DAVI BITTAR DO CARMO

Pasteuria penetrans E COMPOSTOS ORGÂNICOS VOLÁTEIS TÓXICOS A *Meloidogyne* sp. EM CAFEZAIS COMERCIAIS DO SUL DO ESTADO DE MINAS GERAIS

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Orientador

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Dra. Sônia Maria de Lima Salgado	EPAMIG
Dr. Denilson Ferreira de Oliveira	UFLA

Dr. Vicente Paulo Campos

Orientador

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Vivemos esperando o dia em que seremos melhores, melhores no amor, melhores na dor, melhores em tudo...
Vivemos esperando o dia em que seremos para sempre...
Dias melhores pra sempre...

No, we're never gonna survive unless we get a little...bit...crazy...

RESUMO

Nos fatores envolvidos na supressividade natural dos solos, inserem-se os compostos orgânicos voláteis (COVs) e a bactéria *Pasteuria penetrans*, que ainda necessitam de pesquisas. Aldicarbe aplicado em fazendas cafeeiras do Sul de Minas, infestadas por *Meloidogyne exigua* e sem *P. penetrans* (Ibiraci), reduziu significativamente a população de *M. exigua* e aumentou a produção comparada com o controle (sem aplicação do nematicida). Contudo, a aplicação do nematicida nas fazendas com *P. penetrans* (Capetinga) a população de *M. exigua* e a produção foram semelhantes ao controle, mas a população de J_2 parasitados por *P. penetrans* foi reduzida pela aplicação do nematicida. Em solos cafeeiros, tratados por autoclavagem, não foram encontrados endósporos de *P. penetrans,* não ocorrendo com o tratamento do solo por secagem. Contudo, ambos os tratamentos propiciaram a elevação significativa da população de *M. exigua* em pimentão, comparada com solo recentemente colhido e artificialmente infestado, como também reduziram a emissão de COVs tóxicos a *M. exigua.* Em outro ensaio, os J_2 de *M. exigua* foram expostos por 6 a 24 h aos COVs emitidos por solos cafeeiros causando mortalidade de 49 a 98%, respectivamente. Quando o solo foi armazenado em câmara fria (8-10°C) por 2, 8 e 14 dias, a mortalidade J_2 só foi elevada e consistente aos dois dias. Das amostragens feitas no campo em abril, agosto e outubro, os dados mais consistentes de emissão de COVs tóxicos a J_2 de *Meloidogyne* sp., só ocorreram em abril. A umidade do solo amostrado no campo correlacionou-se, positivamente, com a mortalidade causada pela emissão de COVs. Quando o solo com baixa umidade ($\leq$ 20%) e baixa emissão de COVs tóxicos a J_2 recebeu suplementação de água, a emissão de COVs tóxicos foi superior a 75% de mortalidade de J_2.

Palavras-chave: Supressividade. Aldicarbe. Umidade do solo. Microflora.

ABSTRACT

Natural soil suppressiveness involves different factors, including soil microflora, however the roles of volatiles organic compounds (VOCs) and the bacterium *Pasteuria penetrans* are not well understood. Aldicarb applied on farms with *Melodigyne exigua* and without *Pasteuria penetrans* reduced ($P \leq 0.05$) *M. exigua* populations and increased yields when compared to control (without nematicide application). However, nematicide application in farms with *P. penetrans, M. exigua* populations and yield remained similar to the control (without nematicide), but parasitized *M. exigua* J_2 was reduced by aldicarb application. In autoclaved coffee soil, *P. penetrans* endospores were not encoutered, unlike soil dryning where endospores were observed adhered to bioassayed *M. exigua* J_2. Soil drying and autoclaving benefits posterior inoculated *M. exigua* compared to control (recently collected field soil) and reduced significantly the emission of VOCs toxic to *M. exigua* J_2 when compared to the control. In another assay, the time periods from 6 to 24 hours of J_2 exposure to soil VOCs caused mortality from 49% to 98% from a field soil site. Soil stored for 2, 8 and 14 days only furnished consistent high J_2 mortality when stored for 2 days in cold room. However, the sampling time has to be observed. The sampling in April, August and October in coffee fields furnished more consistent evaluations of soil emitted VOCs from April sampling. Soil moisture was positively correlated to the J_2 mortality caused by soil emitted VOCs. When soil with depleted moisture and low VOC emissions had water replacement, the high J_2 toxic VOC emission (above 75% J_2 mortality) occurred.

Keywords: Suppressiveness. Aldicarb. Soil moisture. Microflora.

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CAPÍTULO 1

INTRODUÇÃO GERAL

1 INTRODUÇÃO

A atual pressão mundial pelo aumento de produção agrícola, sem a contaminação de alimentos fibras e outros com resíduos de defensivos agrícolas, tem incentivado o uso de táticas alternativas de controle de doenças e pragas. No caso dos patógenos de plantas, pode-se, por exemplo, selecionar cultivares, fazer rotação de culturas, mudar a densidade e a época de plantio. Além disso, o agricultor pode utilizar de micro-organismos benéficos, via tratamento de semente ou por alterações no solo, resultando no aumento da produtividade ou da defesa da planta (BETTIOL; GHINI, 2001).

Várias estratégias têm sido utilizadas para o controle dos nematoides. Rotação de culturas e o plantio de cultivares resistentes possuem eficácia restrita nos campos infestados com fitonematoides de larga gama de hospedeiros e em populações mistas (ROBERTS, 1992). Nematicidas, apesar da grande eficácia na redução populacional de fitonematoides, poluem o meio ambiente e deixam resíduos tóxicos nos produtos agrícolas, que restringe o seu uso. Por exemplo, o brometo de metila degrada a camada de ozônio da atmosfera e, por isso, foi proibido seu uso, apesar de constituir-se num potente fumigante com capacidades herbicida, nematicida, inseticida e fungicida (RISTAINO; THOMAS, 1997).

O uso de micro-organismos antagonistas a fitonematoides e seus metabólitos tem atraído a atenção como potentes agentes de biocontrole. Vários fungos e bactérias têm sido trabalhados para o desenvolvimento de formulações

comerciais e utilizados, com sucesso, no controle de nematoide em campos de produção (GU et al., 2007).

O solo, por conter metabólitos e resíduos de plantas, suporta população diversificada de micro-organismos, a qual leva à supressividade de populações de patógenos, principalmente do sistema radicular, podendo constituir-se em tática de controle de doença, se bem estudados (BORNEMAN; BECKER, 2007). O manejo de fitonematoides deve ser direcionado para a região onde as raízes irão crescer (25 a 50 cm a partir da base da plântula). A infestação das primeiras raízes afetará, negativamente, o crescimento e reduzirá a produção (SIKORA et al., 2008).

Desta forma, pretende-se estudar a influência da aplicação de aldicarbe em fazendas cafeeiras naturalmente infestadas por *Meloidogyne exigua* e *Pasteuria penetrans* e a emissão de compostos orgânicos voláteis do solo sob diferentes aspectos.

2 REFERENCIAL TEÓRICO

2.1 Supressividade de solos

São considerados solos supressivos aqueles nos quais uma determinada população de patógeno apresenta, na presença de um hospedeiro, um nível baixo comparado com o nível médio de uma infecção. A inospitabilidade natural destes solos aos patógenos pode ser descrita de três formas: o patógeno não se estabelece; ele se estabelece, mas não causa a doença; ou o patógeno se estabelece, causa doença, mas a severidade é reduzida com a monocultura (COOK; BAKER, 1984).

A ocorrência natural de solos supressivos tem sido documentada em vários sistemas de produção e, em várias instâncias, os atributos biológicos, como predadores e parasitas (MCSORLEY et al., 2006; PISKIEWICZ; DUYTS; PUTTEN, 2009), têm sido identificados como fatores contribuintes. O nematoide do cisto *Heterodera avenae* tem sido efetivamente controlado por mais de 20 anos em vários solos do norte europeu. Essa supressividade dependeu de um período inicial de alta população do patógeno, que suportou o desenvolvimento dos fungos *Nematophthora gynophila* e *Verticillium chlamydosporium* (KERRY; CRUMP; MULLEN, 1980). Porém práticas, em vários casos, não se observa comportamento similar em decorrência das agrícolas empregadas, que podem quebrar a supressividade natural de solos (PYROWOLAKIS et al., 2002).

Com o conhecimento dos organismos presentes na microbiota, é possível desenvolver estratégias efetivas e sustentáveis de manejo das doenças, por meio da aplicação de antagonistas ou por práticas agronômicas que influenciem positivamente a densidade da população (BORNEMAN; BECKER, 2007). Em princípio, a combinação de vários agentes de biocontrole tem maior

potencial de supressão do patógeno ou da doença, uma vez que imprime uma colonização mais extensiva da rizosfera, expressa de forma mais consistente seus benefícios sob uma grande variabilidade de condições de solo e possui antagonismo a um maior número de patógenos de plantas do que um único agente (MEYER; ROBERTS, 2002).

As propriedades físicas e químicas do solo agem de forma indireta favorecendo a atividade microbiana ou de forma direta por interferirem no ciclo de vida do patógeno. As principais características do solo envolvidas na supressividade são: teor de matéria orgânica, pH, macro e micronutrientes, estrutura e textura, tipo de argila e retenção de água, entre outras. Rimé et al. (2003) compararam o efeito de solos arenosos supressivos e conducivos nos danos causados por nematoides ectoparasitos da cana e notaram que o solo supressivo possuía maior teor de matéria orgânica, N, Al e Fe e menor teor de P, Ca, Mg, K, Mn e pH. Fatores de solo como teor de matéria orgânica, pH, Al, Fe e Mn fazem parte da supressividade de certos solos a doenças fúngicas, provavelmente, por influenciar a microbiota responsável pela supressividade do solo (HOPER; ALABOUVETTE, 1996).

2.2 A bactéria *Pasteuria penetrans*

O gênero bacteriano *Pasteuria* é composto por importantes parasitas de nematoides de solo. A espécie *P. penetrans* é a mais estudada, sendo encontrada principalmente em solos arenosos.

Seu parasitismo ocorre em duas etapas principais. Primeiro, ocorre adesão de esporos livres na cutícula do nematoide no solo resultante da compatibilidade da ligação entre os esporos bacterianos e o patógeno. Posteriormente, uma infecção interna ocorre depois que os nematoides penetram nas raízes e instalam seus sítios de alimentação. Os esporos germinam,

penetrando pela cutícula e colonizam o pseudoceloma do hospedeiro, formando novos esporos que serão liberados no solo com a morte do nematoide (FOULD et al., 2001).

Sharma e Gomes (1999) relataram que *P. penetrans* foi altamente eficiente no controle de *M. arenaria.* Pimenta e Carneiro (2002) demonstraram grande redução populacional de *M. javanica* em raízes de tomateiro, após incorporação do sistema radicular da cultura anterior (alface) infestado pela bactéria.

2.3 Compostos orgânicos voláteis

Os compostos orgânicos voláteis (COVs) são moléculas com até aproximadamente vinte átomos de carbono, com alta pressão de vapor e baixa polaridade. Podem ser produzidos pelas plantas (DUDAVERA et al., 2006) e pelos micro-organismos, tendo importante influência na química da atmosfera e nos processos biológicos do solo (LEFT; FIERER, 2008).

