SELEÇÃO DE RIZOBACTÉRIAS E DE COMPOSTOS ORGÂNICOS VISANDO O MANEJO DO DECLÍNIO DA GOIABEIRA (*Psidium guajava* L.)

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CAMPOS DOS GOYTACAZES - RJ
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Dissertação apresentada ao Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para obtenção do título de Mestre em Produção Vegetal.

Orientador: Prof. RICARDO MOREIRA DE SOUZA

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Se você sabe sua história,
então você saberia de onde vem.
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RESUMO

ALMEIDA, Alexandre Macedo; M. Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. Dezembro de 2012. Seleção de rizobactérias e de compostos orgânicos visando o manejo do declínio da goiabeira (*Psidium guajava* L.). Orientador: Ricardo Moreira de Souza.

O declínio da goiabeira, causado pela associação sinérgica entre *Meloidogyne enterolobii* e *Fusarium solani*, já causou prejuízos à goiabicultura brasileira estimada em mais de US$ 70 milhões. Até o momento, a utilização de rizobactérias e a aplicação de resíduos orgânicos ao solo infestado, combinada com a adubação correta das plantas, tem se mostrado a melhor estratégia de convívio com esta doença. Em um esforço para elaborar uma estratégia de controle biológico do declínio goiabeira, 44 isolados de rizobactérias, obtidos de árvores assintomáticas em pomares infestados com *Meloidogyne enterolobii*, foram avaliados quanto ao seu potencial na redução da reprodução do nematoide. Para cada isolado testado, seis estacas de goiabeira foram mantidas em contato

com a suspensão bacteriana por oito horas e transplantadas. Após o desenvolvimento das raízes, as plantas foram inoculadas com 2000 ovos de nematoides e deixadas crescendo durante quatro meses em estufa. Mudas sadias e mudas inoculadas com o nematoide serviram como controle. Todos os 44 tratamentos foram equivalentes nas cinco variáveis vegetativas. Várias rizobactérias reduziram a população final do nematoide (Fp), Fp / grama de raiz e fator de reprodução, embora não em níveis significativos. Posteriormente, um experimento de dois anos foi instalado em um pomar de goiaba afetado pelo declínio da goiabeira, onde três isolados mais eficazes de rizobactéria foram comparados com os produtos biológicos Nemat® e Nemaplus® quanto à capacidade de reduzir o parasitismo do nematoide e aumentar a produtividade da goiabeira. Sete aplicações bimestrais destes tratamentos sob a copa das árvores não foram capazes de reduzir o parasitismo do nematoide e aumentar a produtividade. O declínio e a morte de algumas plantas da área experimental forçaram a interrupção do experimento após a primeira colheita. Em conclusão, as aplicações de rizobactérias não reduziram o parasitismo de *M. enterolobii* nas plantas. Não houve também, redução a extensa necrose radicular ou alivio do estresse fisiológico sofrido pelas árvores afetadas com declínio da goiabeira. No segundo trabalho objetivou-se avaliar o efeito de diferentes concentrações (1 a 5% v/v) de farinha de carne e osso (FCO), torta de nim, casca de camarão e quitosana incorporados ao solo, sobre a reprodução do nematoide e desenvolvimento vegetativo de mudas de goiabeiras em casa de vegetação e no campo. Observou-se uma correlação negativa significativa ($P < 0.05$) entre o aumento da concentração de FCO e o número de ovos + J_2/g de raiz e o FR (Pf/Pi), bem como em relação a (altura das plantas, peso fresco da parte aérea e da raiz e volume radicular). Os melhores resultados no controle do nematoide e ao desenvolvimento das mudas foram obtidos com a concentração de 3% v/v. Observaram-se diferenças significativas entre os tratamentos para todas as variáveis avaliadas (equivalente a 3% v/v. As demais fontes de matéria orgânica não proporcionaram controle do nematoide. A aplicação de 12 ton.ha^{-1} de FCO em condições de campo implicou em redução do número de nematoides fitoparasitas no solo em todos os tempos testados. No terceiro trabalho objetivou-se avaliar, em três pomares com níveis tecnológicos distintos, o efeito da incorporação de farinha de carne e osso (FCO) sobre a nematofauna do solo e a

severidade da doença. Testaram-se as doses de 12,5 e 25 kg/planta a cada três meses e 50 kg/planta a cada seis meses. Plantas não tratadas serviram como testemunhas. Os dados obtidos foram analisados por ANOVA e teste de Tukey a 5% de probabilidade. A aplicação de 25 kg/planta a cada três meses reduziu ($p \leq 0,05$) os níveis de J_2 de *M. enterolobii* no solo, nos três pomares testados. Houve também redução na população de *Helicotylenchus* sp. e aumento da população de nematoides bacteriófagos, nas três doses de FCO utilizadas. Nas plantas afetadas pelo declínio da goiabeira, não houve efeito da FCO sobre o peso de raiz, número de galhas/amostragem e número de galhas/g de raiz. No quarto trabalho dando sequência ao trabalho anterior, farinha de carne e ossos (FCO) foram aplicadas ao solo de um pomar comercial de goiaba afetada pelo declínio de goiabeira, em três doses/aplicações e regime: i) aplicações trimestrais de 12,5 kg/planta, ii) aplicações trimestrais de 25 kg/planta, e iii) semestrais aplicações de 50 kg/planta. Este experimento foi conduzido por 24 meses. Um conjunto de variáveis foi avaliado em relação a *M. enterolobii* e outros nematoides fitoparasitas e de vida livre, densidade de *Fusarium* sp. no solo e das raízes da goiabeira, liberação de amônia no solo durante a decomposição da FCO e produtividade nas duas colheitas. A aplicação trimestral de 25 kg/planta foi realizada em três pomares diferentes, que eram claramente distintos em idade e em níveis de severidade da doença e em tratos agronômicos. Os baixos níveis de amoníaco observado no solo após a decomposição MBM em todas as três dosagens/regime aplicações podem explicar a redução modesta densidade de juvenis de segundo estádio de *M. enterolobii* e outros nematoides fitoparasitas no solo. O parasitismo por *M. enterolobii* - expresso como densidade de galhas radiculares e densidade no solo e na raiz de *Fusarium* sp. não foi afetado pelas aplicações MBM, o que levou à progressão do declínio da goiabeira e redução da produtividade. Este trabalho mostra como é difícil o manejo de nematoides fitoparasitas através do uso de compostos orgânicos no solo, especialmente em doença tão agressiva e complexa como o declínio da goiabeira.

Palavras-chave: *Meloidogyne enterolobii,* controle biológico, *Fusarium solani,* torta de nim, quitosana, casca de camarão, controle cultural, farinha de carne e ossos, doença complexa.

ABSTRACT

ALMEIDA, Alexandre Macedo; M.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. December, 2012; Selection of rhizobacteria and of organic amendments for management of guava decline. Advisor: Ricardo Moreira de Souza

