Merr.] (Oliveira et al., 2005) and also several weeds (Lima et al., 1985) have been reported as hosts of this species. The population from rubber tree collected in Rondonópolis, MT, Brazil is very host-specific, parasitizing only rubber tree clones (Lordello & Lordello, 2004).

In this study, some measurements, the stylet morphology of females and males in SEM, the esterase phenotype E2a, the host reaction (race 3) were excellent supporting characters to differentiate *M. exigua* from coffee and rubber tree. Nevertheless, the population from rubber tree was closely related with *M. exigua* from coffee considering several morphological and morphometric features, esterase band E1 (Rm=1.5), cytological and molecular approaches.

Perhaps a new subspecies, *Meloidogyne exigua* subsp. *heveicola* could be suggested, but to introduce the subspecies concept into the genus *Meloidogyne* is not a good proposal for several technical reasons (Karssen, pers.

comm.). Based on the taxonomic review made by Jepson (1987) the subspecies within the genus *Meloidogyne* were synonymized and hence this concept is not recognized.

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References

BERNARDO, E. R. A.; SANTOS, J. M.; SILVA, R. A.; CASSETARI NETO, D.; SANTOS, S. S.; DELMADI, L.; ROCHA, V. F. Levantamento de *Meloidogyne exigua* na cultura da seringueira em São José do Rio Claro, MT, Brasil. **Ciência Rural,** Santa Maria, v. 33, n. 1, p. 157-159, jan./fev. 2003.

CAMPOS, V. P.; VILLAIN, L. Nematode parasites of coffee and cocoa. In: LUC, M.; SIKORA, R. A.; BRIDGE, J. (Ed.). **Plant parasitic nematodes in subtropical and tropical agriculture.** 2.ed. Wallingford, UK: CAB International, 2005. p. 529-579.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A. Caracterização isoenzimática e variabilidade intraespecífica dos nematóides de galhas do cafeeiro no Brasil. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 1., 2000, Poços de Caldas. **Anais...** Poços de Caldas: EMBRAPA, 2000. p. 280-282.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. **Nematologia Brasileira,** Brasília, v. 25, n. 1, p. 35-44, jun. 2001.

CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P.; DICKSON, D. W. Variability among four populations of *Meloidogyne javanica* from Brazil. **Fundamental and Applied Nematology,** Paris, v. 21, n. 4, p. 319-326, Jul./Aug. 1998.

EISENBACK, J. D. Techniques for preparing nematodes for scanning electron microscopy. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University Graphics, 1985. v. 2, p. 79-105.

EISENBACK, J. D.; TRIANTAPHYLLOU, H. H. Root-knot nematodes: *Meloidogyne* species and races. In: NICLE, W. R. (Ed.). **Manual of agricultural nematology**. New York: M. Dekker, 1991. p. 191-274.

FLORES, L.; LÓPEZ, R. Caracterización morfológica del nematodo nodulador del cafeto *Meloidogyne exigua* (Nemata: Heteroderidae). I. Hembras y huevos. **Turrialba,** San José, v. 39, p. 352-360, jul./set. 1989a.

FLORES, L.; LÓPEZ, R. Caracterización morfológica del nematodo nodulador del cafeto *Meloidogyne exigua* (Nemata: Heteroderidae). II. Machos. **Turrialba,** San José, v. 39, p. 361-368, jul./set. 1989b.

FLORES, L.; LÓPEZ, R. Caracterización morfológica del nematodo nodulador del cafeto *Meloidogyne exigua* (Nemata: Heteroderidae). III. Segundos estados juvenils. **Turrialba,** San José, v. 39, p. 369-376, jul./set. 1989c.

FONSECA, H. S.; FERRAZ, L. C. C. B.; MACHADO, S. R. Ultraestrutura comparada de raízes de seringueira parasitadas por *Meloidogyne exigua* e *M. javanica*. **Nematologia Brasileira,** Brasília, v. 27, n. 2, p. 199-206, dez. 2003.

HARTMAN, K. M.; SASSER, J. N. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University Graphics, 1985. v. 2, p. 69-77.

HIRSCHMANN, H. The genus *Meloidogyne* and morphological characters differentiating its species. In: SASSER, J. N.; CARTER, C. C. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University Graphics, 1985. v. l, p. 79-93.

JEPSON, S. B. **Identification of root-knot nematodes (*Meloidogyne* species).** Wallingford, UK: CAB International, 1987. 265 p.

LIMA, R. D.; CAMPOS, V. P.; HUANG, S. P.; MELLES, C. C. A. Reprodutividade e parasitismo de *Meloidogyne exigua* em ervas daninhas que ocorrem em cafezais. **Nematologia Brasileira,** Piracicaba, v. 9, p. 63-72, 1985.

LIMA, R. D.; FERRAZ, S. Análise comparativa das variações morfométricas entre diferentes populações de *Meloidogyne exigua*. **Revista Ceres,** Viçosa, v. 32, n. 183, p. 362-373, set./out. 1985.

LÓPEZ, R. Observaciones sobre la morfologia de *Meloidogyne exigua* con el microscópio electronico de rastreo. **Nematropica,** DeLeon Springs, v. 15, n. 1, p. 27-36, June 1985.

LORDELLO, L. G. E. Contribuição ao conhecimento dos nematóides que causam galhas em raízes de plantas em São Paulo e Estados vizinhos. **Anais da Escola Superior de Agricultura Luiz de Queiroz,** Piracicaba, v. 21, p. 181-218, 1964.

LORDELLO, A. I. L.; LORDELLO, R. R. A. Reação do cafeeiro e de outras plantas a uma população de *Meloidogyne exigua* coletada em seringueira. **Revista de Agricultura,** Piracicaba, v. 79, n. 3, p. 349-352, 2004.

LORDELLO, L. G. E.; ZAMITH, A. P. L. On the morphology of the coffee root-knot nematode, *Meloidogyne exigua* Goeldi, 1887. **Proceedings of the Helminthological Society of Washington,** Beltsville, v. 25, n.2, p. 133-137, July 1958.

MUNIZ, M. F. S.; CAMPOS, V. P.; CASTAGNONE-SERENO, P.; CASTRO, J.M.C.; ALMEIDA, M. R A.; CARNEIRO, R. M. D. G. Diversity of *Meloidogyne exigua* (Tylenchida: Meloidogynidae) populations from coffee and rubber tree. **Nematology,** Leiden, 2008. In press.

MORAES, M. V.; LORDELLO, L. G. E.; PICCININ, O. A.; LORDELLO, R. R. A. Pesquisas sobre plantas hospedeiras do nematóide do cafeeiro, *Meloidogyne exigua*, Goeldi, 1887. **Ciência e Cultura,** São Paulo, v. 24, n. 7, p. 658-660, jul. 1972.

OLIVEIRA, D. S.; OLIVEIRA, R. D. L.; FREITAS, L. G.; SILVA, R. V. Variability of *Meloidogyne exigua* on coffee in the Zona da Mata of Minas Gerais State, Brazil. **Journal of Nematology,** Hanover, v. 37, n. 3, p.323-327, Sept. 2005.

RANDIG, O.; BONGIOVANNI, M.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. **Genome,** Ottawa, v. 45, n. 5, p. 862-870, Oct. 2002.

SANTOS, B. B. Nematóides do gênero *Meloidogyne* Goeldi e algumas plantas hospedeiras do Estado do Paraná. **Revista de Agricultura,** Piracicaba, v. 63, p. 37-43, 1988.

SANTOS, J. M. **Estudos das principais espécies de *Meloidogyne* Goeldi que infectam o cafeeiro no Brasil com descrição de *Meloidogyne goeldii* sp.n**. 1997. 153p. Doutorado (Tese em Proteção de Plantas) - Universidade Estadual Paulista, Botucatu, SP.

SANTOS, J. M.; MATTOS, C.; BARRÉ, L.; FERRAZ, S. *Meloidogyne exigua*, sério patógeno da seringueira nas plantações E. Michelin, em Rondonópolis, MT. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 16., 1992, Lavras. **Anais...** Lavras: Sociedade Brasileira de Nematologia, 1992. p. 75.

SILVA, R. V.; OLIVEIRA, R. D. L.; PEREIRA, A. A.; SÊNI, D. J. Respostas de genótipos de *Coffea* spp. a diferentes populações de *Meloidogyne exigua*. **Fitopatologia Brasileira,** Brasília, v. 32, n. 3, p. 205-212, maio/jun. 2007.

TENENTE, G. C. M. V.; DE LEROY, P.; DE LEROY, I. T.; KARSSEN, G.; VANFLETEREN, J. R. Sequence analysis of the D2/D3 region of the large subunit RDNA from different *Meloidogyne* isolates. **Nematropica**, DeLeon Springs, v. 34, n. 1, p. 1-12, June 2004.