Eles podem atravessar as membranas livremente e são liberados na atmosfera ou no solo, na ausência de uma barreira de difusão (PICHERSKY; NOEL; DUDAREVA, 2006). Também são difundidos pelo movimento de solução aquosa e pelo fluxo em massa de água pelo perfil do solo, proporcionando rápido movimento dos voláteis pelo sistema (WHEATLEY, 2002). São de distribuição eficiente pela porosidade do solo, que aumenta a área de influência dos COVs e melhoram sua eficácia na eliminação de micro-organismos patogênicos sob o ponto de vista de controle. Assim, os COVs atuam na atmosfera acima e abaixo do solo (CAMPOS; PINHO; FREIRE, 2010).

Embora os voláteis sejam comuns nos ecossistemas, os produzidos por micro-organismos, contudo, receberam maior atenção apenas nos últimos anos.

Os COVs emitidos por bactérias e fungos servem como informantes químicos para a comunicação intra e interespecífica, célula-a-célula, além de possível válvula para escape de carbono e agentes promotores ou inibidores de crescimento (KAI et al., 2009), podendo essa última função ser importante na criação de novos métodos de controle de fitopatógenos de solo.

Zou et al. (2007) encontraram 328 isolados bacterianos capazes de produzir COVs antifúngicos, que inibiam a germinação de esporos e crescimento micelial de *Paecilomyces lilacinus* e *P. chlamydosporia.* Fernando et al. (2005) isolaram bactérias, a partir de canola e soja, que produziam COVs antifúngicos. Minerdi et al. (2009) comprovaram a produção de COVs antifúngicos por um isolado não-patogênico de *Fusarium oxysporum* (MSA35) que foram capazes de controlar *formae speciales* patogênicas da mesma espécie, como a f. sp. *lactucae*. Wan et al. (2008) demonstraram que substâncias voláteis produzidas por *Streptomyces platensis* foram capazes de reduzir as populações de *Rhizoctonia solani, Sclerotinia sclerotiorum* e *Botrytis cinerea.*

Além das capacidades fungicidas e bactericidas, os COVs possuem, ainda, importante capacidade nematicida. Gu et al. (2007) constataram a produção de COVs por bactérias do gênero *Bacillus, Serratia* e *Stenotrophomonas* com atividade nematicida contra *Panagrellus redivivus* e *Bursaphelenchus xylophilus.* Huang et al. (2010) constataram que os COVs produzidos por *B. megaterium* YMF3.25 causaram alta mortalidade de J_2 de *M. incognita* e elevada inibição na eclosão e imobilidade total de J_2 após 24 h. *B. megaterium* YMF3.25 reduziu o número de galhas, massas de ovos e quantidade de ovos por massa em relação ao controle, em casa de vegetação. O potencial nematicida de *B. megaterium* já havia sido primariamente comprovado em experimentos com *Meloidogyne chitwoodi* (AL-REHIAYANI et al., 1999) e *Heterodera schachtii* (NEIPP; BECKER, 1999).

Riga, Lacey e Guerra (2008), testanto a produção de COVs pelo fungo *M. albus, in vitro,* evidenciaram mortalidade de *Paratrichodorus allius, Pratylenchus penetrans* e *M. chitwoodi* variando de 82 a 95% e redução de motilidade de 69% para *M. hapla,* mostrando ação nematicida e nematostática. Freire et al. (2010) testaram vários COVs fúngicos contra J_2 de *M. incognita,* que permite observar a alta imobilidade causada por seis isolados de *F. oxysporum* e outros dois causaram alta mortalidade de J_2. O aumento do tempo de exposição dos J_2 de *Meloidogyne* aos COVs causou decréscimo da capacidade de infecção em raízes de tomateiro.

A incorporação de matéria orgânica no solo apresenta-se como outra vertente de produção de voláteis. Resíduos de *Brassicaceae* incorporados no solo tem sido potente prática biofumigante. Aires et al. (2009) constataram supressão de *Globodera rostochiensis* em raízes de batata, com a incorporação de extratos de brassicáceas: couve-flor, nabo e agrião. Além disto, essa incorporação pode favorecer o desenvolvimento da microbiota antagônica aos fitonematoides.

A utilização de micro-organismos como agentes de biocontrole produtores de COVs nematicidas pode produzir efeitos duráveis após a colonização em determinado ambiente. Assim, métodos que combinam adição de resíduos orgânicos com micro-organismos produtores de COVs poderiam servir como alternativa ao uso de químicos (GU et al., 2007).

3 CONSIDERAÇÕES GERAIS

O uso de nematicidas sintéticos é cada vez mais restrito. O brometo de metila, por exemplo, foi retirado do mercado por afetar a camada de ozônio da atmosfera. E outros, ainda, são pouco utilizados por elevada toxicidade, odor forte, ou até mesmo custo elevado. Novos atributos precisam ser agregados às novas moléculas para maior sucesso no mercado de agrotóxicos como, baixo poder residual e menor persistência nos alimentos, aliado à melhoria da eficácia de controle de fitonematoides

Assim, o estudo de atributos do solo, como: a identificação de micro-organismos antagônicos a fitonematoides e a detecção da sua supressividade natural, proporcionará possível manipulação do solo de forma a melhorar o controle de nematoides fitoparasitas. Por exemplo, em áreas de culturas perenes, nas quais o solo sofre pequeno impacto do uso de máquinas agrícolas por grande período de tempo, a influencia nos atributos biológicos do solo será mínima, possibilitando melhor colonização do solo, talvez, por agentes de controle biológico introduzidos. Além disso, o estudo dos COVs produzidos por micro-organismos antagônicos à nematoides ou pela própria microflora do solo pode colaborar para a geração de novos produtos que sejam eficientes no controle dos fitonematoides.

REFERÊNCIAS

AIRES, A. et al. Suppressing potato cyst Nematode, *Globodera rostochiensis*, with extracts of brassicacea plants. **American Journal of Potato Research**, Orono, v. 86, n. 4, p. 327-333, Aug. 2009.

AL-REHIAYANI, S. et al. Effects of *Pratylenchus neglectus*, *Bacillus megaterium*, and oil radish or rapeseed green manure on reproductive potential of *Meloidogyne chitwoodi* on potato. **Nematropica**, Bradenton, v. 29, n. 1, p. 37-49, June 1999.

BETTIOL, W.; GHINI, R. Solos supressivos. In: MICHEREFF, S. J. et al. (Ed.). **Patógenos radiculares em solos tropicais.** Recife: Editora Universitária UFPE, 2001.

BORNEMAN, J.; BECKER, J. O. Identifying microorganisms involved in specific pathogen suppression in soil. **Annual Review of Phytopathology**, Palo Alto, v. 45, p. 153-172, 2007.

CAMPOS, V. P.; PINHO, R. S. C.; FREIRE, E. S. Volatiles produced by interacting microorganisms potentially useful for the control of plant pathogens. **Ciência e Agrotecnologia,** Lavras, v. 34, n. 3, p. 525-535, maio/jun. 2010.

COOK, R. J.; BAKER, K. F. The nature and practice of biological control of plant pathogens. **Nature**, London, v. 309, n. 5970, p. 732-732, June 1984.

DUDAREVA, N. et al. Plant volatiles: recent advances and future perspectives. **Critical Reviews in Plant Sciences**, Brookfield, v. 25, n. 5, p. 417-440, 2006.

ELLENBY, C. Mustard oils and control of the potato-root eelworm, *Heterodera rostochiensis* wollenweber - further field and laboratory experiments. **Annals of Applied Biology**, Warwick, v. 38, n. 4, p. 859-875, Dec. 1951.

FERNANDO, W. G. D. et al. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. **Soil Biology & Biochemistry**, Elmsford, v. 37, n. 5, p. 955-964, May 2005.

FOULD, S. et al. Immunological quantification of the nematode parasitic bacterium *Pasteuria penetrans* in soil. **Fems Microbiology Ecology**, Amsterdam, v. 37, n. 3, p. 187-195, Nov. 2001.

FREIRE, E. S. et al. Volatile substances on the antagonism between fungi, bacteria and *Meloidogyne incognita* and potentially fungi for nematode control. **Journal of Nematology**, College Park, 2010.

GU, Y. Q. et al. Q. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. **Soil Biology & Biochemistry**, Elmsford, v. 39, n. 10, p. 2567-2575, Oct. 2007.

HOPER, H.; ALABOUVETTE, C. Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. **European Journal of Soil Biology**, Montrouge, v. 32, n. 1, p. 41-58, Jan. 1996.

HUANG, Y. et al. Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. **European Journal of Plant Pathology**, Dordrecht, v. 126, n. 3, p. 417-422, Mar. 2010.

KAI, M. et al. Bacterial volatiles and their action potential. **Applied Microbiology and Biotechnology**, Berlin, v. 81, n. 6, p. 1001-1012, Jan. 2009.

KERRY, B. R.; CRUMP, D. H.; MULLEN, L. A. Parasitic fungi, soil-moisture and multiplication of the cereal cyst nematode, *Heterodera avenae*. **Nematologica**, Leiden, v. 26, n. 1, p. 57-68, 1980.

KIRKEGAARD, J. A.; WONG, P. T. W.; DESMARCHELIER, J. M. In vitro suppression of fungal root pathogens of cereals by Brassica tissues. **Plant Pathology**, Oxford, v. 45, n. 3, p. 593-603, June 1996.

LEFT, J. W.; FIERER, N. Volatile organic compound (VOC) emissions from soil and litter samples**. Soil Biology & Biochemistry**, Elmsford, v. 40, n. 7, p. 1629-1636, July 2008.

MCSORLEY, R. et al. Effects of soil type and steam on nematode biological control potential of the rhizosphere community. **Nematropica**, Bradenton, v. 36, n. 2, p. 197-214, 2006.

MEYER, S. L. F.; ROBERTS, D. P. Combinations of biocontrol agents for management of plant-parasitic nematodes and soilborne plant-pathogenic fungi. **Journal of Nematology**, College Park, v. 34, n. 1, p. 1-8, Mar. 2002.

MINERDI, D. et al. Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of *Fusarium oxysporum* strain MSA 35. **Environmental Microbiology**, Oxford, v. 11, n. 4, p. 844-854, Apr. 2009.

NEIPP, P. W.; BECKER, J. O. Evaluation of biocontrol activity of rhizobacteria from *Beta vulgaris* against *Heterodera schachtii*. **Journal of Nematology**, College Park, v. 31, n. 1, p. 54-61, Mar. 1999.

PICHERSKY, E.; NOEL, J. P.; DUDAREVA, N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. **Science**, Washington, v. 10, n. 311, p. 808-811, Feb. 2006.

PIMENTA, C. A. M.; CARNEIRO, R. M. D. G. Controle de *Meloidogyne javanica* por *Pasteuria penetrans* em tomateiro: segundo ensaio. **Nematologia Brasileira**, Campinas, v. 26, n. 1, p. 77-91, June 2002.

PISKIEWICZ, A. M.; DUYTS, H.; PUTTEN, W. H. V. D. Soil microorganisms in coastal foredunes control the ectoparasitic root-feeding nematode *Tylenchorhynchus ventralis* by local interactions. **Functional Ecology**, Oxford, v. 23, n. 3, p. 621-626, June 2009.

PYROWOLAKIS, A. et al. Identification of root-knot nematode suppressive soils. **Applied Soil Ecology**, Amsterdam, v. 19, n. 1, p. 51-56, Jan. 2002.

RIGA, E.; LACEY, L. A.; GUERRA, N. *Muscodor albus*, a potential biocontrol agent against plant-parasitic nematodes of economically important vegetable crops in Washington State, USA. **Biological Control**, Orlando, v. 45, n. 3, p. 380-385, June 2008.

RIME, D. et al. Comparison of sandy soils suppressive or conducive to ectoparasitic nematode damage on sugarcane. **Phytopathology**, Orlando, v. 93, n. 11, p. 1437-1444, Nov. 2003.