The decline of guava caused by the synergistic association between *Meloidogyne enterolobii* and *Fusarium solani*, has already caused losses to Brazilian guava orchards estimated at more than $ 70 million. Until this moment, the use of rhizobacteria and application of organic wastes to the infested soil, combined with correct fertilization of plants, has proved the best strategy to withstand this disease. In attempt to develop a biological control strategy of guava decline, 44 rhizobacteria isolates obtained from asymptomatic trees in orchards infested with *Meloidogyne enterolobii*, were evaluated concerning the potential to reduce nematode reproduction. For each isolate tested, six guava cuttings were inoculated for eight hours with bacterial suspension and transplanted for bag seedling. After the root development, plants were inoculated with 2000 nematode eggs and allowed to grow for four months in a greenhouse. Healthy seedlings and seedlings inoculated with the nematode served as controls. All 44 treatments were equivalent in the five variables that assessed the development of the plant. Several rhizobacteria reduced the final population of the nematode (Fp), Fp / gram

root and reproduction factor, although not in satisfactory levels. Posteriorly, a two-year experiment was installed in a guava orchard affected by the decline of guava, where the three most effective rhizobacteria isolates were compared with organic products and Nemaplus® Nemat® for its ability to reduce the variables related to parasitism of the nematode and to increase productivity of guava. Seven bimonthly applications under the canopy were not able to reduce the nematode parasitism and the increased productivity. The decline and death of some experimental plants forced the trial to be stopped after the first harvest. In conclusion, the application of rhizobacteria seems unable to reduce parasitism of *M. enterolobii* on guava plants, and even less to reduce the extensive root necrosis or relieve physiological stress experienced by trees affected by the decline of guava. The second study aimed to evaluate the effect of different concentrations (1 to 5% v/v) of meat and bone meal (MBM), neem cake, shrimp shell and chitosan incorporated in the soil on nematode reproduction and on the vegetative development of seedlings of guava trees in greenhouse, as well as the effect of the best treatment described above, for the management of *M. enterolobii* under field conditions. We observed a significant negative correlation ($P < 0.05$) between the increase of concentration of MBM and the following variables: number of eggs + J2/g root, FR (Fp/Pi) and vegetative variables (plant height, fresh weight of shoot and root, and root volume). The best results regarding the control of nematode and development of seedlings have been obtained with the concentration of 3% v/v. Were observed significant differences between treatments for all variables evaluated, with the MBM to 12 ton.ha^{-1} (equivalent to 3% v/v) standing out by the reduction of the nematode population and greater growth of guava plants. The other sources of organic matter haven't provided a nematode control. The application of 12 ton.ha-1 MBM at field conditions resulted in the reduction of the numbers of phytoparasitic nematodes in the soil at all-time intervals tested. The third study aimed at evaluating in three orchards with distinct technological levels, the effect of the incorporation of different doses of meat and bone meal (MBM) on nematofauna soil and the disease severity. Were tested the doses of 12.5 and 25 kg / plant every three months and 50 kg / plant every six months. This experiment was conducted during 24 months. A set of variables has been evaluated relative to *M. enterolobii* nematodes and other phytoparasitic and free-living density of *Fusarium* sp. soil and roots of guava, release of ammonia in the soil during decomposition

MBM and productivity in the two crops. The quarterly application of 25 kg / plant was performed in three different orchards, which were clearly distinct in age and levels of severity of the disease and agronomic tracts. The low levels of ammonia found into soil just after the decomposition MBM in all three dosages / applications regime may explain the modest reduction of soil density of second stage juveniles of *M. enterolobii* nematodes and other phytoparasitic. The parasitism by *M. enterolobii* - expressed as root gall density and density in soil and root Fusarium sp. were unaffected by the MBM applications, which led to the progression of the decline of guava and reduced productivity. This work shows how difficult is the management of phytoparasitic nematodes through the use of organic compounds in the soil, especially in complex disease as aggressive as the decline of guava.

Key words: *Meloidogyne enterolobii,* biological control, *Fusarium solani*, neem cake, chitosan, shrimp shell, cultural control, meat and bone meal, disease complex.

1. INTRODUÇÃO

A goiabeira (*Psidium guajava* L.) (Myrtaceae) é uma fruteira rústica, tendo suas origens nos trópicos americanos, com distribuição nas regiões tropicais e subtropicais do mundo, apresentando boa produtividade desde o nível do mar até 1600 m de altitude. No Brasil, a goiabicultura movimenta cerca de R$ 73 milhões ao ano em insumos, ativando as cadeias produtivas de maquinários e agrotóxicos (IBGE, 2009).

Meloidogyne enterolobii Yang & Eisenback, 1983, descrito na China parasitando timbaúva [*Enterolobium consortisiliquum* (VELL.)], vem sendo relatado em diversos hospedeiros selvagens e cultivado em vários países de todos os continentes, exceto Oceania. No Brasil, *M. enterolobii* foi relatado em 19 dos 27 Estados (Silva & Oliveira, 2010; Castro & Santana, 2010), na maioria das vezes parasitando goiabeiras. Este nematoide vem sendo associado a uma doença complexa causada pela associação sinergística entre *M. enterolobii* e *Fusarium solani* (Mart.) Sacc. (Gomes *et al.*, 2011). Nesta doença, goiabeiras imunes a *F. solani* tornam-se suscetíveis às extensas necroses do sistema radicular causadas por esse fungo quanto parasitadas por *M. enterolobii*. Ensaios com amostras de raízes de diferentes regiões do Brasil confirmaram que o declínio da goiabeira foi responsável pelo extermínio de cerca de cinco mil hectares de pomares em todo o Brasil, com perdas diretas estimadas em mais de

US$ 70 milhões (Pereira *et al.*, 2009). Os mecanismos dessa doença complexa parecem envolver alterações químicas nos exsudatos radiculares induzidas pelo nematoide (Gomes *et al*., 2011).

Dentre as estratégias já testadas para o controle de *M. enterolobii* citam-se, com pouca eficácia, até o momento, o controle biológico utilizando fungos, bactéria e nematoides entomopatogênicos, o pousio e o uso de nematicidas (Casassa *et al.*, 1996; Gueye *et al.*, 1997; Duponnois *et al.*, 1998; Moreira *et al.*, 2001; Rodriguez *et al.*, 2003; Brito *et al.*, 2004; Rocha *et al*., 2006; Sousa *et al*., 2006; Carneiro *et al*., 2007; Charchar *et al*., 2007; Acevedo, 2008; Lopes *et al.*, 2009; Oka 2010; Almeida *et al*, 2011). Burla *et al.* (2010), Robaina (2012) e Miranda *et al.,* (2012) obtiveram resultados promissores na busca por fontes de resistência ao nematoide e na produção experimental de porta-enxertos resistente.

Outra opção de controle biológico seriam as rizobactérias, bactérias de vida livre ou associativas predominantemente na rizosfera das plantas, e são conhecidas por apresentarem algum benefício para a planta (Chanway *et al*., 1991). Este efeito benéfico pode ser por meio da fixação de nitrogênio, produção de fitormônios, maior disponibilidade de nutrientes, controle de agentes patogênicos e/ou a indução de resistência sistêmica. Estudos utilizando rizobactérias contra patógenos foliares foram bem-sucedidos em algumas culturas (Chen *et al*., 2000). Igualmente, estudos voltados para o controle de alguns fitonematoides, como *Radopholus similis* (Cobb) Thorne, *Meloidogyne* spp. e *Heterodera* spp. foram realizados (Dias-Arieira *et al*., 2003; Freitas *et al*., 2005; Mendoza & Sikora, 2009).

No entanto, relatos de falta de consistência dos resultados em trabalhos com rizobactérias são frequentes, sendo considerado o principal motivo pelo qual ainda não são produzidos inoculantes de rizobactérias comercialmente viáveis (Shishido & Chanway, 1998). Antoun *et al.* (1998) afirmam que “a variabilidade dos resultados é um dos maiores problemas associados a experimentos de inoculação de rizobactérias e é devida, provavelmente, à complexidade das interações envolvidas na rizosfera entre a planta, a bactéria introduzida e o resto da microbiota rizosférica, neutra ou deletéria” (Silveira & Freitas, 2007). Sendo assim, a utilização de rizobactérias depende de prospecção e seleção do isolado

mais eficiente para o ambiente onde se encontra o problema, devendo ser associada a outros métodos de controle.

A utilização de matéria orgânica e de resíduos agrícolas para o controle de doenças já era conhecida na China, Índia e pela civilização Inca desde os primórdios da humanidade. Contudo, os primeiros relatos para controle de fitonematoides com base científica sob ambientes controlados foram conduzidos por Watson a partir da década 1920, seguidos de inúmeros outros trabalhos (e.g. Watson, 1922; Holtz & Vandecaveye, 1938; Linford *et al*., 1938; Jacobsen, 1997; Cook, 2000; Bridge, 2000; McSorley, 2000, Gomes *et al*., 2010).