TRIANTAPHYLLOU, A. C. Cytogenetics, cytotaxonomy and phylogeny of root –knot nematodes. In: SASSER, J.N.; CARTER, C. C. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University Graphics, 1985a. v. 1, p. 113-126.

TRIANTAPHYLLOU, A. C. Cytological methods for the study of oogenesis and reproduction of root-knot nematodes. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University Graphics, 1985b. v. 2, p.107-114.

ARTIGO 3

Reaction of coffee genotypes to different populations of *Meloidogyne* spp.: detection of a naturally virulent *M. exigua* population breaking resistance of Mex-1 gene

(Preparado de acordo com as normas da revista "Plant Pathology")

M.F.S. Muniz [a], V.P. Campos [b], A.W. Moita [c], W. Gonçalves [d] and R.M.D.G. Carneiro[e]

[a]Universidade Federal de Alagoas, Centro de Ciências Agrárias, 57100-000 Rio Largo, AL, Brazil; [b]Universidade Federal de Lavras, Departamento de Fitopatologia, C.P. 3037, 37200-000 Lavras, MG, Brazil; [c]Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Hortaliças, Km 09 BR 60, 70359-970 Brasília – DF, Brazil; [d]Instituto Agronômico de Campinas, C.P. 28, 13001-970 Campinas, SP, Brazil; and [e]Empresa Brasileira de Pesquisa Agropecuária, Recursos Genéticos e Biotecnologia, C.P. 02372, 70849-979 Brasília, DF, Brazil

The reaction of seven genotypes of *Coffea arabica* to inoculation with 10 *Meloidogyne* spp. populations collected mainly from coffee plantations in Brazil and Costa Rica was evaluated under greenhouse conditions. The inoculum consisted of 10,000 eggs per plant. Evaluations were made 8 months after inoculations considering the root fresh weight, gall and egg mass indices, number of eggs per gram of root and reproductive factor (RF). The cultivars Obatã IAC 1669-20, Sarchimor IAC 4361 and Tupi Amarelo IAC 5111 exhibited susceptibility to the four Brazilian *M. exigua* populations tested. However, cv. Tupi Vermelho IAC 1669-33 revealed resistance (RF value of 0.7) to *M. exigua* population from Lavras, Minas Gerais State, Brazil. The *M. exigua* population from Bom Jesus de Itabapoana, Rio de Janeiro State, Brazil was

highly virulent (RF= 165.7) on cv. IAPAR 59, bearing resistance gene Mex-1, and was also virulent on genotype H 419-5-4-5-2 (RF=396.2). *Meloidogyne* sp. population from coffee, Garça, São Paulo State, Brazil, reproduced at low rates (RF ranging from 0.1 to 3.9) on all genotypes. All tested cultivars were susceptible to *M. incognita* and *M. paranaensis*. *M. mayaguensis* from guava in Paraná State, Brazil, reproduced at low rates (RF ranging from 0.0 to 1.6) in all coffee genotypes. The same species from coffee, Costa Rica was more aggressive and showed RF value that ranged from 0.8 to 12.4. Results of this study point out for the first time the ability of a naturally occurring *M. exigua* population to overcome the resistance of Mex-1 gene in the absence of selective conditions.

Keywords: *Coffea arabica*, resistance, susceptibility, root-knot nematodes

Introduction

Root-knot nematodes of the genus *Meloidogyne* are more widely distributed throughout the world in coffee (*Coffea arabica*) plantations than any other major group of plant-pathogenic nematodes. In Brazil, the most common, damaging and well-known species are *M. exigua*, *M. incognita* and *M. paranaensis* (Campos & Villain, 2005).

Among the most damaging species, *M. exigua* is especially common in Latin America, where it constitutes a major agronomic constraint (Campos & Villain, 2005) and it was the first nematode species described in coffee roots causing damage of economic importance to Brazilian agriculture (Göldi, 1887). *Meloidogyne exigua* is widespread in coffee-producing states in Brazil (Campos & Villain, 2005) and has been found in coffee-growing areas of São Paulo (Lordello & Zamith, 1958), Espírito Santo (Chebabi, 1968), Bahia (Lordello, 1971; Souza et al. 1997), Ceará (Ponte & Senna-Silva, 1972), Minas Gerais (Campos & Melles, 1987), Rio de Janeiro (Barbosa et al., 2004) and in Paraná States (Portz et al., 2006).

Meloidogyne incognita occurs in many coffee-growing areas around the world; however, it is in Brazil that its effects on coffee plantations have become catastrophic (Campos & Villain, 2005). In this country this species was identified in the States of São Paulo (Lordello & Mello-Filho, 1970), Espírito Santo (Lordello & Hashizume, 1971, Lima et al., 2007), Paraná (Lordello & Lordello, 1972), Ceará (Ponte & Castro, 1975), Minas Gerais (Campos & Melles, 1987), and in Bahia (Souza et al., 2000).

In 1996, a new species of the genus *Meloidogyne* was described in the State of Paraná, Brazil and designated as *M. paranaensis* (Carneiro et al., 1996b). This species was also found attacking coffee in Minas Gerais State (Castro et al., 2003; Castro & Campos, 2004). According to Campos & Villain (2005), *M. paranaensis* is as destructive to coffee plantations as *M. incognita*, especially in the states of Paraná and São Paulo, Brazil. This species was also detected in Guatemala and Hawaii (Carneiro et al., 2004).

Meloidogyne mayaguensis seems to be a polyphagous species widespread in many African and America countries (Carneiro, 2003). In Cuba, it is the most damaging species on coffee (Rodríguez et al., 1995). In Brazil, this species is an economically important plant pathogen on guava (*Psidium guajava*) (Carneiro et al., 2001, 2006b; Lima et al., 2003; Torres et al., 2004; 2005; Almeida et al., 2006; Silva et al., 2006; Asmus et al., 2007). In addition, it has been found infecting many other crops (Carneiro et al., 2006a; Nascimento et al., 2006; Gomes et al., 2006; Torres et al., 2007). In greenhouse conditions, one Brazilian population of *M. mayaguensis* from guava infected coffee cv. Mundo Novo (Carneiro, 2003). The host response of coffee cultivars to this species were unknown.

The application of nematicides, crop rotations in areas where old coffee plants have been eradicated, planting in pathogen-free soil, the use of healthy seedlings, resistant rootstocks and resistant cultivars are methods used for

controlling coffee root-knot diseases (Campos & Villain, 2005). According to Roberts (2002), plant resistance has been found and developed mainly for the highly specialized parasitic nematodes that have a sedentary endoparasitic relationship with their host. However, resistance may lack durability due to variability in the nematode population (Starr et al., 2002).

The intraspecific variation of *Meloidogyne* spp. can be expressed in the plant-nematode interaction on three levels: (non-)host status, aggressiveness and virulence. In this context, plant species are good, poor or non-host for a nematode species or group within the species. Aggressiveness reflects the reproductive ability of nematodes on a susceptible good or poor host, whereas virulence is their ability to reproduce on a resistant host (Hussey & Janssen 2002).

Several lines derived from the interspecific cross between *C. arabica* and *C. canephora* (Timor Hybrid) showed resistance to *M. exigua* similar to that observed on *C. canephora* (Bertrand et al., 1997; Gonçalves & Pereira, 1998; Silvarolla et al., 1998; Bertrand et al., 2001; Fazuoli et al., 2006). Recently, a major gene designated Mex-1 locus obtained from *C. canephora* was identified. This gene possibly presented incomplete dominance (Noir et al., 2003). There are no studies showing the spectrum of resistance of the gene Mex-1 to different populations of *M. exigua* (Carneiro, pers. comm.). Resistance or tolerance to *M. incognita* and *M. paranaensis* has been found only in coffee germplasm lines derived from *C. canephora* and Ethiopian *C. arabica* accessions (Carneiro, 1995; Gonçalves et al., 1996; Anzueto et al., 2001; Campos & Villain, 2005).

Considering the great diversity in *Meloidogyne* spp. populations (Randig et al.., 2002; Carneiro et al., 2004; Hernandez et al., 2004; Carneiro et al., 2005; Muniz et al., 2008) on coffee, it is of prime importance to assess the specific pathogenicity of *Meloidogyne* species or types (races or enzymatic phenotypes) to different coffee cultivars, so as to implement integrated management

strategies. The present study investigated the pathogenicity of ten different *Meloidogyne* spp. populations on seven coffee genotypes.

Materials and methods

Nematode populations, multiplication and inoculation

Ten nematode populations taken from infected coffee and guava roots were used (Table 1). The populations were characterized by perineal pattern, races, esterase phenotypes and/or SCAR (sequence-characterized amplified region) markers using the techniques proposed by Hartman & Sasser (1985), Carneiro & Almeida (2001) and Randig et al. (2002).