RISTAINO, J. B.; THOMAS, W. Agriculture, methyl bromide, and the ozone hole: Can we fill the gaps? **Plant Disease**, Saint Paul, v. 81, n. 9, p. 964-977, Sept. 1997.

ROBERTS, P. A. Current status of the availability, development and use of host plant resistance to nematodes. **Journal of Nematology**, College Park, v. 24, n. 3, p. 213-227, Sept. 1992.

SHARMA, R. D.; GOMES, A. C. Controle biológico de *Meloidogyne arenaria* com *Pasteuria penetrans*. **Nematologia Brasileira**, Campinas, v. 23, n. 1, p. 47-52, June 1999.

SIKORA, R. A. et al. Mutualistic endophytic fungi and in-planta suppressiveness to plant parasitic nematodes. **Biological Control**, Orlando, v. 46, n. 1, p. 15-23, July 2008.

WALKER, J. C.; MORELL, S.; FOSTER, H. H. Toxicity of mustard oils and related sulphur compounds to certain fungi. **American Journal of Botany**, Columbus, v. 24, n. 4, p. 536-541, Apr. 1937.

WAN, M. G. et al. Effect of volatile substances of *Streptomyces platensis* F-1 on control of plant fungal diseases. **Biological Control**, Orlando, v. 46, n. 3, p. 552-559, Sept. 2008.

WHEATLEY, R. E. The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek International Journal of General and Molecular **Biological Control**, Orlando, v. 81, n. 1-4, p. 357-364, Dec. 2002.

ZOU, C. S. et al. Possible contributions of volatile-producing bacteria to soil fungistasis. **Soil Biology & Biochemistry**, Elmsford, v. 39, n. 9, p. 2371-2379, Dec. 2007.

CHAPTER 2

Control practices of *Meloidogyne exigua* affecting *Pasteuria penetrans* and volatile organic compounds on soil and coffee fields

ABSTRACT

The effect of using *Meloidogyne* sp. control measures in coffee fields and in nurseries, on beneficial rhizosphere organisms, is not well understood. Aldicarb applied on farms with *Meloidogyne exigua* and without *Pasteuria penetrans* reduced ($P \leq 0.05$) *M. exigua* populations in soil and roots and increased yields when compared to the control (without nematicide application). However, nematicide application in farms with *P. penetrans, M. exigua* populations and yield remained similar to the control (without nematicide), but parasitized *M. exigua* J_2 was reduced by aldicarb application. Coffee soil autoclaving killed *P. penetrans* endospores, unlike soil drying where endospores were observed adhered to bioassayed *M. exigua* J_2. Soil drying and autoclaving benefits posterior inoculated *M. exigua* compared to the control (recently collected field soil) and significantly reduced the emission of VOCs toxic to *M. exigua* J_2 when compared to the control. Therefore, nematicide application on *P. penetrans* and *M. exigua* infested fields should not be recommended until contradictory research data is presented. The reduction of J_2 toxic soil VOC emissions from dried and autoclaved soils reflects on their production by soil microflora which was killed through soil treatment, thereafter permitting the new introduced *M. exigua* inoculum to freely multiply.

Keywords: Aldicarb. Autoclavation. Dryness. Biological control. Soil microbiota.

1 INTRODUCTION

Meloidogyne exigua is the most widespread plant pathogenic nematode in southern Minas Gerais, Brazil (CASTRO et al., 2008), where 25% of the Brazilian coffee were produced in 2011 (COMPANHIA NACIONAL DE ABASTECIMENTO, 2012). Thus, control measures to reduce *M. exigua* populations are used in field and in nursery (CAMPOS; VILLAIN, 2005).

Pasteuria penetrans is known to infest coffee in Brazil (MAXIMINIANO et al., 2001) and has been encountered in four commercial coffee fields in Capetinga city in southern Minas Gerais, Brazil (BOTELHO et al., 2011). Although the nematicide aldicarb presents no reported toxicity to *P. penetrans* (TIMPER et al., 2001) its application on coffee fields infested by both *P. penetrans* and *M. exigua* is of interest in coffee with enhanced vigour by this biological control agent since yield improvement is needed to cover the nematicide application costs.

After the year 2006, when methyl bromide was banished from the Brazilian pesticide market, nursery coffee producers started looking for alternative measures to control nematodes in the soil through substrate used to grow coffee seedlings. One option has been to use soil dried on cement or paved yards or by using sun heat collectors (GHINI; BETTIOL, 1991; GHINI; INOMOTO; SAITO, 1998) to sun heat the soil to be used in growing coffee seedlings. But how is the damage done by the soil drying to the beneficial soil microorganisms is still to be answered. Therefore, it became of interest to study: a) the effect of aldicarb application on coffee yield and in *M. exigua* and *P. penetrans* populations when both occur on the same farm; b) the effect of soil drying and autoclaving to *P. penetrans* population and on emitted VOCs by treated soil as well as *M. exigua* multiplication posteriorly inoculated in treated soils.

2 MATERIAL AND METHODS

2.1 Influence of aldicarb application on *Meloidogyne exigua* control in *Pasteuria penetrans* infested coffee fields

Two coffee fields naturally infested by *P. penetrans* and *M. exigua* located in the town of Capetinga, southern Minas Gerais, Brazil, named Capetinga A and B and in one coffee field located in the town of Ibiraci in the same state but without *P. penetrans* were selected for aldicarb application. The plants selected in the coffee row for experimental units were first checked for the presence of *M. exigua.* Ten plants, side by side from each three rows made the experimental units. The six central plants from the central row were considered for data collecting. A dose of 20 grams of Aldicarb (three grams of active ingredient), was applied using a hand applicator, commonly used by small coffee farmers, at five centimeter depth distributed into four holes made in the soil per plant (five grams per hole), with two holes on the side of each plant in a row. The application was at the edge of the lowest coffee branches. On the control plots, aldicarb was not used. The experiment used a block design with six replicates.

Soil and roots samples were collected from each replicate plots applied with nematicide and the control. The nematicide was applied in February and the sampling was done in April, June and August.

In the laboratory, roots were separated from soil and gently washed. From each field sample, four subsamples (roots and soil) were obtained and considered as replicates. From each replicate we took ten grams of fine roots randomly selected. Then, they were cut into 0.5 cm lengths and placed in a warring blender with hyper chloride 0.5% and eggs were extracted following Hussey and Barker (1973) technique. Under microscopy the egg numbers were estimated and referred as egg/g of root.

Sampled soils were separated from coarse debris. From each replicate, 100 cc of soil was processed by using the Jenkins (1964) and second stage juveniles (J_2) were extracted. The total J_2 per 100 cc of soil, number of *P. penetrans* J_2 with adhered endospores and number of J_2 with more than 15 endospores per J_2 presented in the soil were counted under microscopy.

The amount of either egg/g of root or J_2/100cc of soil evaluated in each sampling time, (April, June and August) were added up for figure presentation.

For coffee yield evaluation, the coffee beans were harvested from six plants (central plants) considered the experimental unit. The amount harvested per experimental units was transformed to 60 kg bag of dried coffee beans per hectare. By comparing nematicide application plots with non-applied plots, the nematicide effect was calculated. The same was done by comparing plots with nematicide and *P. penetrans* with plots without nematicide and *P. penetrans.*

2.2 Autoclaving and drying of soil affecting *Pasteuria penetrans* residual inoculum and nematode inoculum build up inoculated after soil treatment

Coffee fields infested by *M. exigua* and *P. penetrans* located in the town of Capetinga in Minas Gerais, Brazil, denominated Capetinga A and B, along with one coffee field in the town of Varginha in the same state infested with only *M. exigua* were selected for soil sampling. The sampling procedure was the same as previously described. The soil was separated from roots and used in the experiment.

Soils with and without *P. penetrans* were submitted to drying by leaving them scattered in trays on the greenhouse benches by 60 days (FREITAS et al., 2010), performing one treatment. In another treatment the soils were autoclaved for one hour at 120°C per day during two consecutive days. After heat treatments, the soils were placed on benches in the greenhouse to cool down and

stabilize the remaining nutrients. As a control, soil was collected from the field on the same day of establishing the experiment.

The experiment was performed in 300 mL plastic cups filled with soil as described above. The first step was to adjust the soil moisture to 60% field capacity. Then, one pepper (*Capsicum annuum* L.) seedling, of the cultivar Ikeda, of 21 days was transplanted to each soil cup. Five days later 1000 *M. exigua* J_2, obtained from coffee roots, was inoculated per pepper seedling. The transplanted and inoculated pepper seedlings in cups were placed in a temperature controlled room at 28° C and managed properly for good plant growth. The experiment was carried out in a block design with four replicates. Ninety days later the pepper roots were separated from the soil and gently washed. The total soil amount was used for J_2 extraction using the Jenkins (1964). The eggs from total pepper roots were extracted using the Hussey and Barker (1973) technique. The extracted J_2 from soil was counted as well as the number of *P. penetrans* endospores adhered J_2. The experiment was repeated twice.

2.3 Volatile organic compounds toxic to *Meloidogyne exigua* after soil drying and autoclaving

For this assay we used coffee soils from Capetinga (A, B and C), Ibiraci and Varginha, towns in Minas Gerais, Brazil. The field soil moisture was determined through the oven drying method (SERVIÇO NACIONAL DE LEVANTAMENTO E CONSERVAÇÃO DE SOLOS, 1976). Briefly, the soil sample was kept at 105-110°C until maintaining constant weight, where humidity was lost by evaporation. The water lose was determined by calculating the difference between the initial sample weight (P) and weight after exposed to heat (Dw). Where soil moisture (Sm): Sm = [(P-Dw)/Dw]x100 =

[Ww/Dw]x100. The moisture evaluated from sampled fields were: Capetinga A = 31,3%, B = 43,6%, C =33,8%, Ibiraci = 34,5% and Varginha 29,5%. Soils were then submitted to drying or autoclaving as described earlier. For the control, soil was collected from the field on the day of establishing the experiment.

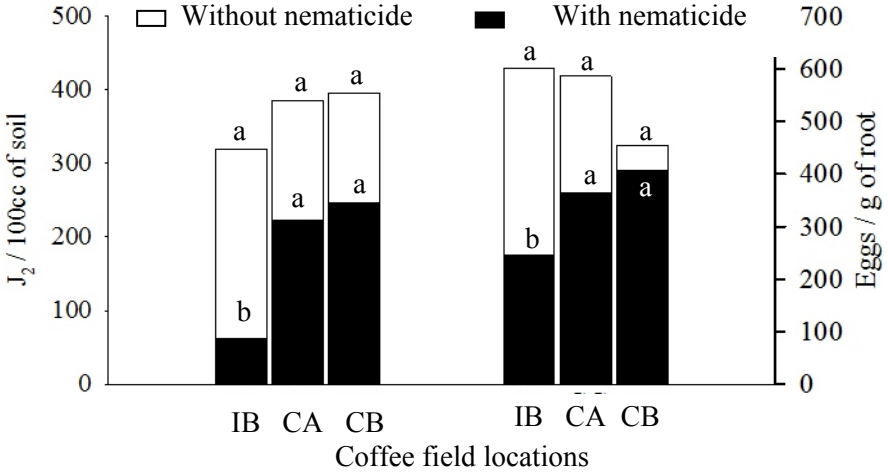
The assay was performed with treated soil by autoclaving or drying and recently collected soil. The volatile organic compounds (VOCs) toxic to *M. exigua* from those soils were bioassayed through the Botelho (2011) technique. Briefly, 25 g of soil from each sample were placed in a Supelco tube and a 1.5 mL eppendorf plastic tube was half buried into the soil and the Supelco tube was closed and kept at 28°C in a growth chamber. As a control the Supelco tube was filled with washed sand. Six days afterwards, 1 mL suspension with 100 J_2 of *M. exigua* was placed into the internal eppendorf microtube, by a syringe. One day later the Supelco tube was opened and the J_2 suspension exposed to soil VOCs was poured onto a polypropylene tray and the number of dead J_2 were counted according to Chen and Dickson (2000), technique. The experiment was repeated twice.