A natureza da matéria orgânica, os microrganismos presentes e as propriedades do solo são fatores-chave que influenciam a população de nematoides no solo e a proteção das culturas (Akhtar & Malik, 2000). Várias fontes de matéria orgânica são hoje em dia incorporadas ao solo para finalidades diversas, tais como: resíduos orgânicos provenientes de indústrias, resíduos de plantas medicinais, produtos resultantes do processamento de resíduos alimentícios, dejetos de origem animal, subprodutos de crustáceos, etc. Mais especificamente, para o controle de nematoides usa-se torta de Nim (A*zadirachta indica* A. Juss) (Silva & Pereira, 2008), materiais quitinosos (Galper *et al.* 1990), quitina, farinha de carne e osso, farinha de chifre, lodo de esgoto, lixo municipal e resíduos de animais (Akhtar & Alam, 1993; Lazarovits, 1999). Entretanto, a recomendação de uso destas fontes de matéria orgânica depende do patossistema em questão e da natureza da cultura (anual ou perene), e do tipo de solo.

Dessa forma, torna-se imprescindível o estudo para a obtenção, multiplicação e avaliação da viabilidade da utilização de rizobactérias, bem como avaliar metodologias de aplicação e sua eficácia. Faz-se necessário também a avaliação de compostos orgânicos promissores no manejo do declínio da goiabeira, avaliando-se a disponibilidade da matéria orgânica local, o custo do volume utilizado, a dosagem eficiente e a rentabilidade da cultura.

2. REVISÃO DE LITERATURA

2.1 Aspectos gerais da cultura da goiabeira

A família *Myrtaceae* reúne cerca de 102 gêneros e 3.024 espécies, distribuídas e cultivadas em diversos países de climas tropical e subtropical, destacando-se o gênero *Eucalyptus* composto de aproximadamente 600 espécies de ocorrência espontânea na Austrália e Indonésia, e é a árvore mais plantada no mundo, no Brasil sua introdução ocorreu em 1868 no Rio Grande do Sul (Dossa, 2003). Espécies fornecedoras de óleos aromáticos, como *Eucalyptus* ou temperos aromáticos, como o cravo da índia (*Syzygium aromaticum*), a canela (*Cinnamomum zeylanicum*), e a pimenta da jamaica (*Pimento officinalis)* apresentam grande importância econômica (Watlington *et al*. 2006).

Outros quatro gêneros se destacam como importantes fruteiras de interesse econômico – *Feijoa* (=*Acca*), *Eugenia*, *Myrciaria* e *Psidium* (Manica *et al*., 2000). O gênero *Psidium* é originário das Américas Tropical e Subtropical e é constituído de cerca de 100 espécies de árvores e arbustos (Landrum & Kawasaki, 1997), das quais a mais importante é a goiabeira pertencente à classe

Magnoliopsida, ordem Myrtiflorae, subordem Myrtineae, família Myrtaceae, gênero *Psidium.* espécie *P. guajava* L.. O gênero engloba também inúmeras outras espécies produtoras de frutos comestíveis, madeireiras e ornamentais, com grande potencial para exploração comercial. Entre essas espécies, os araçazeiros são merecedores de maior atenção, especialmente devido a algumas características específicas de seus frutos, como sabor exótico, teor elevado de vitamina C e boa aceitação pelos consumidores (Manica *et al*., 2000; Pires *et al*., 2002).

Existem mais de 400 espécies de goiaba no mundo, apesar de somente algumas dezenas serem plantadas comercialmente (Pommer; Murakami; Watlington, 2006). Destacando-se como variedades de polpa vermelha a ‘Paluma’, ‘Rica’, ‘Pedro Sato’ e entre as variedades de polpa branca a ‘Ogawa’, e ‘Kumagai’.

A goiabeira é um arbusto de pequeno porte, que, em pomares adultos, pode atingir de três a seis metros de altura. As folhas são opostas e caem após a maturação; as flores são brancas e hermafroditas. O fruto é constituído de uma baga, carnoso, casca verde, amarelada ou roxa, com superfície irregular, de cerca de oito centímetros de diâmetro (Giacomino, 2012). Em seu interior, há uma polpa rosada, branca ou dourada, contendo dezenas de pequenas sementes duras, mas que podem ser ingeridas sem causar problemas à saúde do homem. Os frutos são destinados para os mais diversos fins desde o consumo “*in natura*”, quanto à produção de doces, compotas, fruta cristalizada, molhos “tipo catchup”, bem como na indústria farmacêutica. Seus usos medicinais iniciaram com os índios que usavam tradicionalmente a decocção de folhas, flores e cascas de goiaba para curar a desinteria, dor de garganta, vômitos, problemas de estômago, vertigem e para regularizar períodos menstruais. Seus usos tópicos são utilizados como antisséptico natural para ulcerações e feridas da pele.

2.2 Produção mundial, nacional e no Estado do Rio de Janeiro

Os países que se destacam na produção mundial de goiaba são a Índia, o Paquistão, o Brasil, o Egito, a Venezuela, os Estados Unidos, a África do Sul, o México, a Austrália e o Quênia. A produção de goiaba brasileira se destina principalmente para o mercado interno, sendo exportados pequenas quantidades de goiaba e seus derivados, principalmente para França, Alemanha, Estados Unidos, Argentina, Paraguai e Bolívia (Pereira, 1995; Zambão & Bellintani Neto, 1998; Pereira & Nachtigal, 2002).

Segundo o Instituto Brasileiro de Geografia e Estatística (IBGE, 2012), a área de cultivo de goiabeira na safra 2010 no Brasil foi de 15.677 ha, 316.363 Ton e um valor de produção de 225.104,00 milhões de Reais. As principais regiões produtoras são: Nordeste com 7.021 ha plantados, em seguida vem a região Sudeste com 5.599 ha, o Norte com 1.481 ha e o Centro-Oeste com 570 ha, tendo essas regiões, respectivamente, as seguintes produções: 130.474 Ton., 133.616 Ton., 12.806 Ton. e 16.896 Ton.. No Estado do Rio de Janeiro plantou-se 662 ha, obteve-se 13.059 Ton. de frutos e obteve-se 6.231,00 milhões de Reais em vendas.

2.3 Os fitonematoides

Os nematoides constituem o mais abundante grupo de organismo multicelular em número de indivíduos no mundo, sendo estimado em mais de um milhão de espécies (Viglierchio, 1991).

Os fitonematoides são um grupo de patógenos parasitas de plantas que atacam todas as partes e os órgãos das plantas (caule, folhas, flores, frutos, sementes, raízes). A maioria dos nematoides fitoparasitas induz a formação de alterações morfofisiológicas na planta, prejudicando a absorção e/ou translocação de água e nutrientes, e causam necroses e murchas. No Brasil, a quantificação de perdas não é precisa devido principalmente às interações com danos provocados por pragas e outras doenças, condições climáticas adversas,

presença de plantas invasoras e inadequação de tratos culturais (Ritzinger & Francelli, 2006).

Podem ser endo ou ectoparasitas; sedentário ou migrador. São responsáveis por perdas estimadas na ordem de 10% da produção agrícola mundial (Whitehead, 1998), com perdas na ordem dos US$ 120 bilhões (Nicol *et al.*, 2011). Os grupos de fitoparasitas que causam maior impacto na agricultura mundial são: os que atacam a parte áerea *Anguina, Bursaphelenchus, Ditylenchus* e os que atacam as raízes e tubérculos *Criconemela, Globodera, Helicotylenchus, Heterodera, Hirschmanniella, Hoplolaimus, Pratylenchus, Radopholus, Rotylenchus, Scutellonema, Tylenchus, Meloidogyne* e os vetores de vírus *Xiphinema, Longidorus, Trichodorus.*

2.3.1 *Meloidogyne enterolobii*

O gênero *Meloidogyne* Göeldi, 1887 reúne os nematoides que formam galhas radiculares, mais de 90 espécies são reconhecidas e são conhecidas mais de 2000 plantas hospedeiras (Perry *et al*., 2009). É um gênero de fácil disseminação e de difícil controle, sendo parasitas de culturas de grande importância econômica, resultando em prejuízos que vão desde a redução drástica da produtividade até a morte das plantas, causando grandes prejuízos na agricultura (Melo, 1995).