The populations were multiplied on coffee cv. Catuaí Vermelho IAC 144 or tomato (*Lycopersicon esculentum* group Santa Cruz cv. Kada) roots under greenhouse conditions. To recover the inoculum, 3-month-old tomato roots or 6-month-old coffee roots were cut into 1-2 cm segments and blended for 1 minute in a 0.5% sodium hypochlorite solution (Barker, 1985). Eggs were rinsed thoroughly and counted in 1 ml aliquots in Peter's counting slide. Means of three replicates were used to represent the number of eggs per mL. Single 6-month-old coffee plants grown in 3-liter plastic pots containing 1:1:1 mixture (v/v) autoclaved soil, bovine manure and sand were inoculated with approximately 10,000 eggs of each nematode.

The inoculum in water suspension was pipetted onto the soil surface around the stem base. Plants were arranged in a randomized block, factorial design with eight replicates. Plants were grown with regular watering and fertilization.

Table 1 *Meloidogyne* spp. populations used for coffee genotype evaluations

Population code	**Geographical origin**	**Species/Race**	**Esterase phenotype**
Mexi 1	Lavras – MG, Brazil	*M. exigua* race 1	E1
Mexi 2	Lavras – MG, Brazil	*M. exigua* race 2	E2
Mexi 3	Bom Jesus de Itabapoana – RJ, Brazil	*M. exigua* race 1	E1
Mexi 4	Campinas – SP, Brazil	*M. exigua* race 1	E2
Minc 5	Avilândia – SP, Brazil	*M. incognita* race 1	I1
Minc 6	Londrina – PR, Brazil	*M. incognita* race 3	I2
Msp 7	Garça – SP, Brazil	*Meloidogyne* sp.	S2
Mpar 8	Londrina – PR, Brazil	*M. paranaensis*	P1
Mma 9	Santa Mariana – PR, Brazil	*M. mayaguensis* race 2[a]	M2
Mma 10	Costa Rica	*M. mayaguensis* race 2	M2

[a]Population obtained from guava. The remaining populations were obtained from coffee

Experiment and plant material

The trial was carried out in a greenhouse at temperatures ranging from 22 to 28ºC. Coffee plants derived from Timor Hybrid lines were used. These selections came from Timor Island and were collected by the Coffee Rust Research Center (CIFC), in Portugal. They were formed by coffee plants with high genetic variability in which resistant genes are available for some pathogens (Pereira et al., 2002). This germplasm has been used in *C. arabica* breeding programs. The coffee genotypes tested were the following: Obatã IAC 1669-20, Tupi Vermelho IAC 1669-33, Tupi Amarelo IAC 5111, Sarchimor IAC 4361 and IAPAR 59. All advanced generations were derived from a cross between the 'Villa Sarchi' and the 'Timor Hybrid (CIFC 832/2)'. The genotypes H 419-5-4-5-2 derived from 'Catuaí Amarelo IAC 30 x Timor Hybrid UFV 445-46 (CIFC 2570)' and 'Catuaí Vermelho IAC 144' used as susceptible standard were also included.

Nematode resistance evaluation

Observations were made 8 months after inoculation. The root systems were carefully washed free of soil, blotted onto paper to damp dry, and weighed (RW). The whole root system of each plant was soaked in a solution of Phloxine B (0.015g/L of tap water) for 20 minutes to stain egg masses. Galls and number of egg masses produced by the nematode per root system were counted. Gall Index (GI) and Egg Mass Index (EMI) were calculated according to a scale proposed by Hartman & Sasser (1985), where 0 = no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10, 3= 11-30; 4= 31-100, and 5= over 100 galls or egg masses. Host-plant type symptoms were observed.

Eggs were extracted by root trituration in a blender for 4 minutes in a 1% NaOCl solution according to Hussey & Barker (1973), and the number of eggs per root system (final nematode population = P_f) were counted in triplicate in a Peter's counting slide. This mean value was used to determine the number of eggs per gram of root (eggs/g root) and the reproductive factor (RF), which represents the relation between final and initial nematode population densities for each treatment ($RF=P_f/P_i$), according to Oostenbrink (1966). The genotypes for which $RF \leq 1$ were considered as resistant (R), and those for which $RF > 1$ were considered susceptible (S). The genotypes were scored as highly susceptible (HS), susceptible (S), moderately resistant (MR) and resistant (R), according to the statistical analyses.

Statistical analyses

Analysis of variance was performed for the experiment after a log (x + 1) transformation of the data for the fresh root weight (RW), eggs/g root and RF values, and Scott-Knott test ($P \leq 0.05$) was used to evaluate differences among genotypes within the same population and differences between populations for the same coffee genotype. Pearson correlation coefficients between RW and RF for each genotype and also between GI and EMI versus RF for each nematode population were calculated. All analyses were carried out using the SAS statistical package (SAS Institute, 1988).

Results

Statistical analyses

A statistical analysis of the variables used revealed significant coffee genotypes and *Meloidogyne* spp. population effects, for RW, eggs/g root and RF ($P \leq 0.05$), and interactions effects. Only for the genotypes Catuaí Vermelho IAC 144, Sarchimor IAC 4361, Obatã IAC 1669-20 and H 419-5-4-5-2, was RW significantly correlated to RF, but with low Pearson correlation coefficients (r = 0.25-0.45), showing small influence of root weight on final nematode population. However, GI and EMI were correlated with RF for *M. exigua* (r = 0.59-0.69; 0.36-0.77, respectively), *M. mayaguensis* (r = 0.34-0.51; 0.68-0.73) and *Meloidogyne* sp. (r = 0.42; 0.58), but sometimes with low Pearson correlation coefficient. For *M. incognita* and *M. paranaensis* GI and EMI were not correlated significantly with RF. These pathosystems did not allow the evaluation of nematode infection using these variables**.**

Coffee genotypes x *M. exigua*

The root symptoms observed were small rounded galls mostly on new roots, which usually contained external and internal egg masses. In Table 2, a significant difference between the cultivar Obatã IAC 1669-20 and the remaining genotypes should be noted in relation to the variable RW in population Mexi 1. For populations Mexi 2 and Mexi 4 no difference was observed between the genotypes. In addition, for population Mexi 3 the RW was greater in the genotypes Obatã IAC 1669-20 and Sarchimor IAC 4361.

Variation occurred in index of gall and egg masses among coffee genotypes: the lowest values occurred on 'IAPAR 59' and 'H 419-5-4-5-2' inoculated with Mexi 1, Mexi 2 and Mexi 4 populations, as well as 'Tupi

Vermelho IAC 1669-33' inoculated with Mexi 1. The index value for gall was always equal or higher than egg mass (Table 3).

The cultivars Obatã IAC 1669-20, Sarchimor IAC 4361 and Tupi Amarelo IAC 1669-33 exhibited susceptibility to the four *M. exigua* populations (Tables 4 and 5). Considering the RF value of 0.7, cv. Tupi Vermelho IAC 1669-33 can be classified as resistant to the population Mexi 1 from Lavras, Minas Gerais State, Brazil, and susceptible to the others. However, the RFs are relatively small for this cultivar when compared with the others (Table 5).

No variability occurred with Mexi 1, Mexi 2 and Mexi 4 reproductions on 'IAPAR 59' and genotype H 419-5-4-5-2 based on eggs/gram of root and FR which ranked IAPAR 59 and H 419-5-4-5-2 as resistant to these populations. 'Tupi Vermelho IAC 1669-33' was resistant to Mexi 1 population and the other interactions between coffee genotypes and *M. exigua* were ranked as highly susceptible or susceptible (Tables 4 and 5). However, for the virulent population Mexi 3, collected in Rio de Janeiro State, Brazil, the RF values on some genotypes were very high: IAPAR 59 (RF=165.7) and H 419-5-4-5-2 (RF=396.2). On IAPAR 59, Obatã IAC 1669-20, Sarchimor IAC 4361 and H 419-5-4-5-2 genotypes this population reproduced much more than on Catuaí Vermelho IAC144 (the susceptible control), Tupi Amarelo IAC 5111 and Tupi Vermelho IAC 1669-33 (Tables 3, 4 and 5).