2.4 Data analyses

The data were submitted to a variance analysis and the means were compared by Scott-Knott test (1974) with 5% of significance. Some data (J_2 per 100 cc of soil, eggs per gram of root and total population - eggs and J_2) were transformed to $\sqrt{(x)+0,5}$, using the SISVAR program.

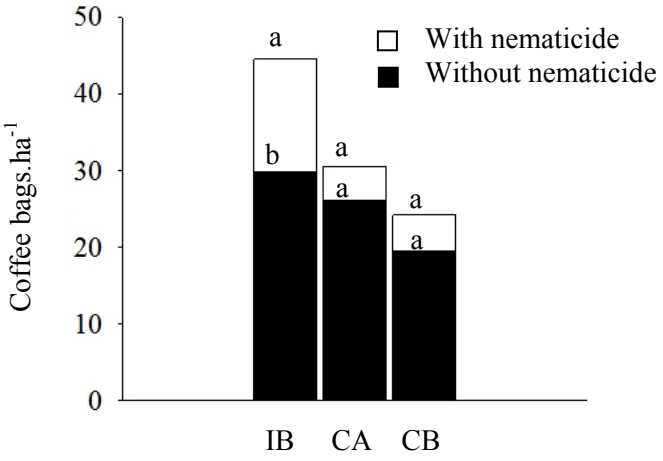
3 RESULTS

The nematicide application on the farm without *P. penetrans* (Ibiraci), reduced *M. exigua* population (eggs/g of root and soil J_2) significantly after application when compared to the control. However, when applied on farms with *P. penetrans* (Capetinga A and B) it caused no reduction of the *M. exigua* population (Graphic 1). But the number of parasitized *M. exigua* J_2 was higher in plots without nematicide when compared to plots applied with nematicide. The number of J_2 with more than 15 endospores/J_2, was low and was not affected by nematicide applications throughout the sampling dates.



Graphic 1 *Meloidogyne exigua* populations on coffee fields infested by *Pasteuria penetrans* (Capetinga A-CA and B-CB) and without *P. penetrans* (Ibiraci-IB) with aldicarb application or none. Effect on second stage juveniles (J_2) per 100 cc of soil and on eggs per gram of root. Coffee field bars followed by different letters indicate a significant difference in the bar ($P \leq 0.05$)

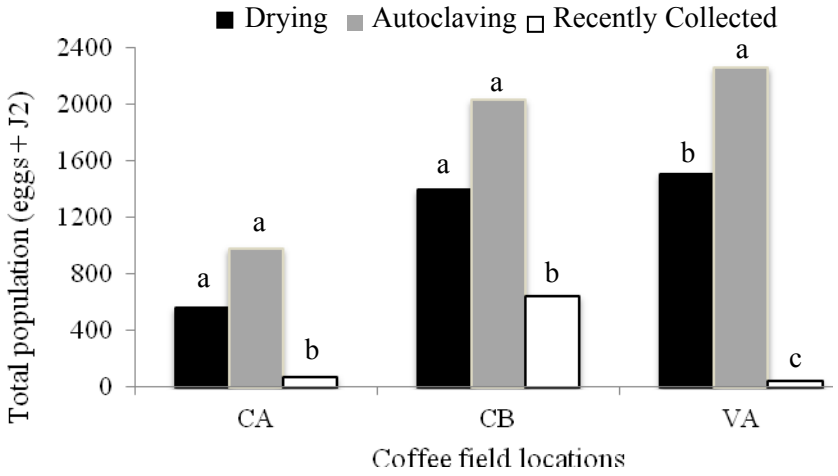
The nematicide application on the farm without *P. penetrans* (Ibiraci) significantly increased coffee yield (48.8%) when compared to the control, unlike the farms with *P. penetrans* (Capetinga A and B), where nematicide application caused similar yield ($P \leq 0.05$) when compared to the control (Graphic 2).



Graphic 2 Coffee Yield (bags.ha^{-1}) from commercial farms of South of Minas Gerais state, Brazil infested with *Meloidogyne exigua* and *Pasteuria penetrans* (Capetinga A-CA and B-CB) or without *P. penetrans* (Ibiraci-IB) with nematicide application or none. Coffee field bars followed by different letters indicate a significant diference in the bar ($P \leq 0.05$)

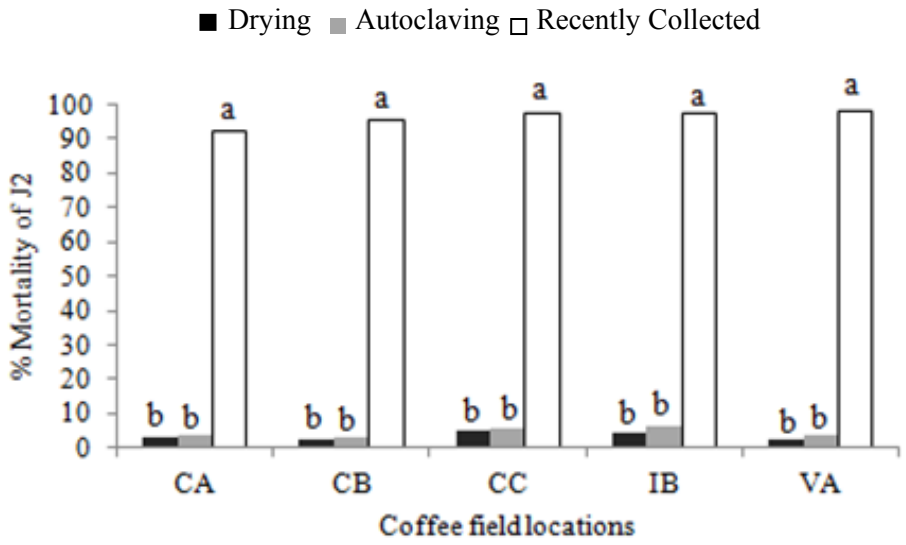
In *P. penetrans* infested soils (Capetinga) treated by autoclaving, no endospores were observed adhered to *M. exigua* J_2 subsequently inoculated by heat treatment at three months after inoculation. On the other hand, some J_2 showed adhering endospores when soil was dried on greenhouse benches (average of 10.8% J_2 with endospores) or recently collected in the field (average of 15.1% J_2 with endospores). But, both dried and autoclaved soil caused a

significant increase in total *M. exigua* population,on pepper roots, resulting from subsequent inoculation after treatments, compared to soil recently collected in the field. Although autoclaved soil allowed higher *M. exigua* population on pepper compared to dried soil, this difference was only significant when soil from the town of Varginha was used (Graphic 3).



Graphic 3 Total population of second stage juveniles (J_2) from soil and eggs from roots of *Meloidogyne exigua* in potted cultivated pepper in soils previously treated by drying or autoclaving and control (recently collected soil), and reinfested by *M. exigua* after treatment including the control. The soils used were collected from town sites Capetinga A-CA and B-CB, both infested with *P. penetrans* and *M. exigua,* and from Varginha-VA, not infested with the bacterium, but all infested by *M. exigua.* Coffee field bars followed by different letters indicate a significant difference ($P \leq 0.05$) among soil treatments and control

Soil treatments by autoclaving and drying significantly reduced the emission of VOCs toxic to *M. exigua* J_2 when compared to recently collected field soils (field moisture between 29.5 and 43.6%). Both soil treatments highly and equally reduced VOCs emissions (Graphic 4).



Graphic 4 Mortality of second stage juveniles (J_2) of *Meloidogyne exigua* by volatile organic compounds of Capetinga A-CA, B-CB, C-CC, Ibiraci-IB and Varginha-VA field soils influenced by the drying and autoclaving treatments and without it (recently collected). Coffee fields bars followed by different letters indicate a significant difference ($P \leq 0.05$) among soil treatments and control

4 DISCUSSION

Nematicide application on coffee farms infested with *M. exigua* has been proved to reduce the nematode population and increases coffee yields (HUANG et al., 1983; LORDELLO et al., 1990). In fact, nematicide is recommended and commonly used by farmers in Brazil to control *M. exigua* in coffee infested fields (CAMPOS; VILLAIN, 2005). However, the failure response on reducing *M. exigua* population and increasing coffee yields on farms naturally infested by *P. penetrans*, poses as a doubt as to the recommendation of nematicide application for the control of *M. exigua* with the presence of this bacterium. *Pasteuria penetrans* may have been responsible for the overall improvement of coffee nutrition by reducing *M. exigua* root damage in Capetinga A and B fields. In Brazil, up to 65% of *M. exigua* J_2 have been infested by *P. penetrans* throughout the year (Maximiniano et al., 2001). Reductions on the nematode population by *P. penetrans* has occurred on other plants in greenhouses and fields (FREITAS et al., 2010; STIRLING, 1984) mostly resulted by research done with introduced inocula. The absence of important biological control agents in the coffee field of Ibiraci such as *P. penetrans,* allowed coffee plants to greatly suffer from *M. exigua* infestation consequently striking the nematicide effect. Once nematicide application reduced the J_2 number with adhering endospores, the *P. penetrans* life cycle will be impaired and future populations reduced. The Capetinga farms with *P. penetrans* have a clay soil texture, which prevents J_2 to quickly move through soil pores and adhere endospores, therefore, the number of J_2 with more than fifteen endospores is low. Fifteen or more endospores per J_2 prevent plant root penetration (DAVIES; KERRY; FLYNN, 1988), interrupting the lifecycle, and consequently no endospores are produced by this infested J_2. Although the soil with an average of fifteen or more endospores per J_2 is considered suppressive to

the nematode (DAVIES; KERRY; FLYNN, 1988), the *P. penetrans* population, in this case, may decline, which in turn should result in worse equilibrium for biological control than in the soil that has the most J_2 with less than 15 endospores which allows plant roots penetrate and produce new endospores.

Soil autoclaving killed endospores of *P. penetrans* from coffee fields. Stirling (1984) working with root-knot nematode showed killing of endospores of *P. penetrans* from grape fields when it was autoclaved. Weibelzahl-Fulton, Dickson e Whitty (1996) also showed killing of *P. penetrans* endospores from naturally infested tobacco field soils by autoclaving, which had not occurred with microwaving and air drying. The increased *M. exigua* population on pepper grown in autoclaved and dried soil compared to recently collected soil samples from the field shows the role of microflora on the antagonism to *M. exigua*. Westphal e Becker (2001), while working with thermal treatment, diagnosed a lesser quantity of cysts and eggs of *Heterodera schachtii* in untreated soils in relation to soil heated to 45°C or more. Pyrowolakis et al. (2002) found a higher number of juveniles of *M. incognita* in fumigated soil with methyl iodide when compared to untreated soil and also the number of eggs was 30 times higher in treated soils, which demonstrates the antagonism caused by the microflora.