O ciclo de vida do *Meloidogyne* começa com a fêmea depositando seus ovos em uma matriz gelatinosa em um ponto da raiz. Cada massa de ovos contém cerca de 400 a 500 ovos e pode-se formar em meio ao parênquima cortical ou sobre a superfície das raízes. O primeiro estádio juvenil (J1) desenvolve-se dentro do ovo, passando por uma ecdise e eclodindo o juvenil de segundo estádio (J2). O J2 eclode do ovo por forças mecânicas de seu estilete e pela ação enzimática de quitinases, produzidas nas glândulas esofagianas e liberadas via estilete (Abad *et al*, 2009). Quando no solo, os J2 migram e são direcionados às raízes das plantas hospedeiras, através de sinalizadores liberados pelas raízes como

exsudatos ou por causa da respiração celular e liberação de CO_2, criando com isso um gradiente de atratividade aos J2. Ao chegar às raízes os J2, liberam compostos enzimáticos, principalmente enzimas degradadoras de parede celular, capazes de romper a parede celular e penetrar nas raízes e em seguida estabelecer modificações celulares, formando células gigantes para criar o seu sítio de alimentação (Taylor *et al*., 1983). Sua reprodução obrigatória é sempre via partenogênese mitótica, sendo a partenogênese meiótica facultativa (Eisenback & Triantaphyllou, 1991).

Os machos mudam de forma no quarto estádio juvenil (J4), o qual passa por uma metamorfose alongando-se e tomando a forma vermiforme, eclodindo inteiramente desenvolvido. Não se alimentam, saem das raízes e movem-se livremente no solo. O acasalamento nas espécies partenogenéticas não ocorre, sendo que o macho permanece no solo até a sua morte (Eisenback & Triantaphyllou, 1991). O ciclo de vida total do gênero dura em torno de 60 dias.

Meloidogyne enterolobii Yang & Eisenback, 1983 (syn. *M. mayaguensis* Rammah & Hischmann, 1988) foi descrito na China parasitando timbaúva [*Enterolobium consortisiliquum* (Vell.)], e vem sendo relatado em diversos hospedeiros selvagens ou cultivados em vários países da África, América do Norte, América Central, América do Sul e Europa.

No Brasil, *Meloidogyne enterolobii* foi relatado pela primeira vez parasitando goiabeira (*Psidium guajava*) no vale do submédio São Francisco, nos estados de Pernambuco e Bahia, causando prejuízos com a destruição de pomares daquela região (Carneiro *et al*., 2001). Sendo este relatado em 19 dos 27 estados (Silva & Oliveira, 2010, Castro & Santana, 2010). Apresenta, além da goiabeira, diversos hospedeiros, incluindo hortaliças, frutíferas, essências florestais, ornamentais e plantas daninhas (Brito *et al*., 2003; Brito *et al*., 2007; Guimarães *et al*. 2003; Kaur *et al*., 2007; Lima *et al*., 2003; Rodriguez *et al*., 2003).

Bitencourt & Silva (2010) testaram a susceptibilidade de olerícolas ao *M. enterolobii* e concluíram que Tomate-cereja ‘Carolina’, pepino, melão, berinjela, melancia, coentro e tomate ‘Santa Cruz’ mostraram-se muito suscetíveis. Quiabo, pimentão, maxixe, abóbora, pimenta-malagueta, alface americana e alface crespa comportaram-se como levemente ou muito resistentes. Vinagreira, couve, cebolinha, cenoura e coentro-tapuio foram altamente resistentes. Lima *et al*., 2003, encontrou susceptibilidade em mamão (*Carica papaya*), acerola (*Malpighia*

punicifolia), fedegoso (*Senna* spp.), serralha (*Emilia sonchifolia*), beldroega-pequena (*Chamaesyce prostata*), urtiga (*Cnidoscolus urensi*) e maracujá-do-mato (*Passiflora mucronata).*

Cantu *et al*. (2009) avaliaram a resistência a *M. enterolobii* de oito porta-enxertos de tomate considerados resistentes a M. enterolobii, *M. incognita*, *M. javanica* e *M. arenaria*, foram suscetíveis a *M. enterolobii*, demonstrando com isso a sua capacidade de quebra da resistência do gene Mi em tomate e em outras espécies vegetais antes resistente a *Meloidogyne spp.*

Dentre os não hospedeiros experimentais, tem-se o algodão, o amendoim (Rammah & Hirschmann, 1988), o milho, fruta-do-conde, chirimóia (*Anona cherimolia*), laranja azeda, *grapefruit* (*Citrus paradisi*), cinamomo (*Melia azedarach*), timo (*Thymus vulgaris*) e alho (Rodriguez *et al.,* 2003). Moura *et al.* (2003) relatam o abacateiro, jambeiro, coqueiro, mangueira, pinheira e gravioleira, como não hospedeiros.

2.4 O declínio da goiabeira

O estudo das interações patogênicas entre fungos e nematoides foi destaque nos anos sessenta e oitenta. As publicações eram frequentes e culminaram em simpósios (APS, 1963) e revisões (Powell, 1971; Webster, 1985). A interação mais estudada foi envolvendo o *Meloidogyne* Göeldi, 1887 e formas especiais do fungo *Fusarium oxisporum* Schlecht, principalmente nos complexos da murcha fusariana (Cooper & Brodie, 1963; Johnson & Littell, 1969; Moura & Powell, 1977; Webster, 1985), atuando o nematoide como fator predisponente a doença.

O declínio da goiabeira é uma doença complexa causada pela associação sinergística entre *M. enterolobii* e *Fusarium*. Gomes *et al*., (2011) através da amplificação da região rDNA ITS dos isolados de *Fusarium* das regiões brasileiras, confirmaram homologia com as da espécie *Fusarium solani* e foram depositadas no Genbank, com os respectivos registros JN006807 a JN006818.

Goiabeiras em declínio apresentam clorose, necrose das bordas foliares, murchamento, queda das folhas e necroses radiculares. Estes sintomas estão associados à deficiência foliar de nitrogênio, potássio, fósforo, cálcio e magnésio e a um acumulo de cloro, manganês e sódio (Gomes *et al*., 2010). As plantas em declínio têm seu sistema radicular apodrecido ao longo da evolução da doença, não se recuperando e morrendo dentro de alguns meses. Nesta doença, goiabeiras imunes a *F. solani* tornam-se susceptíveis a este fungo quando parasitadas por *M. enterolobii*.

Gomes *et al.* (2010) demonstraram que existe efeito sinérgico entre *M. enterolobii* e *F. solani* causando o declínio da goiabeira, demonstrando com isso a natureza complexa da doença e sugerem que existe um fator fisiológico envolvido na indução de susceptibilidade de goiabeiras parasitadas por *M. enterolobii ao F. solani*, uma vez que experimentos em que foram feitos ferimentos físicos em mudas, não apresentaram podridão radicular induzida pelo fungo, consequentemente não houve caracterização do declínio. Demonstrando que o declínio da goiabeira não é causado por *M. enterolobii* apenas, sendo *F. solani*, o agente que acentua os danos causados à planta.

Ensaios com amostras de raízes de diferentes regiões do Brasil confirmaram que o declínio da goiabeira está presente nas regiões sul, sudeste, nordeste e centro-oeste. Os mecanismos dessa doença complexa parecem envolver alterações químicas nos exsudatos radiculares induzidas pelo nematoide (Gomes *et al.*, 2012).

A utilização de exsudatos radiculares coletados de plantas não parasitadas para irrigar plântulas, com raiz exposta entre duas camadas de papel germiteste, mostrou que as mesmas se desenvolvem normalmente, e que o uso de exsudato coletado de plantas parasitadas por *M.* enterolobii, as plântulas apresentaram colonização e necrose radicular, quando na presença do *F. solani*. Existe, portanto efeito local dos exsudatos radiculares de plantas parasitadas por *M. enterolobii* na patogenicidade de *F. solani* às raízes de goiabeira (Gomes *et al*., 2011).