Table 2 Fresh weight of roots (g) of seven coffee genotypes eight months after inoculation with 10,000 eggs of *Meloidogyne* spp. per plant

Coffee genotypes	***Meloidogyne* populations**									
	Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma10**
Catuaí Vermelho IAC 144	106.0 Bb	96.9 Ab	101.3 Ab	83.4 Ab	65.7 Aa	99.0 Cb	122.3 Bb	80.9 Ab	67.9 Aa	38.4 Aa
IAPAR 59	89.9 Bc	124.7 Ad	87.3 Ac	116.5 Ad	140.1 Bd	25.7 Aa	140.4 Bd	93.6 Ac	78.1 Bc	57.8 Bb
Obatã IAC 1669-20	53.1 Aa	145.0 Ab	162.1 Bb	103.9 Ab	58.4 Aa	102.1Cb	85.9 Aa	79.1Aa	118.1 Cb	157.1 Db
Sarchimor IAC 4361	102.7 Ba	98.0 Ab	146.0 Bb	92.3 Aa	112.9 Bb	101.1 Cb	130.8 Bb	59.1 Aa	66.7 Aa	67.9 Ba
H 419-5-4-5-2	98.3 Bc	107.6 Ac	108.0 Ac	88.9 Ac	115.0 Bc	49.3 Ba	133.9 Bc	79.9 Ab	79.2 Bb	57.3 Ba
Tupi Amarelo IAC 5111	107.3 Ba	122.3 Ab	93.3 Aa	94.2 Aa	110.9 Bb	87.9 Ca	153.6 Bb	89.0 Aa	80.4 Ba	85.6 Ca
Tupi Vermelho IAC 1669-33	100.9 Bb	118.6 Ab	104.1 Ab	88.0 Ab	59.1 Aa	77.4 Cb	137.7 Bb	90.2 Ab	49.2 Aa	45.4 Aa

Values within columns followed by same capital letter or row followed by same lower letter represent data not significantly different from each other at 5% probability using the Scott Knott test.
Values are means of eight replicate root systems. Population codes are given in Table 1.

Table 3 Mean values of gall index and egg mass index produced by 10 *Meloidogyne* spp. populations on seven coffee genotypes

Coffee genotypes		***Meloidogyne* populations**									
		Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma 10**
Catuaí Vermelho IAC 144	GI	5.0	5.0	4.9	3.1	2.8	3.3	0.9	3.6	1.4	3.0
	EMI	5.0	5.0	4.4	3.1	5.0	5.0	1.1	5.0	1.4	4.3
IAPAR 59	GI	0.0	0.0	5.0	0.1	3.6	3.5	0.3	3.3	3.0	2.8
	EMI	0.0	0.0	5.0	0	4.9	5.0	0.5	5.0	3.6	2.3
Obatã IAC 1669-20	GI	2.1	2.9	5.0	0.6	3.6	4.3	1.6	3.6	0.0	3.6
	EMI	2.0	2.9	4.8	0.3	5.0	5.0	3.3	5.0	0.0	4.6
Sarchimor IAC 4361	GI	5.0	5.0	4.9	3.4	2.9	3.5	0.8	4.0	0.1	1.8
	EMI	4.4	5.0	4.5	3.3	5.0	4.3	2.9	4.9	0.3	0.9
H 419-5-4-5-2	GI	0.0	0.0	5.0	0.0	2.5	4.0	0.0	4.1	0.0	2.8
	EMI	0.0	0.0	4.4	0.0	5.0	4.9	1.9	5.0	0.0	1.6
Tupi Amarelo IAC 5111	GI	2.6	2.9	4.9	3.1	2.3	3.0	0.0	3.9	0.0	2.9
	EMI	1.6	2.1	3.6	2.1	4.9	4.9	0.3	4.6	1.6	1.1
Tupi Vermelho IAC 1669-33	GI	1.0	3.8	5.0	2.5	3.3	4.4	0.0	3.4	0.1	2.3
	EMI	0.0	2.1	1.4	1.6	5.0	4.9	0.0	4.6	0.4	1.5

Values are means of eight replicate root systems. Population codes are given in Table 1.
Gall Index (GI) and Egg Mass Index (EMI) were based on a 0 – 5 scale, where 0 = no galls or egg masses and 5 = over 100 galls or egg masses.

Table 4 Mean values of the number of eggs per gram of root system for ten *Meloidogyne* spp. populations on seven coffee genotypes

Coffee genotypes	***Meloidogyne* populations**									
	Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma10**
Catuaí Vermelho IAC 144	11314.3 Dc	6077.5 Dc	10354.4 Ac	3373.0 Bb	6626.3 Bc	1476.4Ab	89.9 Ba	2358.2Bb	48.4Ba	2565.0 Bb
IAPAR 59	3.4 Aa	20.6 Ab	17770.5 Be	42.3 Ab	2983.6 Ad	4550.0 Bd	14.7Ab	2336.6Bd	195.2Cc	3014.8Bd
Obatã IAC 1669-20	5116.5 Cb	4085.9 Cc	16148.6 Bd	638.0 Aa	9650.4 Bd	1509.6 Ac	695.4 Bb	1741.9 Ac	10.5 Aa	1666.1Bc
Sarchimor IAC 4361	8110.5 Dc	4404.5 Dc	12016.8 Bc	4167.6 Bb	3150.0 Ac	1469.7 Ac	372.6 Bb	2145.9 Bc	20.2 Aa	339.8 Ab
H 419-5-4-5-2	26.3 Bb	25.3 Ab	37466.2 Bf	35.4 Ab	2191.8 Ae	2806.8 Be	98.5 Bc	3851.2 Be	5.8 Aa	751.4 Bd
Tupi Amarelo IAC 5111	2034.9 Cc	913.2 Bc	13742.8 Ad	1737.9 Ab	6968.8 Bd	1565.1 Ad	17.6 Aa	1614.1Ad	183.6 Bb	339.7 Ac
Tupi Vermelho IAC 1669-33	70.9 Bb	1154.1 Bc	2065.3 Ad	1040.1 Ab	4925.6 Bd	1125.4 Ad	21.7 Aa	1013.0 Ad	27.8 Ab	389.7 Ac

Values within columns followed by same capital letter or row followed by same lower letter represent data not significantly different from each other at 5% probability using the Scott-Knott test.
Values are means of eight replicate root systems. Population codes are given in Table 1.

Table 5 Reproduction factor (RF) and type of reaction on seven coffee genotypes assessed eight months after inoculation with 10,000 eggs per plant of 10 *Meloidogyne* populations in greenhouse conditions

Coffee genotypes	***Meloidogyne* populations**									
	Mexi 1	**Mexi 2**	**Mexi 3**	**Mexi 4**	**Minc 5**	**Minc 6**	**Msp 7**	**Mpar 8**	**Mma 9**	**Mma 10**
Catuaí Vermelho IAC 144	119.5 C HS	58.6 C HS	112.8 A S	31.7 B HS	36.2 A S	14.1 A S	1.1 B MR	19.3 B HS	0.3 A R	4.7 B S
IAPAR 59	0.1 A R	0.3 A R	165.7 B HS	0.4 A R	41.7 A S	10.4 A S	0.1 A R	20.7 B HS	1.6 B MR	7.0 B S
Obatã IAC 1669-20	30.7 B S	73.4 C HS	265.1 B HS	9.8 A S	61.9 B HS	16.5 A S	3.2 B MR	13.9 A S	0.1 A R	12.4 C S
Sarchimor IAC 4361	84.0 C HS	45.3 C HS	167.0 B HS	42.8 B HS	35.3 A S	14.0 A S	3.9 B MR	11.3 A S	0.1 A R	1.1 A MR
H 419-5-4-5-2	0.2 A R	0.3 A R	396.2 B HS	0.3 A R	25.5 A S	13.3 A S	1.4 B MR	31.5 B HS	0.0 A R	2.0 A MR
Tupi Amarelo IAC 5111	26.2 B S	9.3 B S	97.5 A S	16.4 A S	76.5 B HS	13.5 A S	0.2 A R	13.0 A S	1.3 B MR	1.2 A MR
Tupi Vermelho IAC 1669-33	0.7 A R	13.2 B S	18.9 A S	11,8 A S	29.0 A S	8.6 A S	0.4 A R	8.8 A S	0.2 A R	0.8 A R

R: resistant; MR: moderately resistant; S: susceptible; HS: highly susceptible

Values within columns followed by same capital letter represent data not significantly different from each other at 5% probability using the Scott-Knott test.

Values are means of eight replicate root systems. Population codes are given in Table 1.

Coffee genotypes x *M. incognita*

Coffee plants inoculated with this species showed swellings on the root and galls, and in some of them, necrosis of the tap roots was observed. Egg masses were produced on the root surface. In general, the genotypes of coffee evaluated responded differently to the attack of the two populations. In fact, for population Minc5 race 1, from São Paulo State, Brazil, the smallest values for RW were observed for Catuaí Vermelho IAC 144, Obatã IAC 1669-20 and Tupi Vermelho IAC 1669-33 cultivars, whereas for population Minc 6 race 3 from Paraná State, Brazil, it should be noted that the smallest values for this variable were obtained for the genotypes IAPAR 59 and H 419-5-4-5-2 (Table 2).