The reduction of toxic VOCs to *M. exigua* by soil treatments resulted from the absence of soil microflora which is naturally antagonistic to the nematode, strengthening the relationship between VOCs measurement (toxic to J_2) and microflora mass. In addition, VOCs emitted by soil microflora turn into other soil fungi and bacteria mode of action on the antagonism to *M. exigua* in field inocula. Soil microflora (fungi and bacteria) are most responsible for soil VOCs emission (FREIRE et al., 2010; GU et al., 2007; LEFT; FIERER, 2008; MINERDI et al., 2009).

Although soil drying and autoclaving is required to eliminate plant pathogenic organisms including soil nematodes to be used as plant substrate,

they also eliminate beneficial organisms involved in naturally controlling certain population levels of plant pathogens. Thus, the introduced population of pathogens benefits from the soil treatment and the application of the results from the research become impossible to use in the field.

5 CONCLUSIONS

a) nematicide application was only effective on reducing *M. exigua* population and increase coffee yield on farm without *P. penetrans,* but, reduced parasitized J_2 on farm infested by *P. penetrans;*

b) autoclaving reduces the suppressive effect caused by *P. penetrans;*

c) drying and autoclaving reduce VOCs toxic to J_2 emitted by coffee soils and increase *M. exigua* population by subsequent inoculation on pepper compared to recently collected soil.

6 FINAL CONSIDERATIONS

Nematicide field application assay on farms with both *M. exigua* and *P. penetrans* should be repeated to better support the recommendation on avoiding pesticide use on the presence of this beneficial bacterium, likewise, to obtain better insights on the effect of nematicide on *P. penetrans* population through reduction of *M. exigua* J_2.

REFERENCES

BOTELHO, A. O. et al. Fatores de supressividade a *M. exigua* na rizosfera cafeeira no campo: nova técnica para avaliação de compostos orgânicos voláteis tóxicos a fitonematoides. **Biological Control,** Orlando, 2011.

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: CABI, 2005. p. 529-580.

CASTRO, J. M. C. et al. Levantamento de fitonematoides em cafezais do sul de Minas Gerais. **Nematologia Brasileira,** Campinas, v. 32, n. 1, p. 56-64, June 2008.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira café:** safra 2012: primeira estimativa. Brasília: CONAB, 2012. Disponível em:<http:// www.conab.gov.br/OlalaCMS/uploads/arquivos/12_01_10_10_54_22_boletim_cafe_1a_estimativa.pdf>. Accesso em: 01 fev. 2012.

CHEN, S. Y.; DICKSON, D. W. A technique for determining live second-stage juveniles of *heterodera glycines*. **Journal of Nematology**, College Park, v. 32, n. e, p. 117-121, Mar. 2000.

DAVIES, K. G.; KERRY, B. R.; FLYNN, C. A. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. **Annals of Applied Biology**, Warwick, v. 112, n. 3, p. 491-501, June 1988.

FREIRE, E. S. et al. Volatile substances on the antagonism between fungi, bacteria and *Meloidogyne incognita* and potentially fungi for nematode control. **Journal of Nematology**, College Park, 2010.

FREITAS, L. G. et al. Supressividade de solo a *Meloidogyne* por *Pasteuria penetrans* nos Estados do Maranhão e Santa Catarina. In: BETTIOL. W.; MORANDI. M. A. B. (Ed.). **Biocontrole de doenças de plantas:** uso e perspectivas. Jaguariúna: Embrapa, 2010. p. 153-172.

GHINI, R.; BETTIOL, W. Coletor solar para desinfestação de substratos. **Summa Phytopathologica**, Jaguariúna, v. 17, n. 3-4, p. 281-286, set. 1991.

GHINI, R.; INOMOTO, M. M.; SAITO, E. S. Coletor solar no controle de Meloidogyne arenaria em substratos para produção de mudas. **Fitopatologia Brasileira**, Brasília, v. 23, n. 1, p. 65-67, Mar. 1998.

GU, Y. Q. et al. Q. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. **Soil Biology & Biochemistry**, Elmsford, v. 39, n. 10, p. 2567-2575, Oct. 2007.

HUANG, S. P. et al. Effect of aldicarb, ethoprop and carbofuran on control of root-knot nematode, *Meloidogyne exigua*. **Journal of Nematology**, College Park, v. 15, n. 4, p. 510-514, Oct. 1983.

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

JENKINS, W. R. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant Disease Reporter,** Saint Paul, v. 48, n. 9, p. 692, Sept. 1964.

LEFT, J. W.; FIERER, N. Volatile organic compound (VOC) emissions from soil and litter samples. **Soil Biology & Biochemistry**, Elmsford, v. 40, n. 7, p. 1629-1636, July 2008.

LORDELLO, R. R. A. et al. Plantio de cafezal em área infestada por *Meloidogye exigua***. Nematologia Brasileira**, Campinas, v. 14, n. 1, p. 18-19, June 1990.

MAXIMINIANO, C. et al. Flutuação populacional de *Meloidogyne exigua* em cafezal naturalmente infestado por *Pasteuria penetrans***. Nematologia Brasileira**, Campinas, v. 25, n. 1, p. 63-69, June 2001.

MINERDI, D. et al. Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of *Fusarium oxysporum* strain MSA 35. **Environmental Microbiology**, Oxford, v. 11, n. 4, p. 844-854, Apr. 2009.

PYROWOLAKIS, A. et al. Identification of root-knot nematode suppressive soils. **Applied Soil Ecology**, Amsterdam, v. 19, n. 1, p. 51-56, Jan. 2002.

SCOTT, A. J.; KNOTT, M. A. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Washington, v. 30, n. 2, p. 507-512, June 1974.

SERVIÇO NACIONAL DE LEVANTAMENTO E CONSERVAÇÃO DE SOLOS. **Manual de métodos de análise de solo.** Rio de Janeiro: SNLCS, 1979.

STIRLING, G. R. Biological control of *Meloidogyne javanica* with *Bacilluspenetrans.* **Phytopathology**, Orlando, v. 74. p. 55-60, June 1984.

TIMPER, P. et al. Influence of cropping systems on stem rot (Sclerotium rolfsii), *Meloidogyne arenaria*, and the nematode antagonist *Pasteuria penetrans* in peanut. **Plant Disease**, Saint Paul, v. 85, n. 7, p. 767-772, Jul. 2001.

WEIBELZAHL-FULTON, E.; DICKSON, D. W.; WHITTY, E. B. Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. **Journal of Nematology**, College Park, v. 28, n. 1, p. 43-49, Mar. 1996.

WESTPHAL, A.; BECKER, J. O. Components of soil suppressiveness against *Heterodera schachtii*. **Soil Biology & Biochemistry**, Elmsford, v. 33, n. 1, p. 9-16, Jan. 2001.

CHAPTER 3

Sampling time, storage and moisture on the soil emissions of volatile organic compounds toxic to *Meloidogyne* spp.

ABSTRACT

In response to the exposure time to volatile organic compounds (VOCs), second stage juveniles (J_2) of *Meloidogyne* spp. showed mortality and immobility. The time periods from 6 to 24 hours of J_2 exposure to soil VOCs caused mortality from 49% to 98% from a field soil site. This test type is useful for different research proposals when biotests for the presence of soil emitted VOCs is required. Soil stored for 2, 8 and 14 days only furnished consistent high J_2 mortality when stored for 2 days in cold room. However, the sampling time has to be observed. The sampling in April, August and October in coffee fields furnished more consistent evaluations of soil emitted VOCs from the April sampling. Soil moisture was positively correlated to the J_2 mortality caused by soil emitted VOCs. When soil with depleted moisture and low VOC emissions had water replacement, the high J_2 toxic VOC emission (above 75% J_2 mortality) occurred. Soil emission of VOCs toxic to *Meloidogyne* spp. varies with J_2 exposure time, storage time in cold room and soil moisture.

Keywords: *Meloidogyne exígua.* *Meloidogyne incognita.* Microflora. Environment.

1 INTRODUCTION

As a result of many interacting organisms, fertilizers and crop residues, soil becomes suppressive or not to a plant pathogenic organism depending on the quality and quantity of the suppressive factors (BETTIOL; GHINI, 2001; BORNEMAN; BECKER, 2007; KERRY; CRUMP; MULLEN, 1980; WELLER et al., 2002). Natural soil suppressiveness does occur in the field (BACKER; COOK, 1974; HUBER; SCHNEIDER, 1982; YIN et al., 2003). The contribution to this soil suppressiveness by the soil volatile organic compounds (VOCs) is still to be studied.

VOCs are molecules with up to 20 carbon atoms with high vapor pressure and low polarity. They freely cross membranes and are released into the atmosphere or soil in the absence of a diffusion barrier (PICHERSKY; NOEL; DUDAREVA, 2006), as well as diffusion through an aqueous solution movement and mass flow of water through the soil profile causing rapid movement of these VOCs throughout the system (WHEATLY, 2002). Easy membrane penetration and efficient distribution through soil porosity by VOCs enlarge the volatile area of influence and improves death efficacy of the target microorganisms from the control point of view.

In ecosystems, soils receive VOCs from plant roots (DURADEVA et al., 2006), bacteria, fungi and litter (ISIDOROV; JDANOVA, 2002; LEFT; FIERER, 2008). The biocide potential of VOCs to bacteria, fungi and nematode has been proved (ZOU et al., 2007; FERNANDO et al., 2005; MINERDI et al., 2009; GU et al., 2007; HUANG et al., 2010). So the interacting microorganism in soil, including plant parasitic nematodes will suffer from VOCs toxic effect (CAMPOS; PINHO; FREIRE, 2010).

Although, the naturally suppression of plant parasitic nematode by soil has been studied (PISKIEWICZ; DUYTS; PUTTEN; 2008; WESTPHAL;

BECKER, 2000; WESTPHAL; BECKER, 2001), the contribution of VOCs to the overall suppressiveness needs to be studied. Soil microflora produces the most soil VOCs (LEFT; FIERER, 2008), but, to differentiate VOCs from microorganisms types and from nematode antagonistic microflora still needs to be investigated. Soil microflora isolated fungi and bacteria produce VOCs toxic to nematodes (GUet al., 2007). Since soil is a complex system also influenced by the air condition (temperature and humidity, among others), a field study needs to be done to establish procedures for future research on VOCs toxic to nematodes. Moreover, the feasible idea arises in using the nematode mortality as a laboratory analysis for the levels of suppressive soil potential to the plant parasitic nematodes avoiding the use of nematicide in certain levels of soil suppressivness. As a result, the following subjects will be of interest to study: a) the exposure times of *Meloidogyne exigua* J_2 to VOCs emitted by soils; b) the influence of soil storage periods in cold room on soil VOC emissions toxic to *Meloidogyne* spp. J_2; c) correlation of soil moisture with toxic VOCs emitted by soils.

2 MATERIAL AND METHODS

2.1 Inoculum of second stage juveniles of *Meloidogyne incognita* and *M. exigua* for biotests

Meloidogyne incognita was grown on tomato roots in a greenhouse and *M. exigua* was obtained from the coffee roots of infested fields. The galled roots were carefully cut into 2 cm pieces, placed in a warring blender with 200 mL of 0.5% hyper chloride for 40 seconds. Afterwards, the eggs were collected through sieves according to the technique described by Hussey and Barker (1973). The extracted eggs were cleaned of root debris by using kaolin with the technique described by Coolen and Herde (1972). The eggs were placed in hatching chambers and left to hatch in incubators at 28°C. The J_2 were only collected for the assays at 48 and 72 hours after placed in hatching chambers.