Em outro experimento utilizando sistema radicular bipartido de mudas de goiabeiras e isolado em dois vasos, Gomes *et al.* (2011) demonstraram que não existe fator sistêmico que permita a colonização das raízes da goiabeira por *F. solani.*

2.5 Rizobactérias

Os microrganismos estão diretamente associados à qualidade ambiental, tendo papel fundamental na manutenção dos ecossistemas, bem como na sensibilidade a variações nos muitos fatores que compõem os ambientes, sendo estes de grande interesse agronômico. As bactérias encontradas na rizosfera de plantas são conhecidas como rizobactérias (Schoroth e Hancock, 1982), e geralmente denominadas Rizobactérias Promotoras de Crescimento de Plantas ou RPCPs. Vivem na rizosfera que é denominada a região do solo ao redor da raiz com influência imediata do sistema radicular, zona rica em nutrientes por liberação e deposição de compostos orgânicos sintetizados e exsudado pela planta.

As rizobactérias podem conferir às plantas: maior resistência a condições de estresse, alterações nas condições fisiológicas, proteção contra organismos patogênicos, produção de fitormônios, aumento da disponibilidade de nutrientes pela fixação assimbiótica de nitrogênio, solubilização de fosfato, dentre outros (Oliveira, 2009). Podem ser benéficas, deletérias ou neutras (Dobbelaere *et al*., 2003). As bactérias não estão distribuídas aleatoriamente na rizosfera, mas sim agregadas, principalmente nas regiões intercelulares da epiderme por serem áreas de ativa exsudação (Bowen & Rovira, 1999).

A utilização destas rizobactérias se dá principalmente por aplicação via microbiolização de sementes, tratamento das sementes em suspensão bacteriana, ou diretamente adicionado ao substrato. Sua colonização se dá de forma gradativa acompanhando o crescimento da raiz e a liberação de exsudatos ou no momento da germinação da semente onde carboidratos e aminoácidos são liberados. Sendo que a capacidade de colonizar e multiplicar na rizosfera é chamada de “competência de rizosfera”.

Grande parte das rizobactérias com capacidade de biocontrole de fitopatógenos tem a capacidade de produzir substâncias tóxicas e outros metabólicos, com atividade nematicida. Os principais mecanismos de ação das rizobactérias responsáveis pela redução na taxa de infecção da raiz são: produção de metabólitos e diminuição da comunicação entre a planta hospedeira

e o nematoide e a degradação de exsudatos provenientes da raiz que regulam o comportamento do fitonematoide (Siddiqui & Mahmood, 1999).

As rizobactérias usadas como antagonistas de fitonematoides podem ser classificadas como bactérias parasitas e não parasitas. Os principais gêneros para biocontrole de nematoides são *Pasteuria, Bacilos, Pseudomonas, Serratia, Clostridium, Streptomyces, Agrobacterium* (Siddiqui & Mahmood, 1999).

2.6 Uso de compostos orgânicos no controle de nematoides

Na China, Índia e civilização Inca desde os primórdios da humanidade, já utilizavam matéria orgânica e resíduos agrícolas na agricultura. Durante séculos, os utilizavam para manipular o solo e produzir seus alimentos. A matéria orgânica é conhecida por afetar a aeração do solo, estrutura, drenagem, capacidade de retenção de umidade, disponibilidade de nutrientes e sua fauna e flora microbiana (Davey, 1996).

Linford *et al*. (1938) sugeriram que as alterações orgânicas do solo estimularam a atividade de antagonistas de ocorrência natural de pragas e nematoides e argumentaram que a atividade destes organismos atua no controle de fitonematoides. O conceito de que as alterações orgânicas geralmente agem desta forma tem sido considerado promissor para o controle biológico de fitonematoides.

Após a segunda guerra mundial, a população abandonou gradativamente a adubação orgânica em detrimento da adubação química, bem como se ampliou a monocultura e reduziu-se a rotação de culturas. Consequentemente as doenças e pragas causaram mais danos nas plantações levando a uma corrida por defensivos agrícolas químicos. Meio século depois, a necessidade de se voltar a um equilíbrio orgânico na agricultura e por inúmeros casos de intoxicação, biomagnificação de resíduos químicos, fez-se necessário voltar às origens para buscar novamente o equilíbrio com o meio ambiente.

Os primeiros relatos de manejo de fitonematoides com o uso de matéria orgânica são de Watson nas décadas de 20/30. A natureza da matéria orgânica, os microrganismos presentes no solo e as propriedades do solo são fatores-chave que determinam a eficiência de controle de fitonematoides (Akhtar & Alam, 1993; Akhtar & Malik, 2000; Ferraz, *et al*.2010).

Várias fontes de matéria orgânica já foram utilizadas com sucesso, como torta de Nim (A*zadirachta indica* A. Juss) (Silva & Pereira, 2008), materiais quitinosos (Galper *et al*. 1990), quitina, farinha de carne e osso, farinha de chifre, lodo de esgoto, lixo municipal e resíduos de animais (Akhtar & Alam, 1993, Lazarovits, 1999). Entretanto, a recomendação de uso destas fontes de matéria orgânica depende do patossistema em questão, seja envolvendo uma cultura anual ou perene, nematoides ecto- ou endoparasitas, e com ou sem o envolvimento de outros patógenos de solo. A origem, fonte e disponibilidade da matéria orgânica afetam diretamente sua aplicação, seu manejo e sua viabilidade financeira da cultura.

3. TRABALHOS

3.1- Greenhouse and field assessment of rhizobacteria to control guava decline[1][2]

Avaliação de rizobactérias em casa de vegetação e em campo visando ao controle do declínio da goiabeira

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ABSTRACT

In an effort to devise a biological strategy to control guava decline, 120 rhizobacteria isolates were obtained from symptomless guava trees located in *Meloidogyne enterolobii*-infested orchards. Of those isolates, 44 were assessed for their potential to reduce nematode's reproduction: for each isolate, six guava stem cuttings were embedded for eight hours with bacterial suspension and transplanted. Upon development of the roots, the plants were inoculated with 2000 nematode eggs and allowed to grow for four months under greenhouse. Seedlings embedded with water, inoculated or not with the nematode, served as controls. All treatments were equivalent in the five variables that assessed plant development. Several rhizobacteria reduced ($p<0.05$) the final nematode population (Fp), Fp/gram of root and reproduction factor, although not to satisfactory levels. Subsequently, a two-year experiment was set up in a guava orchard affected by guava decline, in which three of the most effective rhizobacterial isolates were compared with the biological products Nemat® and Nemaplus® for their ability to reduce variables related to nematode parasitism and increase guava productivity. Seven bimonthly applications of these treatments under the tree canopy were unable to reduce nematode parasitism and increase productivity. The decline and death of some plants forced the experiment to be stopped after the first harvest. In conclusion, rhizobacteria applications seem unable to reduce the parasitism of *M. enterolobii* on guava plants, and even less to reduce the extensive root decay or alleviate the physiological stress suffered by trees affected by guava decline.

Key words: *Psidium guajava, Meloidogyne enterolobii,* biological control, *Fusarium solani*.