Unlike *M. exigua*, populations of *M. incognita* (Minc 5 and Minc 6) had EMI greater than GI. But EMI and GI for both Minc 5 and Minc 6 were high in all genotypes (Table 3). Even though differences ($P \leq 0.05$) occurred among coffee genotypes evaluated by number of eggs per gram of roots when inoculated by either Minc 5 or Minc 6 (Table 4). The pathogenicity reaction types according to RF ranked all coffee genotypes as highly susceptible or susceptible when inoculated with both populations (Table 5). However, considering our RF values, *M. incognita* race 1 seems to be more aggressive to coffee cultivars than race 3**.**

Coffee genotypes x *Meloidogyne* sp.

The inoculated plants had slight symptoms of root destruction and some egg masses were observed in some cultivars. For this population, the RW values were hardly variable among the tested cultivars (Table 2). Both indices (EMI and GI) in each genotype were lower than in coffee inoculated with *M. incognita* races 1 and 3, but values varied from 0.0 to 3.3 for EMI (Table 3). The number of eggs per gram of roots was higher ($P \leq 0.05$) in Catuaí Vermelho IAC 144, Obatã IAC 1669-20, Sarchimor IAC 4361 and H 419-5-4-5-2 than other coffee genotypes (Table 4). The RF were low in all coffee genotypes, ranking the coffee genotypes as resistant and moderately resistant (Table 5). We can consider the population Msp 7 as a weak parasite of coffee.

Coffee genotypes x *M. paranaensis*

The symptoms observed in the inoculated plants were swellings on the roots, and they did not produce typical root knot nematode galls. Several developed egg masses were observed on the roots. Coffee genotypes showed low RW and high number of eggs per gram of roots. The EMI values were always higher than GI. The RFs were high, ranking all coffee genotypes tested as highly susceptible or susceptible (Tables 2, 3, 4 and 5).

Coffee genotypes x *M. mayaguensis*

The root symptoms included swellings on the root tips and necrosis. There was no formation of typical galls. Egg masses were observed on the roots. In the population Mma 9 from guava (Santa Mariana, Paraná State, Brazil), the smallest values for RW values were observed on the cvs. Catuaí Vermelho IAC 144, Sarchimor IAC 4361 and Tupi Vermelho IAC 1669-33 (Table 2).

The EMI and GI values were low in almost all coffee genotypes except in IAPAR 59 with EMI higher than GI when coffee plants were inoculated with Mma 9 (Table 3). When coffee genotypes were inoculated with Mma 10 the values of EMI and GI were higher than Mma 9 inoculated coffee, but in only two genotypes (Obatã IAC 1669-20 and Catuaí Vermelho IAC 144) EMI was greater than GI, showing differences in host susceptibility (Table 3). A higher ($P \leq 0.05$) number of eggs per gram of roots was observed when each cultivar was inoculated with Mma 10, with variation also among genotypes, within the same nematode population (Table 4). The RF values were always lower and less variable for coffee cultivars inoculated with Mma 9 compared to Mma 10, ranking the coffee genotypes inoculated with Mma 9 from moderately resistant to highly resistant, but when these genotypes were inoculated with Mma 10 they were ranked from susceptible to resistant. In fact, differences and similarities among coffee genotypes inoculated with both populations of *M. mayaguensis* were observed. Tupi Vermelho IAC 1669-33, Tupi Amarelo IAC 5111, H 419-5-4-5-2 and Sarchimor IAC 4361 were resistant to both Mma 9 and Mma 10, but Catuaí Vermelho IAC 144, IAPAR 59 and Obatã IAC 1669-20 were resistant to Mma 9 and susceptible to Mma 10 (Table 5). We can consider the population Mma 9 as a weak parasite of coffee.

In the entire experiment, there were frequently contrasting responses between the replicates. For example, on cv. IAPAR 59, population 3 of *M. exigua* from Bom Jesus de Itabapoana, Rio de Janeiro State, Brazil, the RF values ranged from 51.0 to 413.8. It was also observed that gall and egg-masses indices were not good variables to evaluate coffee infection with *M. incognita*, *M. paranaensis*, *Meloidogyne* sp. and *M. mayaguensis*. These pathosystems did not allow the production of typical root galls and sometimes the egg-masses' position is internal (*M. exigua* and *M. paranaensis*) and impossible to be stained with Phloxine B.

Discussion

The galling and egg mass index were not reliable indicators of nematode multiplication rates because the symptoms of damage caused by different species of *Meloidogyne* on coffee are variable and very difficult to quantify. Based on these findings, the best parameter is number of eggs per g of roots or the reproduction factor (RF). This result disagrees with the observations made by Hernandez et al. (2004), who considered galling index as a relatively good indicator of nematode multiplication rate.

Most information on virulence in *Meloidogyne* spp. is known with regard to the Mi resistant gene in tomato. In the 1950s the occurrence of resistance-breaking isolates was noticed in *M. incognita*, *M. arenaria* and *M. javanica* and designated as B-races (Riggs & Winstead, 1959). Selection experiments under laboratory conditions have shown that the proportion of virulent nematodes gradually increases after each successive generation on resistant tomato plants (Netscher, 1977). The same was observed for *M. exigua* on resistant IAPAR 59 plants (data not included).

Our results showed the first naturally resistance–breaking field populations of *M. exigua* on the cultivar IAPAR 59 derived from crossing '*C. arabica* Villa Sarchi x Timor Hybrid' and carrying gene Mex-1. In addition, this virulent population has been observed even when it was not previously exposed to resistant cultivars. This resistance-breaking was also observed in the genotype H 419-5-4-5-2. Since *M. exigua* is a meiotic parthenogenetic species (Triantaphyllou, 1985), mechanisms of genetic recombination or other must be responsible for the increasing virulence. According to Cook & Evans (1987) meiotic parthenogenesis maintains the opportunity for sexual reproduction and this permits recombination between homologous chromosomes. These authors suggested that parthenogenesis does not reduce mutation rates and this may

generate atypical populations. Triantaphyllou (1987) suggested that the action of a high frequency of mutations in minor genes affects virulence. Castagone-Sereno et al. (1994a) hypothesized a gene amplification system of genomic regions or chromosomes carrying virulence alleles, but they also assumed different mechanisms involved in the acquisition of virulence between field and laboratory–selected virulent nematodes due to the observed differences in stability and spectrum of their virulence (Roberts et al., 1990; Castagnone-Sereno et al., 1994b).

The results of this study were partially consistent with earlier findings by Bertrand et al. (1998; 2000) and Salgado et al. (2002, 2005). These authors detected the resistance of the cultivar IAPAR-59 to different populations of *M. exigua.* This resistance was also observed in H 419-5-4-5-2 coffee genotype for the three populations of *M. exigua*. Their ability to multiply on such cultivars and their uniformly higher rate of reproduction compared with other *M. exigua* populations used in this study has to be viewed as very important and potentially dangerous characteristics of this population. Other reports of differential behavior of populations of *M. exigua* in Brazil have been suggested (Barbosa et al., 2007); however, the FR value for 'IAPAR 59' was very small (0.75) and it was impossible to characterize the virulence. This virulence variability shows the presence of vertical resistance. This type of resistance is only effective against certain variants of a particular parasite (Van Der Plank, 1963).

The nematode resistance originated from Timor Hybrid germplasm and its derivatives has been considered of an incomplete dominance and monogenic (Noir et al., 2003), and also postinfectional (Salgado et al., 2005). According to Anthony et al. (2005) resistance conferred by the Mex-1 gene is strongly associated with a hypersensitive reaction (HR). Recently, Alzipar et al. (2007) concluded that Mex-1 could have incomplete dominant expression because most

of the F2 populations showed a gall index higher than the mean value of the resistant parent.

In the present work, all coffee cultivars were susceptible to two *M. incognita* populations, but race 1 (EST I1) from São Paulo was more aggressive than race 3 (EST I2) from Paraná State. Several studies have been made of the reaction of *C. arabica* or *C. canephora* genotypes *to M. incognita* under greenhouse or field conditions in Brazil, and other countries (Carneiro, 1995; Gonçalves et al., 1996; Bertrand et al., 2000; Anzueto et al., 2001; Hernandez et al., 2004; Tomazini et al., 2005). In these studies, some progeny of *C. canephora* or Ethiopian *C. arabica* accessions were proved to be effective. However, in these studies, the *Meloidogyne* species were sometimes incorrectly identified.

Meloidogyne sp. collected from coffee in Garça, São Paulo State, presented low aggressiveness to coffee genotypes in greenhouse and field conditions (W. Gonçalves, pers. comm.). This population displaying the esterase phenotype S1 (= S2), presented a perineal pattern resembling *M. incognita* (Oliveira et al., 2006). Other morphological characters using scanning electron microscopy and SCAR markers should be used to clarify the characterization of this species.