2.2 Field soil sampling.

Four towns in Minas Gerais, Brazil, were selected for sampling. In one of them (Capetinga), four fields were selected, named Capetinga A, B, C and D, and two others in Ibiraci and Varginha, totaling six fields. But not all of them were always used in the assays. In each field, soils were collected beneath the coffee branches at 20 cm from the stem and from 0 to 30 cm of depth. Six plants were used for sampling, from obtaining 3 Kg of rhizosphere soil.

The sampling was done in April, August and October and stored in a cold room at 8 – 10°C until used.

2.3 Mortality and Immobility of *Meloidogyne exigua* second stage juveniles after exposure to different time periods to volatile organic compounds emitted by soils

This assay was carried out to define the best J_2 exposure time to VOCs from field soils. To do this, soils from the two towns of Capetinga and Varginha were used. They were sieved and separated from coarse debris. From each field, twenty-five grams of soil were placed in a Supelco tube and a 1.5 mL eppendorf plastic tube was half buried into the soil and the Supelco tube was closed and kept at 28°C in a growth chamber, following the technique described by Botelho et al., 2011. Six days later, 1 mL suspension with 100 J_2 of *M. exigua* was placed into the internal eppendorf microtube by a syringe.

Next, the J_2s were exposed to the Supelco vial internal VOCs for 0.7, 1.5, 3, 6, 12 or 24 hours. The experiment was organized in a completely randomized design with 4 replicates. After the J_2 exposure time to VOCs the Supelco vial was opened and the eppendorf plastic tube was removed and the J_2 suspension was poured into polipropilene tray wells. The number of immobile and mobile J_2s was estimated. The number of dead J_2s was evaluated, among the immobile ones by using the Chen and Dickson (2000), test.

2.4 Volatile organic compounds toxic to *Meloidogyne incognita* and *M. exigua* from stored soils

Soils from six coffee fields (Capetinga A, B, C and D, Ibiraci and Varginha) were sampled in April, August and October and the soils were separated from roots and debris, sieved and kept in cold room (8-10°C) for 2, 8 and 14 days. From each field, twenty-five grams of soil were placed in a Supelco tube and a 1.5 mL eppendorf plastic tube was half buried into the soil and the

Supelco tube was closed and kept at 28°C in a growth chamber, following the technique described by Botelho et al. (2011). As a control the Supelco tube was filled with washed sand. Six days afterwards, 1 mL suspension with 100 J_2 of *M. incognita* or *M. exigua* was placed into the internal eppendorf microtube, by a syringe. One day later, the Supelco tube was opened and the J_2 solution exposed to VOCs was poured onto polipropilene tray wells and the number of dead J_2 was counted according to the Chen and Dickson (2000), technique. In each sampling, the field soil moisture was determined by the oven drying method (SERVIÇO NACIONAL DE LEVANTAMENTO E CONSERVAÇÃO DE SOLOS, 1979). The soil sample was kept at 105-110°C so the humidity was lost by evaporation until maintaining constant weight. The water lost was determined by the difference between the initial sample weight (P) and weight after the temperature exposed sample (Dw). Then, soil moisture (Sm) was:

$$Sm = [(P-Dw)/Dw]x100 = [Ww/Dw]x100.$$

The VOCs biotest was performed by using *M. incognita* and *M. exigua*. But for the August soil sample test, inoculum of *M. exigua* was scarce, then, only *M. incognita* was used.

2.5 Volatile organic compounds toxic to *Meloidogyne exigua* emitted by coffee field soils before and after water replacement

For this assay, coffee soils from three fields were sampled in August at the towns: Capetinga, Ibiraci, and Varginha, all in Minas Gerais, Brazil. The field soil moisture was determined as previously described.

For the experiment, we used soils with both natural humidity and also by adding 3.5 mL or 7.0 mL of water into the Supelco vial with 25 g of soil. The

soils with or without water addition were checked for the % of humidity according to the procedure previously described. The soils with the humidity percentages (Table 1) were employed for testing the emission of VOCs by using *M. exigua* according to Botelho technique, 2011, as previously described.

Table 1 Humidity percentage of sampled coffee soils readily after sampling (natural) and after addition of 3.5 or 7.0 mL of water

Coffee field sites	**Soil moisture**		
	Natural	**Water replacement**	
		3.5 mL	**7.0 mL**
Capetinga	22.8	43.9	64.9
Ibiraci	5.9	21.8	37.7
Varginha	15.3	32.3	49.4

2.6 Data analyses

The data were submitted to variance analysis and the means were compared by Scott-Knott test (1974) with 5% of significance. The Pearson correlation was calculated with Excel program.

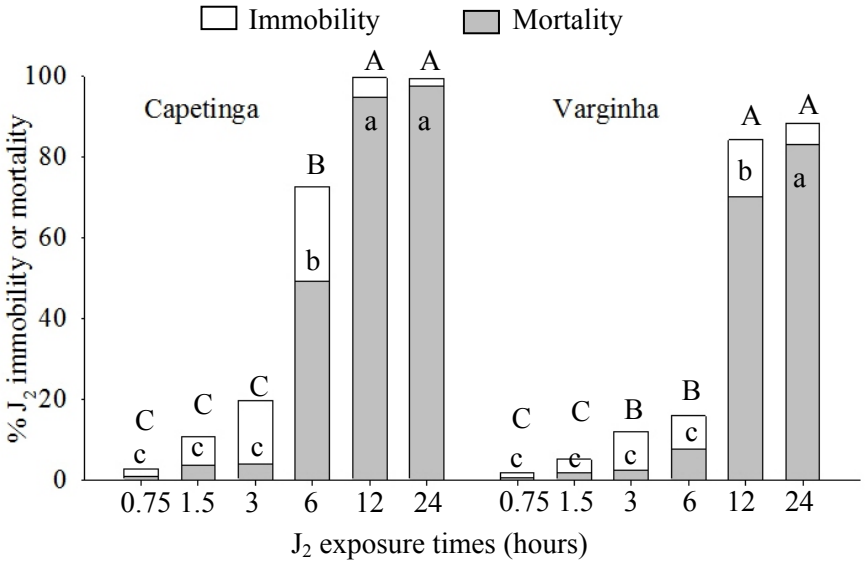
3 RESULTS

3.1 Mortality and Immobility of *Meloidogyne exigua* second stage juveniles after exposure to different time periods to volatile organic compounds emitted by soils

The technique used (BOTELHO et al., 2011) permits the insertion of *Meloidogyne* sp. J_2 into the gas chamber emitted by the soil six days after hermetically closing the Supelco vial cover. In this way, it was possible to evaluate the sensibility of the J_2 to the accumulate gases in the gas chamber over time.

The volatile organic compounds (VOCs), emitted by the two coffee soils tested, always caused higher J_2 immobility than mortality in any VOC exposure time. Short exposure times (from 0.75 to 3 hours) had low and similar VOCs effect on J_2 mortality emitted by both tested coffee soils. During this time period (from 0.75 to 3 hours) only 3 hours of VOCs exposure from Varginha coffee soil showed a higher J_2 immobility difference ($P \leq 0.05$) when compared to other exposure times. By 6 hours of exposure time, the VOCs emitted from Varginha field soil caused the same and low (7.5%) J_2 mortality when compared to shorter time periods. At this exposure time (6 hours), the VOCs emitted by Capetinga soil caused significant higher J_2 mortality (49%) and immobility (72.5%). However, 12 and 24 hours of J_2 exposure to VOCs emitted by both soils mostly increased the J_2 mortality and immobility compared to any other time periods. But, VOCs emitted by Varginha soil caused less ($P \leq 0.05$) mortality (70.1%) at 12 hours exposure than 24 hours (83.0%). The J_2 immobility was similar at 12 and 24 hours exposure to the emitted soil VOCs (Graphic 1).

Since J_2 mortality and immobility were above 80% at 24 hours exposure time for both soils, this exposure period was used on the following assays.



Graphic 1 Immobility and mortality of *Meloidogyne exigua* second stage juveniles (J_2) by volatile organic compounds (VOCs) from two coffee farm soils (Capetinga and Varginha) during different time periods of exposure to VOCs. Exposure time bars followed by different capital and regular letters indicate a significant difference ($P \leq 0.05$) in immobility and mortality, respectively in each coffee farm soil

3.2 Volatile organic compounds toxic to *Meloidogyne incognita* and *M. exigua* from stored soils

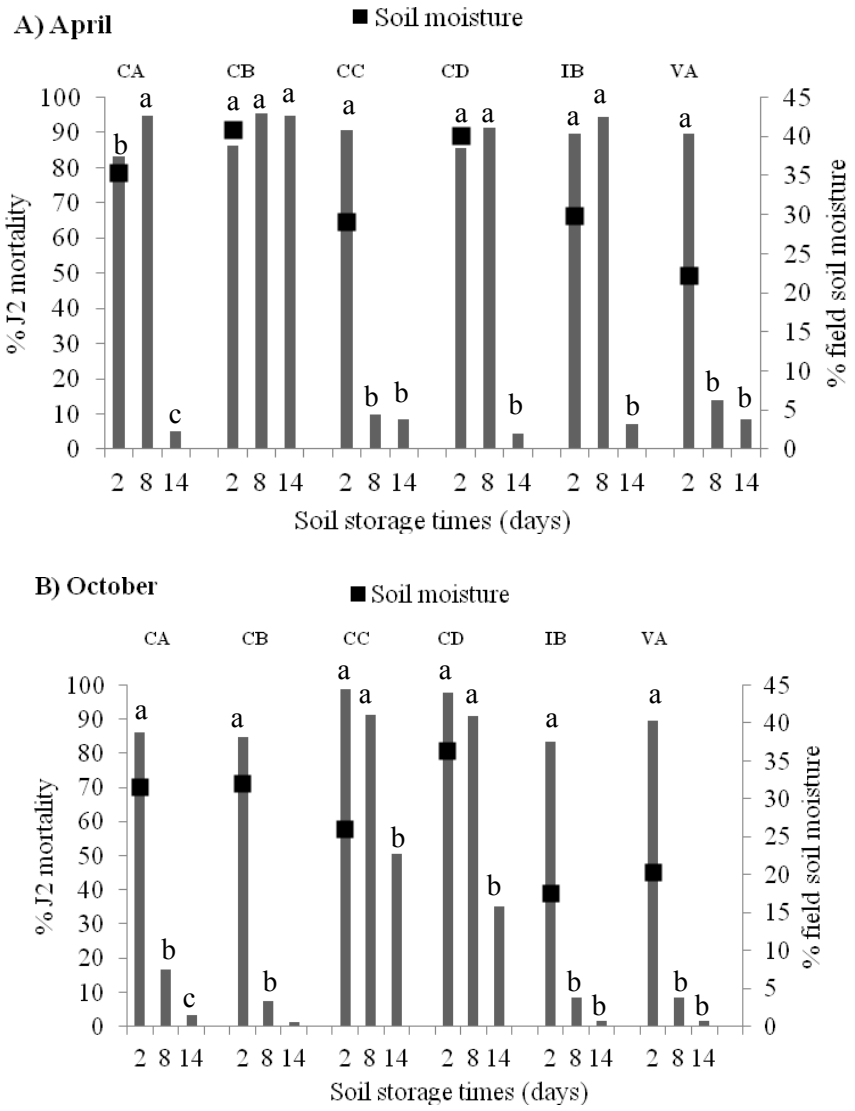
The emission of VOCs from sampled coffee field soils was highly influenced by storage time period. Field soils sampled in April emitted VOCs which caused the highest J_2 mortality (above 80%) either to *M. exigua* or *M. incognita* when stored for two days in a cold room (8-10°C) and lowest (below 15%) when stored for 14 days, in the same conditions, with only one exception that occurred for soil from Capetinga B (CB) sampled in April and tested with *M. exigua.* Soils collected in April and stored for 8 days in a cold room (8-10°C) had low J_2 mortality by emitted VOCs from some fields but from most of the

field soils the J_2 mortality continued to be high when compared to two day storage data (Graphics 2 and 3). The soil moisture from the April sample was the highest among all sampling times and varied from 41% to 22%.