RESUMO

Buscando o controle biológico do declínio da goiabeira, foram obtidos 120 isolados de rizobactérias de goiabeiras assintomáticas, localizadas em pomares infestados por *M. enterolobii*. Dos 120 isolados, 44 foram avaliados em seu potencial para reduzir o nível populacional do nematoide. Para cada isolado, seis estacas vegetativas de goiabeira foram embebidas com suspensão bacteriana por 8 horas e transplantadas para sacolas de 5 L. Após o desenvolvimento das raízes, as mudas foram submetidas à inoculação com 2.000 ovos de *M. enterolobii* e mantidas por quatro meses em casa de vegetação. Mudas submetidas à inoculação com água, com ou sem *M. enterolobii,* serviram como controles. Os 46 tratamentos foram equivalentes nas cinco variáveis que avaliaram o desenvolvimento das plantas. Várias rizobactérias reduziram ($p<0,05$) a população final do nematoide (PF), PF/g de raiz e fator de reprodução, embora em níveis insatisfatórios. Posteriormente, um experimento bianual foi estabelecido em um pomar afetado pelo declínio da goiabeira, no qual três rizobactérias foram comparadas com os produtos biológicos Nemat® e Nemaplus® em sua capacidade de reduzir o parasitismo pelo nematoide e aumentar a produtividade da goiabeira. Sete aplicações bimensais desses tratamentos não reduziram o parasitismo pelo nematoide e não houve aumento de produtividade. A morte de algumas plantas levou à finalização antecipada do experimento após a primeira colheita. Em conclusão, aplicações de rizobactérias parecem incapazes de diminuir o parasitismo por *M. enterolobii,* e menos ainda reduzir a extensa necrose radicular e o estresse fisiológico ocorrido nas árvores afetadas pelo declínio de goiabeira.

Palavras-chave: *Psidium guajava, Meloidogyne enterolobii,* controle biológico, *Fusarium solani*.

1. INTRODUCTION

Guava trees (*Psidium guajava* L.) (Myrtaceae) are robust fruit-bearing plants that originated in the American tropics and are distributed throughout tropical and subtropical regions worldwide (GONZAGA NETO and SOARES, 1994). In Brazil, guava crops turn over about 73 million reais (in 2010, the equivalent of 43 million US dollars) per year, affecting productive chains in the area of machinery, pesticides and fertilizers. They also have an important social impact in that they strengthen family-scale agriculture involving orchards of 3 to 5 hectares (ha) on average (NATALE *et al*., 1996; ROSANE *et al*., 2003; IBGE, 2006).

Guava decline, caused by the synergistic association between *Meloidogyne enterolobii* Yang & Eisenback and the fungus *Fusarium solani* (Mart.) Sacc., has wiped out about 5000 ha of orchards in various regions of Brazil, causing direct damage estimated at 112.7 million reais and the unemployment of 3,703 rural workers (PEREIRA *et al*., 2009). In this disease, the *F. solani*-immune guava plants become susceptible to extensive root decay caused by the fungus upon parasitism by *M. enterolobii* (GOMES *et al*., 2011). The mechanisms of this complex disease are presently under study, and it seems to involve nematode-induced alterations in root exudates. Although this disease may be managed relatively successfully by applying certain types of organic compost to the soil (Gomes *et al*., 2010), a number of attempts at control have failed, such as the use of nematicides, antagonistic plants, nematophagous fungi and bacteria, genetic resistance and fallowing (Rocha *et al*., 2000; Rodriguez *et al*., 2003; Rocha *et al*., 2004; Souza *et al*., 2006; Lopes *et al*., 2007).

Currently, society in general and the scientific community in particular are prioritizing environmental sustainability, stimulating the discovery of bioactive compounds for integrated management of pests and diseases (Pires, 2008). The relative inefficiency of pesticides and the restrictions on their use have increased interest in biological control as an alternative tool for integrated disease management in a number of crops (Mafia *et al*., 2009). Free-living or associative bacteria predominate in the plant rhizosphere, and are known as rhizobacteria if

they present some benefit to the plant (Chanway *et al*., 1991). This beneficial effect may take place by means of nitrogen fixation, phytohormone production, greater availability of nutrients, pathogens control and/or induction of systemic resistance. Studies using rhizobacteria against leaf pathogens have been successful in some crops (Chen *et al*., 2000). Equally, studies directed toward the control of some plant nematodes are taking place, such as *Radopholus similis* (Cobb) Thorne, *Meloidogyne* spp. and *Heterodera* spp. (Dias-Arieira *et al*., 2003; Freitas *et al*., 2005; Mendoza and Sikora, 2009).

This study reports on efforts to obtain rhizobacteria isolates associated with commercial guava orchards and to test them in the greenhouse and in the field, with a view to reducing the population of *M. enterolobii*. It is believed that a smaller population of this nematode causes less physiological stress to the guava tree, allowing it to resist opportunistic action from *F. solani*, which causes root rot. In the field, some rhizobacteria isolates were compared with commercial products based on biological agents (Nemat® and Nemaplus® produced by Ballagro Agro Tecnologia, Brazil).

2. MATERIAL AND METHODS

Isolation and selection of rhizobacteria isolates in greenhouse

Fifteen samples, each with one kilogram of rhizosphere soil, were collected from a number of healthy guava trees in orchards infested with *M. enterolobii*, in the municipalities of Cachoeiras de Macacú (22°34'37"S and 42°43'12"W) and São João da Barra (21°39'21"S and 41°02'07"W; 21^{0}41'22"S and 41^{0}3'20"W).

The 15 samples were individually homogenized and aliquots of 10 g of soil were diluted in 100 mL of aqueous solution of NaCl at 0.85%. The suspensions were shaken at room temperature for 10 min in a Tecnal® shaker at 100 rpm. Next, serial dilutions were made from 10^{-4} to 10^{-9} of the suspensions of each sample, removing aliquots of 100 µl from each dilution, to be transferred to Petri

dishes with medium 523 (KADO and HESKETT, 1970). The dishes were incubated in an incubator at 28 °C for 24 hours. The 120 rhizobacteria colonies that appeared on the Petri dishes were transferred to test tubes of 10 mL containing medium 523 and incubated in an incubator at 28 °C for 24 hours. To preserve the colonies, the tubes were put in a refrigerator, adding autoclaved mineral oil.

The 120 rhizobacteria isolates were grouped according to morphological and color similarities in the colonies. The diversity seen in the colonies was reflected in the choice of 44 isolates for the first experiment, which took place in the greenhouse. For this experiment, each isolate was cultivated separately in Petri dishes in medium 523 for 24 h at 28 °C in the dark, scraping the plate using an aqueous solution of NaCl at 0.85% for the preparation of rhizobacteria suspensions. Bacterial density of the suspensions was adjusted to 0.4 Å in 540 nm with the use of the SP-22 Biospectro® spectrophotometer.

Two hundred and sixty-four herbaceous cuttings of guava 'Paluma' were planted in trays with burned rice husks. Thirty days later, the region of the callus was washed and immersed for 8 hours in the rhizobacteria suspensions (six cuttings per isolate). As a control, the calluses were immersed in distilled water. Next, the cuttings were transplanted separately into 0.5 L bags, with a substrate based on *Pinus* sp. bark and coconut fiber (3:1).

Ninety days later, the rooted cuttings were transplanted into 5 L plastic bags filled with the same substrate, with Osmocot® fertilizer added at a dose of 3 kg/m^3 of substrate. Fifteen days later, the plants were individually inoculated with 2000 *M. enterolobii* eggs in 20 mL of water applied to orifices around the plant. As a control 20 mL of distilled water was applied on the plants. The experimental design was in randomized blocks with 46 treatments and six repetitions per treatment (one plant per pot).

The population of *M. enterolobii* used in this study was obtained from an orchard in São João da Barra (21°39'21"S and 41°02'07"W) and was kept on guava plants in a greenhouse. For extraction of nematode eggs, roots were washed and put in 3 L glass vials containing 1.5 L of an aqueous solution at 6% of QBoa® commercial bleach (sodium hypochlorite concentration at 2.5%), and submitted to shaking for 4 minutes at 130 cycles per minute using the shaker TE-240 Tecnal®. The suspension was poured through layered sieves with mesh 60

and 500, and the nematode concentration obtained through counting on a Peters slide in three aliquots of 1 mL/plant.

Four months after inoculation, the following variables were measured: total number of leaves, total leaf area, fresh weight of the aerial part, and the root and chlorophyll index (using the portable chlorophyll measure, SPAD Minolta®). Reproduction of the nematode was also measured using the following variables: final nematode population (Fp) = number of eggs + second-stage juveniles (J_2), Fp/gram of root, and reproduction factor (RF) = Fp/2000. The nematodes were extracted and counted as described above. The non-transformed data were analyzed by ANOVA and Scott-Knot test at $P< 0.05$ using the statistical program SAEG (Ribeiro Júnior, 2001).