Meloidogyne mayaguensis is considered the most dangerous species on coffee fields in Cuba (Rodríguez et al., 1995; 2001). The two populations of *M. mayaguensis* (from guava, Brazil and from coffee, Costa Rica) revealed differences in aggressiveness suggesting a physiological specialization of this species on coffee. Moreover, the low values of RF indicate that coffee is a poor host for *M. mayaguensis* in the Brazilian conditions.

In Brazil, *M. mayaguensis* was reported for the first time in the semi-arid zone of the northeastern region in the States of Pernambuco and Bahia causing severe damage in guava plantations (Carneiro et al., 2001) and later in the States of Rio de Janeiro (Lima et al., 2003), Rio Grande do Norte (Torres et

al., 2004), Ceará (Torres et al., 2005), São Paulo (Almeida et al., 2006), Paraná (Carneiro et al., 2006b), Piauí (Silva et al., 2006), Mato Grosso do Sul (Asmus et al., 2007), and Espírito Santo (Lima et al., 2007) on the same host. In addition, this species was also detected parasitizing papaya plants (*Carica papaya*) (Lima et al., 2003); tomato (Carneiro et al., 2006a); pumpkin (*Cucurbita moschata*) (Nascimento et al., 2006); tobacco (*Nicotiana tabacum*) (Gomes et al., 2006), pepper (*Capsicum annuum*) (Carneiro et al., 2006a; Torres et al., 2007), and also on weed hosts (Lima et al. 2003; Carneiro et al., 2006b). However, despite its pathogenicity and distribution, there is no report of this species being a potential coffee parasite in Brazil.

Considering the diversity of *Meloidogyne* species able to parasite coffee in Brazil and Central America (Carneiro et al., 2004; Hernandez et al., 2004, Muniz et al., 2008) and in terms of virulence, priority must be given to conduct experiments with more than one population of each *Meloidogyne* species when evaluating resistance of new coffee genotypes. Such information will be of considerable interest for the development of integrated management programs and, especially, for the development of durable resistant cultivars adapted to the different parasitic situations in coffee-growing areas.

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References

ALMEIDA E. J.; SOARES P. L. M.; SANTOS, J. M.; MARTINS, A. B. G. Ocorrência de *Meloidogyne mayaguensis* na cultura da goiaba (*Psidium guajava*) no Estado de São Paulo. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006. p. 85.

ALZIPAR, E.; ETIENNE, H.; BERTRAND, B. Intermediate resistance to *Meloidogyne exigua* root-knot nematode in *Coffea arabica*. **Crop Protection,** Oxford, v. 26, n. 7, 903-910, July 2007.

ANTHONY, F.; TOPART, P.; MARTINEZ, A.; SILVA, M.; NICOLE, M. Hypersensitive-like reaction conferred by the Mex-1 resistance gene against *Meloidogyne exigua* in coffee. **Plant Pathology,** Avenel, v. 54, n. 4, p. 476-482, Aug. 2005.

ANZUETO, F.; BERTRAND, B.; SARAH, J. L.; ESKES, A. B.; DECAZY, B. Resistance to *Meloidogyne incognita* in Ethiopian *Coffea arabica* accessions. **Euphytica,** v. 118, n. 1, p. 1-8, Mar. 2001.

ASMUS, G. L.; VICENTIN, E.M.; CARNEIRO, R. M. D. G. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado de Mato Grosso do Sul. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 61.

BARBOSA, D. H. S. G.; VIEIRA, H. D.; SOUZA, R. M.; SILVA, C. P. Survey of root-knot nematode (*Meloidogyne* spp.) in coffee plantations in the State of Rio de Janeiro, Brazil. **Nematologia Brasileira,** Brasília, v. 28, n. 1, p. 43-47, jun. 2004.

BARBOSA, D. H. S. G.; VIEIRA, H. D.; SOUZA, R. M.; DIAS, P. D.; VIANA, A. P. Desenvolvimento vegetativo e reação de genótipos de *Coffea* spp. a uma população de *Meloidogyne exigua* virulenta a cultivares resistentes. **Nematologia Brasileira,** Piracicaba, v. 31, n. 1, p. 1-6, abr. 2007.

BARKER, K. R. Nematode extraction and bioassays. In: BARKER, K.R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University Graphics, 1985. v. 2, p. 19-35.

BERTRAND, B.; AGUILAR, G.; BOMPARD, E.; RAFINON, A.; ANTHONY, F. Comportement agronomique et résistance aux principaux déprédateurs des lignées de Sarchimor et Catimor au Costa Rica. **Plantations, recherche, développement**, Montpellier, v. 4, n. 5, p. 312-318, Sept./Oct. 1997.

BERTRAND, B; ANTHONY, F.; LASHERMES, P. Breeding for resistance to *Meloidogyne exigua* in *Coffea arabica* by introgression of resistance genes of *Coffea canephora*. **Plant Pathology,** Avenel, v. 50, n. 5, p. 637-643, Oct. 2001.

BERTRAND, B.; CILAS, C.; HERVÉ, G.; ANTHONY, F.; ETIENNE, H.; VILLAIN, L. Relations entre les populations des nematodes *Meloidogyne exigua* et *Pratylenchus* sp., dans les racines de *Coffea arabica* au Costa Rica. **Plantations, recherche, développement,** Montpellier, v. 5, n. 4, p. 279-284, juillet/août 1998.

BERTRAND, B.; ETIENNE, H.; SANTACREO, R.; ANZUETO, F.; ANTHONY, F. El mejoramiento genético en América Central. In: SEMINÁRIO INTERNACIONAL SOBRE BIOTECNOLOGIA NA AGROINDÚSTRIA CAFEEIRA, 3., 1999, Londrina. **Anais...** Londrina: UFPR/IAPAR, 2000. p. 231-243.

CAMPOS, V. P.; MELLES, C. C. A. Ocorrência e distribuição de espécies de *Meloidogyne* em cafezais dos Campos das Vertentes e do Sul de Minas. **Nematologia Brasileira,** Piracicaba, v. 11, p. 233-241, mar. 1987.

CAMPOS, V. P.; VILLAIN, L. Nematode parasites of coffee and cocoa. In: LUC, M.; SIKORA, R. A.; Bridge, J. (Ed.). **Plant parasitic nematodes in subtropical and tropical agriculture.** 2.ed. Wallingford, UK: CAB International, 2005. p. 529-579.

CARNEIRO, R. G. Reação de progênies de Café 'Icatu' a *Meloidogyne incognita* raça 2, em condições de campo. **Nematologia Brasileira,** Campinas, v. 19, n. 1/2, p. 53-59, 1995.

CARNEIRO, R. M. D. G. Uma visão mundial sobre a ocorrência e patogenicidade de *Meloidogyne mayaguensis* em goiabeira e outras culturas. **Nematologia Brasileira,** Brasília, v. 27, n. 2, p. 229-230, dez. 2003.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. **Nematologia Brasileira,** Brasília, v. 25, n. 1, p. 35-44, jun. 2001.

CARNEIRO, R. M. D. G.; ALMEIDA, M. R. A.; BRAGA, R. S, ALMEIDA, C. A, GIORIA R. Primeiro registro de *Meloidogyne mayaguensis* parasitando plantas de pimentão (*Capsicum annuum*) e tomate (*Lycopersicum esculentum*), resistentes à meloidoginose no Estado de São Paulo. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006a. p. 86.

CARNEIRO, R. M. D. G.; CARNEIRO, R. G.; ABRANTES, I. M. O.; SANTOS, M. S. N. A.; ALMEIDA, M. R. A. *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. **Journal of Nematology**, Hanover, v. 28, n. 2, p. 177-189, June 1996b.

CARNEIRO, R. G.; MÔNACO, A. P. A.; MORITZ, M. P.; NAKAMURA, K. C.; SCHERER, A. Identificação de *Meloidogyne mayaguensis* em goiabeiras e em plantas invasoras, em solo argiloso, no Estado do Paraná. **Nematologia Brasileira,** Brasília, v. 30, n. 3, p. 293-298, dez. 2006b.

CARNEIRO, R.M.D.G.; RANDIG, O.; ALMEIDA M. R. A.; GONÇALVES, W. Identificação e caracterização de espécies de *Meloidogyne* em cafeeiro nos Estados de São Paulo e Minas Gerais através dos fenótipos de esterase e SCAR-Multiplex-PCR. **Nematologia Brasileira,** Brasília, v. 29, n. 2, p. 233-241, dez. 2005.

CARNEIRO, R. M. D. G.; MOREIRA, W. A.; ALMEIDA, M. R. A.; GOMES, A. C. M. M. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. **Nematologia Brasileira,** Brasília, v. 25, n. 2, p. 223-228, dez. 2001.

CARNEIRO, R. M. D. G.; TIGANO, M. S.; RANDIG, O.; ALMEIDA, M. R. A.; SARAH, J. L. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida:Meloidogynidae) on coffee from Brazil, Central America and Hawaii. **Nematology,** Leiden, v. 6, n. 2, p. 287-298, Mar. 2004.