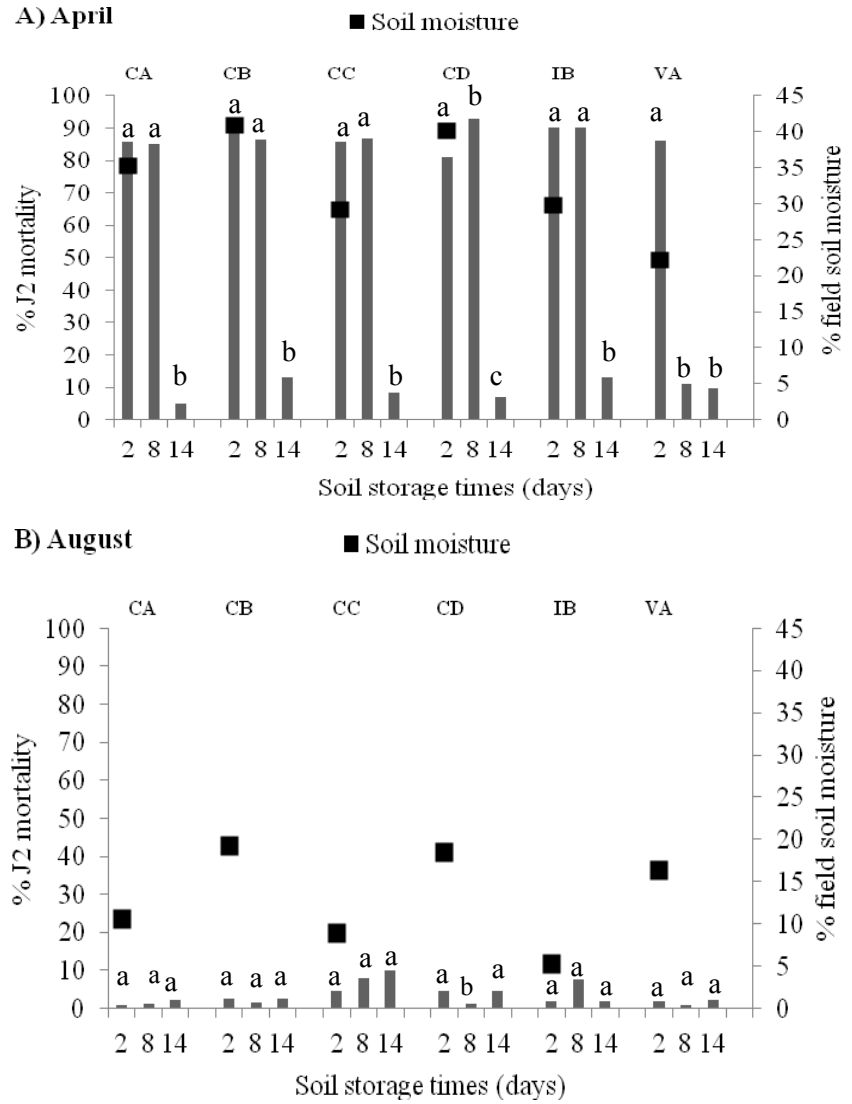
From the August sampling, the soil emitted VOCs expressed by J_2 mortality were below 10% or almost nil, thus J_2 mortality was low and similar ($P \leq 0.05$), regardless of the storage time period, in contrast to the April emitted VOCs test. The soil moisture varied from 19% to 5% which is the lowest level among all sampling dates (Graphic 3). Because of poor field inoculum quality and low quantity of *M. exigua* inoculum, insufficient to run the assay, only *M. incognita* was used in the biotest.

Although the J_2 mortality caused by emitted soil VOCs from the October sampling at Capetinga B-CB, Ibiraci and Varginha fields varied when bioassayed by *M. exigua* and *M. incognita*, the soil storage for 14 days remained the same as the April sampling, with lower values for J_2 mortality significantly different from mortality resulting from two days storage. In the October sampling, the variation among soil field moistures was the highest among all sampling dates, varying from 36% to 17%. But, it is worthy to mention that the J_2 mortality below 12% from the October sampling and biotested with *M. incognita* by the emitted VOCs from Ibiraci and Varginha soils in all storage periods, coincides with low soil moisture (below 20%) as it occurred on all tested field soils in the August sampling which also had low soil moisture at the same level (Graphics 2 and 3).

Soil moisture percentage of all tested fields positively correlated by 0.44 and 0.77 with J_2 mortality of *M. exigua* and *M incognita*, respectively.



Graphic 2 Second stage juveniles (J_2) mortality of *Meloidogyne exigua* by volatile organic compounds of six coffee farms (Capetinga A-CA, B-CB, C-CC and D-CD, Ibiraci-IB and Varginha-VA) stored in cold room (8-10°C) for different time periods. Sampling months: A) April; B) October. In control, mortality was between 3.6 and 4.3% in April and 10.0% in October. Soil storage time bars followed by different letters indicate significant difference ($P \leq 0.05$) among soil storage times in each coffee field



Graphic 3 Second stage juveniles (J_2) mortality of *Meloidogyne incognita* by volatile organic compounds of six coffee farms (Capetinga A-CA, B-CB, C-CC and D-CD, Ibiraci-IB and Varginha-VA) stored in cold room (8-10°C) for different time periods. Sampling months: A) April; B) August; C) October. In control, mortality varied between 3.3 and 5.6% in April, 0.9 and 5.4% in August and 1.9 and 4.9% in October. Soil storage time bars followed by different letters indicate significant difference ($P \leq 0.05$) among soil storage times in each coffee field

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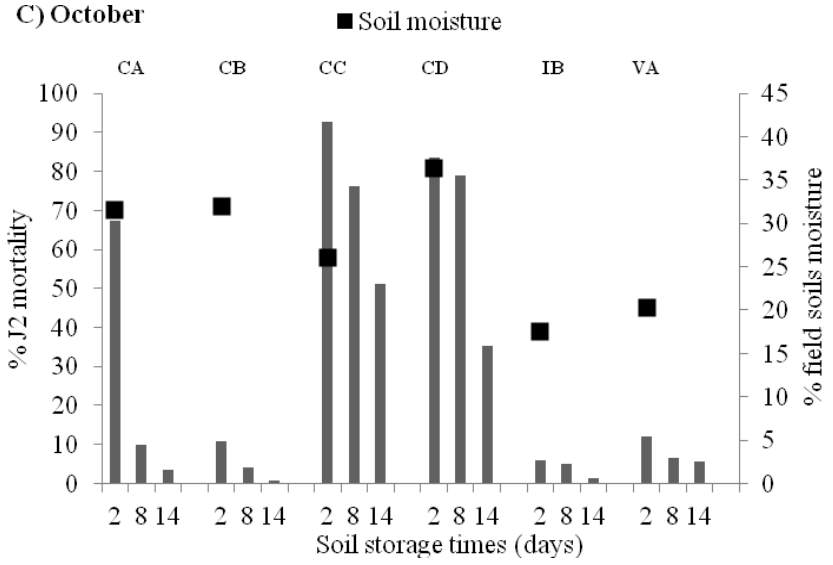
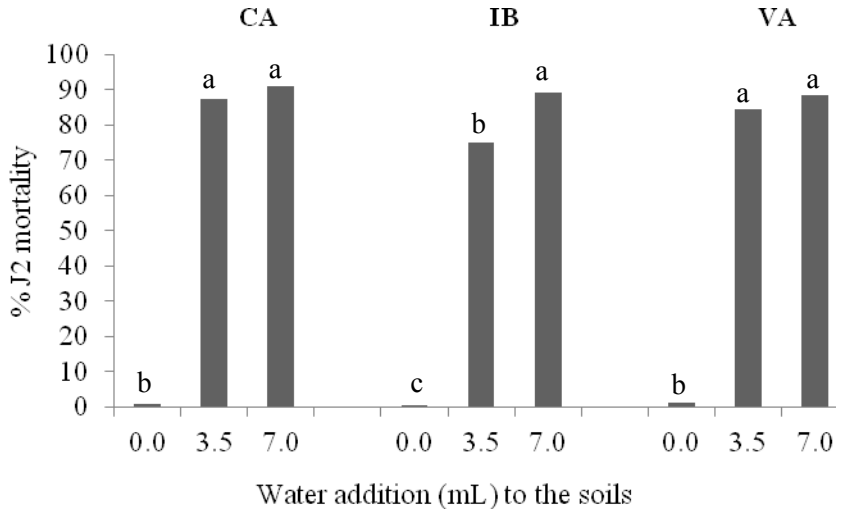


3.3 Volatile organic compounds toxic to *Meloidogyne exigua* emitted by coffee field soils before and after water replacement

The soils from Capetinga, Ibiraci and Varginha fields collected in August and the replaced water, had raised VOCs emission and caused above 74% *M. exigua* J_2 mortality. The effect of water replacement by 3.5 mL and 7.0 mL was only different from each other when regarding the toxic VOC emitted to *M. exigua* J_2 when Ibiraci soil was tested. The J_2 mortality by the VOCs emitted by all soils immediately after sampling was the lowest (Graphic 4). Soil moistures immediately after sampling ranged from 5.9 to 22.8% among the coffee fields sampled. After water replacement by 3.5 mL and 7.0 mL the moisture content raised and ranged from 21.8 to 43.9 and 37.7 to 64.9%, respectively (Table 1).



Graphic 4 Second stage juveniles (J_2) mortality of *Meloidogyne exigua* by volatile organic compounds of three coffee farms (Capetinga-CA, Ibiraci-IB and Varginha-VA) influenced by the soil moisture increase through the addition of water. Water addition bars followed by different letters indicate significant difference ($P \leq 0.05$) among water addition to the soils in each coffee field

4 DISCUSSION

The J_2 toxicity difference throughout the exposure time test shows the need to establish a time to place the J_2 into the gas chamber which is possible when the test is done by the Botelho et al. (2011), technique. Depending on the VOC emitted test proposals, the use of VOC exposure by 6 hours will differentiate soil antagonism to nematode with high VOC emission capacities. Twenty-four hours of exposure will be appropriate to study different soils influenced by environmental factors (moisture, temperature, sampling time soil, texture, organic matter and others) on the antagonism. Six hours of J_2 exposure time to the soil VOCs seems to be the breaking point amongst the time periods studied, which may be called the J_2 sensitivity threshold. If the soil, during cropping holds the VOCs by this period (6 hours) or longer the J_2 will be drastically affected. Gu et al., 2007, tested the effect of bacterial VOCs to nematodes by exposing them for 24 hours. However, they used a compartmental Petri dish, in which the target nematode is placed into the side compartment of the bacterial colony at the moment the Petri dish is sealed with parafilm. In our assay, VOCs were accumulated before placing the target nematode (*Meloidogyne* spp. J_2).

The reduction of toxic VOC emissions to *M. exigua* J_2 over the longer period of soil storage coincides to the decreasing activity of soil microflora when soil samples are stored for fourteen days. Soil drying and storage affected the detectable community structure, microbial biomass and activity (TZENEVA et al., 2008; DENOBILI; CONTIN; BROOKES, 2006).