Biannual assessment of three rhizobacteria isolates, Nemat® and Nemaplus® in a commercial orchard affected by guava decline

The experiment was established in October 2008 in a commercial 'Paluma' guava orchard, with trees that were about five years old and spaced 4 × 4 meters, in São João da Barra ($21^0$39'21"S and $41^0$02'07"W). Previous samples indicated an average nematode density of 38 J_2/100 cm^3 of soil, and in the orchard there was a low incidence of guava decline, with some trees presenting root rot, chlorosis, scorching of margin, wilting and falling of leaves. Orchard management consisted of daily irrigation by spraying for two hours, organic fertilization with 60 kg of mature cow manure per plant twice a year and chemical fertilization with 300 g/plant using the 20-5-20 formulation, every three weeks during the period between pruning and the start of harvesting. Management of pests and diseases, mainly psyllids (*Triozoida* sp.) and leaf rust caused by *Puccinia psidii* Winter, was carried out with pesticides as recommended.

The tested treatments were Nemat® (product based on the fungi *Paecilomyces* sp. and *Arthrobotrys* sp.), Nemaplus® (product based on rhizobacteria) and rhizobacteria isolates selected in the greenhouse experiment (see results): 108, 117 (both belonging to genus *Pseudomonas*) and 164 (*Bacillus* sp.). In the bimonthly applications of commercial products (c.p.) dosages followed manufacturers' recommendations: Nemat® at a dosage of 0.5 g of c.p. applied in a volume of 2 L/plant, and Nemaplus® at a dosage of 50 ml of c.p. applied in a

volume of 2 L/plant. Rhizobacteria 108, 117 and 164 were cultivated separately on Petri dishes in medium 523, scraped with a saline solution and calibrated in a suspension as described previously, applying 2 L of bacterial suspension bimontly. All treatments were sprayed uniformly under the tree canopy with a Jacto® backpack sprayer. Tap water was used as a control, at the same volume/plant. A random block design was used, with six treatments and six repetitions (trees)/treatment. To avoid interference due to horizontal movement of the products or rhizobacteria in the soil, two barrier plants were placed between each test plant and a barrier row was used between blocks.

Population density of *M. enterolobii* was evaluated just before every application of products and rhizobacteria (seven sampling dates in total). In every sampling date, the 36 guava trees were sampled individually, collecting soil and roots from the two sub-samples on opposite sides of the plant, under the canopy, at 0-20 cm depth, with a ≈ 500 cm^3 soil capacity auger. The 36 compound samples were individually homogenized and aliquots of 100 cm^3 of soil were processed for extraction of J_2 in accordance with Jenkins (1964). The density of J_2/100 cm^3 of soil was calculated from three counts of 1 ml/plant. For each compound sample, the roots were separated and weighed, obtaining the variable root mass/sampling. After weighing, the roots were examined under a magnifying glass to count the number of galls, whose density was expressed as number of galls/sampling and number of galls/g of root. The epidemiological relevance of all these variables for guava decline has been assessed by Gomes *et al*. (2010).

Yield was obtained by weighing the fruits of each plant individually per replication of each treatment and expressed in kg of fruit/plant. Data were analyzed by ANOVA and Tukey test at $P < 0.05$ using the statistical program SAEG (Ribeiro Jínior, 2001).

3. RESULTS AND DISCUSSION

Selection of rhizobacteria isolates in the greenhouse

There was no difference ($P < 0.05$) between the 46 treatments in terms of the variables total number of leaves, total leaf area, fresh weight of the aerial part and the root and chlorophyll index (data not shown). Based on the experimental conditions tested, it was concluded that *M. enterolobii* did not reduce the development of guava plants and the rhizobacteria isolates did not promote the development of plants that were parasitized by the nematode. When conducting two six-month-microplot experiments, Gomes *et al*. (2011) also did not observe damage caused by *M. enterolobii* alone, but only when associated with *F. solani*. These results suggest that *M. enterolobii* may be a mild pathogen to guava when it is on its own.

Many rhizobacteria isolates reduced ($P < 0.05$) the variables Fp, Fp/gram of root and RF in relation to the control inoculated with *M. enterolobii* alone (Table 1). Nonetheless, this reduction was considered not satisfactory because damage to guava trees and high yield loss have been associated with fairly low nematode population densities (Gomes *et al*., 2010). In addition to reducing Fp and RF, the isolates 108, 117 and 164 were associated with greater plant development, although not significantly ($P > 0.05$) (data not shown). Therefore, these isolates were chosen for the field experiment in the expectation that repeated inundative applications would have a more antagonist effect on *M. enterolobii.*

Table 1. Absolute and relative final nematode population (Fp) and reproduction factor (RF) of *Meloidogyne enterolobii* (*M.e.*), four months after inoculation of guava seedlings that had been inoculated with one of 44 isolates of rhizobacteria in greenhouse.

Treatments	Fp (× 1000)[1]	Fp/gram of root (× 100)	RF[2]
Control plants (non-inoculated)	0 b	0 b	0 b
Inoculation with *M.e.*	237 a	20 a	118 a
Inoculation with *M.e.* + isolate 07.v	211 b	23 a	105 b
M.e. + 22	90 b	14 b	45 b
M.e. + 24	236 a	22 a	118 a
M.e. + 25	127 b	14 b	63 b
M.e. + 28	270 a	21 a	135 a
M.e. + 35	305 a	29 a	152 a
M.e. + 41.v	113 b	10 b	56 b
M.e. + 48	207 b	19 a	103 b
M.e. + 49	148 b	13 b	88 b
M.e. + 63	176 b	18 b	88 b
M.e. + 65	146 b	13 b	73 b
M.e. + 66	202 b	18 b	101 b
M.e. + 74	196 b	14 b	98 b
M.e. + 80	195 b	20 a	97 b
M.e. + 85	310 a	26 a	155 a
M.e. + 86	465 a	47 a	232 a
M.e. + 92	236 a	19 a	118 a
M.e. + 97	168 b	16 b	84 b
M.e. + 100	176 b	15 b	88 b
M.e. + 106	309 a	27 a	154 a
M.e. + 107	127 b	11 b	63 b
M.e. + 108	103 b	8 b	51 b
M.e. + 109	257 a	23 a	128 a
M.e. + 110	145 b	14 b	72 b
M.e. + 112	363 a	28 a	181 a
M.e. + 113	172 b	21 a	86 b
M.e. + 116	163 b	12 b	76 b
M.e. + 117	107 b	10 b	53 b
M.e. + 120	196 b	21 a	98 b
M.e. + 121	170 b	20 a	85 b
M.e. + 122	255 a	27 a	127 a
M.e. + 123	151 b	12 b	75 b
M.e. + 124	123 b	9 b	61 b
M.e. + 128	159 b	16 b	79 b
M.e. + 133	183 b	27 a	91 b
M.e. + 137	199 b	20 a	99 b
M.e. + 142	97 b	9 b	48 b
M.e. + 143	340 a	39 a	170 a
M.e. + 146	191 b	17 b	95 b
M.e. + 149	282 a	28 a	141 a
M.e. + 151	221 b	16 b	110 b

M.e. + 159	154 b	16 b	77 b
M.e. + 164	96 b	93 b	48 b
M.e. + 165	155 b	31 a	77 b
CV%	64.6	76.3	64.4

[1] Fp= total eggs + J_2 extracted from the root system
[2] RF= Fp/inoculum of 2000 eggs
Values are average of six plants per treatment. Values followed by the same letter in the column are not different according to Scott-Knot test at $P < 0.05$.

Studies aiming to control plant-parasitic nematodes by using rhizobacteria show that only a small proportion of the tested isolates have an antagonistic effect on these nematodes (Freitas *et al*., 2005; Medeiros *et al*., 2009). Therefore, increasing the number of rhizobacteria isolates assessed could conceivably reveal more promising isolates against *M. enterolobii.* Also, repeated inoculations of the rhizobacteria during the tests could increase their effectiveness against the nematode and or *F. solani*.