CASTAGNONE-SERENO, P.; WAJNBERG, E.; BONGIOVANNI, M.; LEROY, F.; DALMASSO, A. Genetic variation in *Meloidogyne incognita* virulence against the tomato Mi resistance gene: evidence from isofemale line selection studies. **Theoretical and Applied Genetics,** Heidelberg, v. 88, n. 6/7, p. 749-753, Aug. 1994a.

CASTAGNONE-SERENO, P.; BONGIOVANNI, M.; DALMASSO, A. Reproduction of virulent isolates of *Meloidogyne incognita* on susceptible and Mi-resistant tomato. **Journal of Nematology,** Hanover, v. 26, n. 3, p. 324-28, Sept. 1994b.

CASTRO, J. M. C.; CAMPOS, V.P. Ocorrência de *Meloidogyne paranaensis* em cafeeiros na região Sul de Minas Gerais. **Fitopatologia Brasileira,** Brasília, v. 29, p. 89, 2004. Suplemento.

CASTRO, J. M. C.; CAMPOS, V. P.; NAVES, R. L. Ocorrência de *Meloidogyne paranaensis* em cafeeiros na região do Alto Paranaíba em Minas Gerais. **Fitopatologia Brasileira,** Brasília**,** v. 28, n. 5, p. 565, set./out. 2003.

CHEBABI, A. Ocorrência de nematóides em cafezais do estado do Espírito Santo. **Revista de Agricultura,** Piracicaba, v. 43, p. 140, 1968.

COOK, R.; EVANS, K. Resistance and tolerance. In: BROWN, R. H.; KERRY, B.R. (Ed.). **Principles and practice of nematode control in crops.** Marrickville, Australia: Academic, 1987. p. 179-231.

FAZUOLI, L. C.; GONÇALVES, W.; BRAGHINI, M. T.; SILVAROLLA, M. B. 'Tupi RN IAC 1669-13': cultivar de café (*Coffea arabica*) resistente a *Hemileia vastatrix* e a *Meloidogyne exigua*. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006. p. 95.

GÖLDI, E. A. **Relatório sobre a moléstia do cafeeiro na Província do Rio de Janeiro, 1887.** Recife: UFRPE. Fadurpe, 1998. 121p. (Reeditado por Romero Marinho Moura).

GOMES, C.B.; LIMA, D. L.; CARNEIRO, R. M. D. G. Ocorrência de *Meloidogyne mayaguensis* em fumo (*Nicotiana tabacum*) em Santa Catarina. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006. p. 88.

GONÇALVES, W.; FERRAZ, L. C. C. B.; LIMA, M. M. A.; SILVAROLLA, M. B. Reações de cafeeiros às raças 1, 2 e 3 de *Meloidogyne incognita*. **Summa Phytopathologica,** Jaboticabal, v. 22, n. 2, p.172-177, abr./jun.1996.

GONÇALVES, W.; PEREIRA, A. A. Resistência do cafeeiro a nematóides IV – Reação de cafeeiros derivados do Híbrido de Timor a *Meloidogyne exigua*. **Nematologia Brasileira,** Brasília, v. 22, n. 1, p. 39-50, jun.1998.

HARTMAN, K. M.; SASSER, J. N. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: BARKER, K. R.; CARTER, C. C.; SASSER, J. N. (Ed.). **An advanced treatise on *Meloidogyne***. Raleigh, NC: North Carolina State University, 1985. v. 2, p. 69-77.

HERNANDEZ, A.; FARGETTE, M.; SARAH, J. L. Pathogenicity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) isolates from Central America and Brazil on four genotypes of *Coffea arabica*. **Nematology,** Leiden, v. 6, n. 2, p. 205-213, Mar. 2004.

HUSSEY, R. S.; BARKER, K. R. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. **Plant Disease Reporter,** St. Paul, v. 57, n. 12, p. 1025-1028, Dec. 1973.

HUSSEY, R.S.; JANSSEN, G. J. W. Root-knot nematodes: *Meloidogyne* species. In: STARR, J. L.; COOK, R.; BRIDGE, J. (Ed.). **Plant resistance to parasitic nematodes.** Wallingford, UK: CAB International, 2002. p. 43-70.

LIMA, I. M.; DOLINSKI, C. M.; SOUZA, R. M. Dispersão de *Meloidogyne mayaguensis* em goiabais de São João da Barra (RJ) e relato de novos hospedeiros dentre plantas invasoras e cultivadas. **Nematologia Brasileira,** Brasília, v. 27, n. 2, p. 257-258, dez. 2003. Resumo.

LIMA, I. M.; MARTINS, M. V. V.; SERRANO, L. A. L.; CARNEIRO, R. M. D. G. Ocorrência de *Meloidogyne mayaguensis* em goiabeira 'Paluma' no Estado do Espírito Santo. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 96.

LORDELLO, L. G. E. Bahia: nematóides atacam o cafeeiro. **Revista de Agricultura,** Piracicaba, v. 46, n. 4, p. 175, 1971.

LORDELLO, L. G. E.; HASHIZUME, H. Suscetibilidade da variedade Kouillou de *Coffea canephora* a um nematóide. **Revista de Agricultura,** Piracicaba, v. 46, n. 4, p. 157-158, 1971.

LORDELLO, L. G. E.; LORDELLO, R. R. A. *Meloidogyne incognita* ataca o cafeeiro no Paraná. **O Solo,** Piracicaba, v. 64, n.2, p. 27, nov. 1972.

LORDELLO, L. G. E.; MELLO FILHO, A. T. Mais um nematóide ataca o cafeeiro. **Revista de Agricultura,** Piracicaba, v. 45, n.2/3, p. 102, 1970.

LORDELLO, L. G. E.; ZAMITH, A. P. L. Nematódeos atacando cafeeiro no Estado de São Paulo. **Revista de Agricultura**, Piracicaba, v. 33, n. 1, p. 59-62, 1958.

MUNIZ, M. F. S.; CAMPOS, V. P.; CASTAGNONE-SERENO, P. CASTRO, J. M. C.; ALMEIDA, M. R. A.; CARNEIRO, R. M. D. G. Diversity of *Meloidogyne exigua* (Tylenchida: Meloidogynidae) populations from coffee and rubber tree. **Nematology,** 2008. In press.

NASCIMENTO, R. R. S.; PIMENTEL, J. P.; POZZER, L.; GISMOND, A. S.; SILVA, S. C.; BRIOSO, P. S. T. Infecção natural de abóbora (*Cucurbita moschata*) por *Meloidogyne mayaguensis*, no Estado do Rio de Janeiro. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 26., 2006, Campos dos Goytacazes. **Anais...** Campos dos Goytacazes: Sociedade Brasileira de Nematologia, 2006. p. 90.

NETSCHER, C. Observations and preliminary study on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. **Cahiers ORSTOM Series Biologie,** Bondy, v. 11, p. 173-178, 1977.

NOIR, S.; ANTHONY, F; BERTRAND, B.; COMBES, M.C.; LASHERMES, P. Identification of a major gene (Mex-1) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. **Plant Pathology**, Avenel, v. 52, n. 1, p. 97-103, Feb. 2003.

OLIVEIRA, D. S.; OLIVEIRA, R. D. L.; GONÇALVES, W. Fenótipo S1 de esterase em *Meloidogyne incognita* no Brasil. **Fitopatologia Brasileira,** Brasília, v. 31, n. 2, p. 207, mar./abr. 2006.

OOSTENBRINK, M. **Major characteristics of the relation between nematodes and plants.** Meded: Landbouwhogesch. Wageningen, 1966. 46p.

PEREIRA, A. A.; MOURA, W. M.; ZAMBOLIM, L.; SAKIYAMA, N. S.; CHAVES, G. M. Melhoramento genético do cafeeiro no Estado de Minas Gerais – cultivares lançadas e em fase de obtenção. In: ZAMBOLIM, L. (ed). **O estado da arte de tecnologias na produção de café.** Viçosa: UFV, 2002. p. 253-295.

PONTE, J. J.; CASTRO, E. F. Lista adicional de plantas hospedeiras de nematóides das galhas, *Meloidogyne* spp., no Estado do Ceará (Brasil), referente a 1969/74. **Fitossanidade,** Fortaleza, v. 1, n. 2, p. 29-30, 1975.

PONTE, J. J.; SENA-SILVA, M. S. *Meloidogyne exigua* atacando cafeeiros no Ceará. **Pesquisa Agropecuária Brasileira Série Agronomia,** Rio de Janeiro, v. 7, p. 93-94, 1972.