Fungi and bacteria isolated from soil produce toxic VOCs to nematode (FREIRE et al., 2010; GU et al., 2007). In the soil, the lack of moisture reduces microflora soil activity (GUNADI; HERMAN; BEDAUX, 1998; El SEBAI et al., 2010). In our assay, the soil moisture and *Meloidogyne* J_2 mortality were

positively correlated, demonstrating that moisture is required for toxic VOCs emission by soil. Burns (1989) suggested that films of water are the main sites of microbial activity. Badin et al. (2011), demonstrated the effect of soil moisture to the overall mass of soil fungi and bacteria and informed that the bacterial communities are driven by the hydro dynamics, although no such trend was found for fungal communities.

The lowest toxicity on J_2 from VOC emitted by all field soils sampled in August and from Ibiraci and Varginha fields in October and biotested with *M. incognita* demonstrates that humidity below 20% is the major factor for the drastic J_2 mortality reduction expressing the microflora mass reduction. Under well-watered conditions (60% of soil capacity), the microbial biomass carbon was 63% higher for the same soil in dry conditions and also higher levels of some soil enzymes and water-soluble nitrogen contents were achieved (HUESO; HERNANDEZ; GARCIA, 2011).

The month of August occurs in the winter season in Brazil (from June 21 to September 20) and most of the coffee leaves fall or the plant is defoliated by harvesting the mature beans which exposes the soil to the sun. In addition, the winter is dry and cold. These harsh environmental conditions decrease the soil microflora activity (SARDANS; PEÑUELAS, 2005), and consequently VOCs emitted by the soil during this time period, in our assay, were very low. El Sebai et al. (2010), have demonstrated that although soil microbiota can adapt to the herbicide diuron mineralisation, its activity is strongly dependent on climatic conditions, such as temperature and soil moisture.

The October sampling represented the transition period from winter (very dry) to summer (December 21 to March 20) which is the rainy season. In October the field soil moisture varies among locations because rain is scattered and scarce, which explains the VOC emission variation including pools of soil moisture variation inside the collected sample.

The soil moisture replacement by water addition in the August soil samples raised the high VOCs emission capacity. Again the water replacement on soil increases the microflora activity. Hueso, Hernandez e Garcia (2011), experienced a rapid recovery, after re-watering the soil, in microbiological and biochemical parameters, by evaluating the growth and activity of the microbiota of a semiarid soil.

The difference in quantity of soil VOCs emitted throughout the sampling dates (April, August and October) demonstrates that a specific sampling period has to be established if one wants to compare the VOCs emission from different coffee farms.

All tested soil in this work has antagonistic microflora to *M. exigua* because the produced VOCs were toxic to the nematode. However, antagonism levels observed here may not be enough to efficiently reduce coffee damaged by nematode population during all the plant phenological development.

Since most soil VOCs are produced by the microflora (LEFT; FIERER, 2008), soil handling, storage and guidelines for sampling including collecting date must follow the recommendations for soil microflora total mass in the field. However, part of the total soil VOCs is regarded to the *Meloidogyne* sp. J_2 mortality, thus expressing the antagonism to this nematode.

If total soil microflora mass, or the nematode antagonistic part of it were measured consistently and it was acceptable by the emitted VOCs, a new, faster diagnostic method for soil antagonism to nematode could be created for laboratory analysis of farm samples in the future as far as the new research data support is efficacy.

5 CONCLUSIONS

a) the J_2 mortality increases drastically after 6 hours exposure to soil emitted VOCs;
b) two days stored samples gave the most consistent data on J_2 mortality by VOCs from different soils;
c) soil moisture was positively correlated to J_2 mortality by the soil VOCs;
d) high J_2 toxic VOC emission was raised by soil water replacement.

6 FINAL CONSIDERATIONS

More field soil data on VOC emission should be generated from different crops and environmental conditions. The relationship between soil microbiota biomass and VOC emission needs to be studied on different aspects. For example, to look for specific molecule from microbiota emitted VOC and from cultivated isolated fungi and bacteria VOC of soil microbiota. Once they have the same molecule structure the VOC may become a diagnostic test for microbiota presence in the soil.

REFERENCES

BACKER, K. F.; COOK, R. J. **Biological control of plant pathogens**. San Francisco: Freeman, 1974.

BADIN, A. L. et al. P. Structural stability, microbial biomass and community composition of sediments affected by the hydric dynamics of an urban stormwater infiltration basin. **Microbial Ecology**, New York, v. 61, n. 4, p. 885-897, May 2011.

BETTIOL, W.; GHINI, R. Solos Supressivos. In: MICHEREFF, S. J. et al (Ed.). **Patógenos radiculares em solos tropicais**. Recife: Editora Universitária UFPE, 2001.

BORNEMAN, J.; BECKER, J. O. Identifying microorganisms involved in specific pathogen suppression in soil. **Annual Review of Phytopathology**, Palo Alto, v. 45, p. 153-172, 2007.

BOTELHO, A. O. et al. Fatores de supressividade a ***M. exigua*** na rizosfera cafeeira no campo: nova técnica para avaliação de compostos orgânicos voláteis tóxicos a fitonematoides. **Biological Control,** Orlando, 2011.

BURNS, R. G. Microbial and enzymatic activities in soil biofilms. In: CHARACKLIS, W. G.; WILDERER, P. A. (Ed.). **Structure and function of biofilms**. London: John Wiley, 1989. p. 333-350.

CAMPOS. V. P.; PINHO. R. S. C.; FREIRE. E. S. Volatiles produced by interacting microorganisms potentially useful for the control of plant pathogens. **Ciência e Agrotecnologia,** Lavras, v. 34. n. 3. p. 525-535. maio/jun. 2010.

CHEN. S. Y.; DICKSON. D. W. A technique for determining live second-stage juveniles of *Heterodera glycines*. **Journal of Nematology**, College Park, v. 32. n. 1. p. 117-121, Feb. 2000.

COOLEN, W. A.; HERDE, C. J. **A method for the quantitative extraction of nematodes from plant tissue.** Ghent: State Agriculture Research Centre, 1972.

DENOBILI, M.; CONTIN, M.; BROOKES, P.C. Microbial biomass dynamics in recently air-dried and rewetted soils compared to others stored air-dry for up to 103 years. **Soil Biology & Biochemistry**, Elmsford, v. 38, n. 9, p. 2871-2881, Sept. 2006.

DUDAREVA. N. et al. Plant volatiles: recent advances and future perspectives. **Critical Reviews in Plant Sciences**, Brookfield, v. 25. n. 5. p. 417-440, 2006.

EL SEBAI, T. et al. Diuron mineralisation in a Mediterranean vineyard soil: impact of moisture content and temperature. **Pest Management Science, Sussex,** v. 66, n. 9, p. 988-995, Sept. 2010.

FERNANDO, W. G. D. et al. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. **Soil Biology & Biochemistry**, Elmsford, v. 37. n. 5. p. 955-964, May 2005.

FREIRE, E. S. et al. Volatile substances on the antagonism between fungi, bacteria and *Meloidogyne incognita* and potentially fungi for nematode control. **Journal of Nematology**, College Park, 2010.

GU, Y. Q. et al. Q. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. **Soil Biology & Biochemistry**, Elmsford, v. 39. n. 10. p. 2567-2575. Oct. 2007.

GUNADI, B.; HERMAN, A. V.; BEDAUX, J. J. M. Seasonal dynamics of decomposition of coniferous leaf litter in a forest plantation (*Pinus merkusii*) in Central Java, Indonesia. **Soil Biology & Biochemistry**, Elmsford, v. 30, n. 7, p. 845-852, July 1998.

HUANG, Y. et al. Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. **European Journal of Plant Pathology**, Dordrecht, v. 126, n. 3, p. 417-422, Mar 2010.

HUBER, D. M.; SCHNEIDER, R. W. The description and occurrence of suppressive soils. In: SCHNEIDER, R. W. (Ed.). **Suppressive Soils and Plant Disease**. Saint Paul: APS Press, 1982. p. 1-7.

HUESO, S.; HERNANDEZ, T.; GARCIA, C. Resistance and resilience of the soil microbial biomass to severe drought in semiarid soils: The importance of organic amendments. **Applied Soil Ecology,** Amsterdam, v. 50, p. 27-36, Oct. 2011.

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

ISODOROV. V.; JDANOVA, M. Volatile organic compounds from leaves litter. **Chemosphere**, Davis, v. 48, n. 9, p. 975-979, Sept. 2002.

KERRY, B. R.; CRUMP, D. H.; MULLEN, L. A. Parasitic fungi, soil-moisture and multiplication of the cereal cyst nematode, *Heterodera avenae*. **Nematologica**, Leiden, v. 26, n. 1, p. 57-68, 1980.

LEFT. J. W.; FIERER. N. Volatile organic compound (VOC) emissions from soil and litter samples. **Soil Biology & Biochemistry**, Elmsford, v. 40, n. 7, p. 1629-1636, July 2008.

MINERDI. D. et al. Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of *Fusarium oxysporum* strain MSA 35. **Environmental Microbiology**, Oxford, v. 11. n. 4. p. 844-854, Apr. 2009.

PICHERSKY, E.; NOEL, J. P.; DUDAREVA, N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. **Science**, Washington, v. 10, n. 311, p. 808-811, Feb. 2006.

PISKIEWICZ, A. M.; DUYTS, H.; PUTTEN, W. H. V. D. Multiple species-specific controls of root-feeding nematodes in natural soils. **Soil Biology & Biochemistry**, Elmsford, v. 40, n. 11, p. 2729-2735, Nov. 2008.

SARDANS, J.; PEÑUELAS, J. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. **Soil Biology & Biochemistry**, Elmsford, v.3 7, n. 3, p.455–461, Mar. 2005.

SCOTT, A. J.; KNOTT, M. A. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Washingtomnv. 30, n. 2, p. 507-512, June 1974.

SERVIÇO NACIONAL DE LEVANTAMENTO E CONSERVAÇÃO DE SOLOS. **Manual de métodos de análise de solo.** Rio de Janeiro: SNLCS, 1979.

TZENEVA, V. A. et al. Effect of soil sample preservation, compared to the effect of other environmental variables, on bacterial and eukaryotic diversity. **Research in Microbiology,** Paris, v. 160, n. 2, p. 89-98, Mar. 2009.

WELLER, D. M. et al. Microbial populations responsible for specific soil suppressiveness to plant pathogens. **Annual Review of Phytopathology**, Palo Alto, v. 40, p. 309, 2002.

WESTPHAL, A.; BECKER, J. O. Components of soil suppressiveness against *Heterodera schachtii*. **Soil Biology & Biochemistry**, Elmsford, v. 33, n. 1, p. 9-16, Jan. 2001.

WESTPHAL, A.; BECKER, J. O. Transfer of biological soil suppressiveness against *Heterodera schachtii*. **Phytopathology**, Orlando, v. 90, n. 4, p. 401-406, Apr. 2000.

WHEATLEY, R. E. The consequences of volatile organic compound mediated bacterial and fungal interactions. **Antonie Van Leeuwenhoek**, Amsterdam, v. 81, n. 1-4, p. 357-364, 2002.

YIN, B. et al. Identification of fungal rDNA associated with soil suppressiveness against *Heterodera schachtii* using oligonucleotide fingerprinting. **Phytopathology**, Orlando, v. 93, n. 8, p. 1006-1013, Aug. 2003.

ZOU, C. S. et al. Possible contributions of volatile-producing bacteria to soil fungistasis. **Soil Biology & Biochemistry**, Elmsford, v. 39, n. 9, p. 2371-2379, Dec. 2007.