PORTZ, R. L.; STANGARLIN, J. R.; FRANZENER, G.; BALBI-PEÑA, M. I.; FURLANETTO, C. *Meloidogyne* spp. associadas à cafeicultura em municípios do oeste do Paraná. **Nematologia Brasileira,** Brasília, v. 30, n. 1, p. 23-27, abr. 2006.

RANDIG, O.; BONGIOVANNI, M.; CARNEIRO, R. M. D. G.; CASTAGNONE-SERENO, P. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. **Genome,** Ottawa, v. 45, n.5, p. 862-870, Oct. 2002.

RIGGS, R. D.; WINSTEAD, N. N. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. **Phytopathology,** Baltimore, v. 49, n. 11, p.716-724, Nov. 1959.

ROBERTS, P. A. Concepts and consequences of resistance. In: STARR, J. L.; COOK, R.; BRIDGE, J. (Ed.). **Plant resistance to parasitic nematodes.** Wallingford, UK: CAB International, 2002. p. 23-41.

ROBERT, P. A.; DALMASSO, A.; CAP, G. B.; CASTAGNONE-SERENO, P. Resistance in *Lycopersicon peruvianum* to isolates of Mi gene–compatible *Meloidogyne* populations. **Journal of Nematology,** Lawrence, v. 22, n. 4, p. 585-589, Oct. 1990.

RODRÍGUEZ, M. G.; RODRÍGUEZ, I.; SÁNCHEZ, L. Especies del genero *Meloidogyne* que parasitan el cafeto en Cuba. Distribución geográfica y sintomatología. **Revista de Protección Vegetal,** San José de las Lajas, v. 10, p.123-128, 1995.

RODRÍGUEZ, M. G.; SÁNCHEZ, L.; AROCHA, Y.; PETEIRA, B.; SOLORZANO, E.; ROWE, J. Identification and characterization of *Meloidogyne mayaguensis* from Cuba. **Nematropica,** DeLeon Springs, v. 31, n. 2, p.152, Dec. 2001. Abstract.

SALGADO, S. M. L.; CAMPOS, V.P.; RESENDE, M. L. V.; KRZYZANOWSKI, A. A.; Reprodução de *Meloidogyne exigua* em cafeeiros 'Iapar-59' e 'Catuaí'. **Nematologia Brasileira,** Brasília, v. 26, n. 2, p. 205-207, dez. 2002.

SALGADO, S. M. L.; RESENDE, M. L. V.; CAMPOS, V. P. Reprodução de *Meloidogyne exigua* em cultivares de cafeeiros resistentes e suscetíveis. **Fitopatologia Brasileira,** Brasília, v. 30, n. 4, p. 413-415, jul./ago. 2005.

SAS INSTITUTE INC. **SAS/STAT user's guide**. Release 6.03 ed. Cary, NC: SAS Institute, 1988.1028 p.

SILVA, G. S.; ATAÍDE SOBRINHO, C.; PEREIRA, A. L.; SANTOS, J. M. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado do Piauí. **Nematologia Brasileira,** Brasília, v. 30, n. 3, p. 307-309, dez. 2006.

SILVAROLLA, M. B.; GONÇALVES, W.; LIMA, M. M. A. Resistência do cafeeiro a nematóides V – reprodução de *Meloidogyne exigua* em cafeeiros derivados da hibridização de *Coffea arabica* com *C. canephora*. **Nematologia Brasileira,** Brasília, v. 22, n. 1, p. 51-59, jun. 1998.

SOUZA, S. E.; SANTOS, J. M.; MATOS, R. V.; RAMOS, J. A.; SANTOS, F. S.; FERRAZ, R. C. N.; CARVALHO, G. S.; OLIVEIRA, C. A. Levantamento preliminar de *Meloidogyne* em cafeeiros do Estado da Bahia Planalto de Vitória da Conquista e Chapada Diamantina. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 1., 2000, Poços de Caldas. **Anais...** Poços de Caldas: EMBRAPA, 2000. p. 167-170.

SOUZA, S. E.; SOUZA, L. H.; MAIA, J. S.; LIMA, E.; CASTRO, J.; SANTOS, F. G.; SANTOS, F. S.; SILVA, R. V. Ocorrência de nematóide das galhas em cafeeiros no município de Barra do Choça, Bahia. **Nematologia Brasileira,** Brasília, v. 21, n. 1, p. 24. dez. 1997. Resumo.

STARR, J. L.; BRIDGE, J.; COOK, R. Resistance to plant-parasitic nematodes: history, current use and future potential. In: STARR, J. L.; BRIDGE, J.; COOK, R. (Ed.). **Plant resistance to parasitic nematodes.** Wallingford, UK: CABI, 2002. p. 1-22.

TOMAZINI, M. D.; SILVA, R. A.; OLIVEIRA, C. M. G.; GONÇALVES, W.; FERRAZ, L. C. C. B.; INOMOTO, M. M. Resistência de genótipos de cafeeiros a *Pratylenchus coffeae* e *Meloidogyne incognita*. **Nematologia Brasileira,** Brasília, v. 29, n. 2, p. 193-198, dez. 2005.

TORRES, G. R. C.; COVELLO, V. N.; SALES JÚNIOR, R.; PEDROSA, E. M. R. MOURA, R. M. *Meloidogyne mayaguensis* em *Psidium guajava* no Rio Grande do Norte. **Fitopatologia Brasileira,** Brasília, v. 29, n. 5, p. 570, set./out. 2004.

TORRES, G. R. C.; SALES JÚNIOR, R.; MEDEIROS, H. A. Ocorrência de *Meloidogyne mayaguensis* associado a pimentão no município de Baraúna, Rio Grande do Norte. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, 27., 2007, Goiânia. **Anais...** Goiânia: Sociedade Brasileira de Nematologia, 2007. p. 80.

TORRES, G. R. C.; SALES JÚNIOR, R.; REHN, V. N. C.; PEDROSA, E. M. R.; MOURA, R. M. Ocorrência de *Meloidogyne mayaguensis* em goiabeira no Estado do Ceará. **Nematologia Brasileira,** Brasília, v. 29, n. 1, p. 105-107, jun. 2005.

TRIANTAPHYLLOU, A. C. Cytogenetics, cytotaxonomy and phylogeny of root –knot nematodes. In: SASSER, J. N.; CARTER, C. C. (Ed.). **An advanced treatise on *Meloidogyne*.** Raleigh, NC: North Carolina State University, 1985. v. 1, p. 113-126.

TRIANTAPHYLLOU, A. C. Genetics of parasitism on plants. In: VEECH, J. A.; DICKSON, D.W. (Ed.). **Vistas on Nematology.** Hyattsville, Maryland: Society of Nematologists, 1987. p. 354-363.

VAN DER PLANK, J. E. **Plant diseases:** epidemics and control. New York: Academic, 1963. 349p.

CONSIDERAÇÕES FINAIS

Dentre as principais espécies de *Meloidogyne* que afetam o cafeeiro no Brasil, *M. exigua* é a mais disseminada. No estado do Mato Grosso, ela é considerada importante em seringueira. Com a utilização de eletroforese, os perfis de esterase confirmaram ser específicos. O método SCAR-multiplex–PCR permitiu diferenciar *M. exigua* de outras espécies parasitas do cafeeiro, constituindo em uma importante ferramenta para diagnose. A técnica de RAPD mostrou-se uma ferramenta importante no estudo de diversidade genética de *M. exigua*.

Estudos morfológicos utilizando microscopia de luz e eletrônica de varredura, incluindo os três estádios do nematóide (machos, fêmeas e J2), são necessários para a descrição precisa de uma espécie e devem ser baseados no maior número possível de caracteres. Outros tipos de caracteres taxonômicos, tais como dados citológicos, bioquímicos e fisiológicos, devem ser incluídos.

Os resultados apresentados neste estudo evidenciaram a grande diversidade de *M. exigua*. O fato é que essa espécie reproduz-se por partenogênese meiótica facultativa, o que permite cruzamento. É importante ressaltar que, na natureza, encontram-se populações consideradas atípicas em termos de morfologia, de padrões eletroforéticos, de virulência e de gama de hospedeiras, dentre outras características.

A cultivar IAPAR 59, considerada resistente a *M. exígua*, mostrou-se suscetível a uma população desse nematóide coletada em Bom Jesus de Itabapoana, RJ. Assim sendo, a variabilidade do nematóide deve ser considerada em programas de melhoramento do cafeeiro visando à obtenção de cultivares resistentes. As informações obtidas neste trabalho representam uma contribuição para o estudo de importantes espécies do gênero *Meloidogyne* que atacam o referido hospedeiro.

Em futuros trabalhos de pesquisa, recomenda-se ênfase na realização de levantamentos nas principais regiões produtoras brasileiras, visando estimar a presença de populações virulentas de *M. exigua* e avaliação de perdas, para obter melhor visualização da importância econômica e distribuição dessas populações.