Biannual assessment of three rhizobacteria isolates, Nemat® and Nemaplus® in a commercial orchard affected by guava decline

Seven bimonthly applications of Nemat®, Nemaplus® or the rhizobacteria isolates 108, 117 or 164 were incapable of reducing ($P < 0.05$) the density of J_2 of *M. enterolobii* in the soil (Table 2), nor did they affect the density of root galls. Guava decline is characterized by progressive rotting of the root system, among other symptoms. Therefore, it is believed that an effective control would benefit expansion of plant root system. This effect was not seen because there was no increase in root mass obtained in the samplings. Consequently, none of the treatments increased the productivity per plant in the first harvest. These not satisfactory results, along with the death of five experimental plants, forced the experiment to be stopped after the first harvest.

Table 2. Variables related to parasitism by *Meloidogyne enterolobii*, severity of guava decline, and fruit yield (one harvest) in a commercial guava orchard in which the trees were treated bimonthly with commercial products or rhizobacteria, in São João da Barra, Brazil.

Treatments	Number of J_2/ 100 cm^3 of soil	Number of root galls/sampling	Number of root galls/g of root	Root mass/ sampling	Yield (kg of fruit/plant)
Untreated control	46.4[ns]	204.5 [ns]	11.8 [ns]	15.2 [ns]	123.4 [ns]
Nemat®	68.0	133.2	9.5	13.5	112.3
Nemaplus®	66.6	172.4	11.1	13.2	171.6
Rhizobacteria isolate 108	44.9	232.1	11.9	13.4	178.9
Rhizobacteria isolate 117	28.9	104.1	6.4	14.0	170.9
Rhizobacteria isolate 164	80.8	147.1	10.6	14.4	247.4
CV (%)	183.5	218.8	139.9	60.0	64.3

Values are average of six trees per treatment in seven evaluations, except for the productivity which was evaluated in one harvest
[ns] Not different according to Tukey`s test at $P < 0.05$.

Rhizobacteria applications seemed unable to significantly antagonize the parasitism of *M. enterolobii* on guava plants, and even less to reduce the extensive root decay, or alleviate the physiological stress, suffered by trees affected by guava decline. Actually, the aggressive nature of this disease, which may kill a tree within a few months upon the start of the decline, may preclude the effectiveness of any biological control approach. Indeed, other biological approaches have been tested with no definite success (Carneiro *et al*., 2004; Molina *et al*., 2007). Management strategies based on proper fertilization of the trees and application of organic soil amendments have been devised (Gomes *et al*., 2010), while a few research groups in Brazil and elsewhere are working to develop guava decline-resistant cultivars or rootstocks.

4. CONCLUSION

In greenhouse, none of the rhizobacteria isolates promoted the growth of *M. enterolobii*-parasitized guava plants. Although several rhizobacteria isolates reduced significantly the nematode population, the values obtained for Fp and RF were nonetheless considerably high. In the field, neither the three rhizobacteria isolates nor the products Nemat® and Nemaplus® controlled the nematode or increased guava yield.

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3.2- Greenhouse and field assessment of different organic compounds against guava-parasitic *Meloidogyne enterolobii*[3][4]

Avaliação em casa de vegetação e em campo de diferentes compostos orgânicos contra *Meloidogyne enterolobii*

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ABSTRACT

Guava decline is a complex disease involving *Meloidogyne enterolobii* and *Fusarium solani* and it has caused major direct losses to Brazilian growers. Although several strategies have been sought to control the nematode, the use of organic soil amendments is currently the best approach to manage this disease. To assess the best amount of meat and bone meal (MBM) to be incorporated into the soil, guava seedlings inoculated with *M. enterolobii* were treated with 1-5% v/v of the MBM. Ninety days later variables related to nematode reproduction and plant development were evaluated, which indicated a potential nematicidal effect of the MBM at 3%. Another experiment assessed nematode- and plant-related variables 90 days after treatment of the seedlings with MBM, chitosan, shrimp shell or neem cake at 3%, 0.05%, 2% and 0.1% v/v, respectively. The MBM ranked first, reducing nematode reproduction. This MBM rate was converted to 25 kg/tree and assessed in three application regimes (monthly, bimonthly or trimonthly), for six months, in an orchard affected by guava decline. The variables assessed were soil density of colony forming units (CFU) of bacteria and fungus, and soil and/or root density of *M. enterolobii, Helicotylenchus* sp., and of different nematode trophic groups. In all three application regimes the MBM reduced all plant-parasitic nematodes in the soil and the fungus CFUs. It also promoted an increase in bacterial CFU and bacterivorous nematodes.

Key words: *Psidium guajava, Fusarium solani,* neem cake, chitosan, shrimp shell, cultural control, guava decline, meat and bone meal.

RESUMO

O declínio da goiabeira, uma doença complexa envolvendo *Meloidogyne enterolobii* e *Fusarium solani*, tem causado grandes prejuízos diretos para os produtores brasileiros. Apesar de várias estratégias terem sido discutidas para o controle do nematoide, a utilização de matéria orgânica adicionada ao solo é atualmente a melhor abordagem para conviver com essa doença. Para avaliar a dose adequada de farinha de carne e ossos (FCO) a ser incorporada ao solo, mudas de goiabeira inoculadas com *M. enterolobii* foram tratadas com 1-5% v/v da FCO. Noventa dias após foram avaliadas variáveis relacionadas à reprodução do nematoide e ao desenvolvimento das plantas, indicando um possível efeito nematicida da FCO a 3%. Outro experimento avaliou variáveis relacionadas ao nematoide e à planta 90 dias após o tratamento das mudas com FCO, quitosana, casca de camarão e torta de nem a 3%, 0,05%, 2% e 0,1% v/v, respectivamente. A FCO reduziu a reprodução do nematoide, destacando-se em relação aos demais tratamentos. Esta dosagem de FCO foi convertida para 25 kg planta^{-1} e avaliada em três regimes de aplicação (mensal, bimestral ou trimestral), por seis meses, em pomar de goiaba acometido pelo declínio. As variáveis avaliadas foram densidade no solo de unidades formadoras de colônia (UFC) de bactérias e fungos, e a densidade no solo e/ou raiz de *M. enterolobii, Helicotylenchus* sp., e de diferentes grupos tróficos de nematoides. Em todos os três regimes de aplicação a FCO reduziu todos os nematoides parasitas de plantas no solo e o número de UFC de fungos, e promoveu aumento no número de UFC de bactérias e nematoides bacteriófagos.

Palavras-chave: *Psidium guajava, Fusarium solani,* torta de nim, quitosana, casca de camarão, controle cultural, declínio da goiabeira, farinha de carne e ossos.

1. INTRODUCTION

The guava (*Psidium guajava* L.) (Myrtaceae) is a robust fruit-bearing tree that originated in the American tropics; its current distribution covers all the tropical and subtropical regions of the world (GONZAGA NETO and SOARES, 1994). In Brazil, guava cultivation is typically practiced by smallholders, with annual turnover of about US$ 38 million, involving many agro-industries and productive chains for machinery and pesticides (IBGE, 2009).

Guava decline is a complex disease caused by the synergistic association between *Meloidogyne enterolobii* Yang and Eisenback, 1983 and *Fusarium solani* (Mart.) Sacc. (GOMES *et al*., 2011). In this disease, *F. solani*-immune guava trees become susceptible to extensive necrosis of the root system caused by this fungus upon parasitism by *M. enterolobii*. Assays conducted with *F. solani* isolates from different Brazilian regions confirmed that guava decline is responsible for exterminating about 5000 hectares of orchards throughout Brazil, with direct losses estimated to stand at more than US$ 70 million (PEREIRA *et al*., 2009).

As an agent that predisposes the plant to guava decline, *M. enterolobii* has been the target of various control strategies, as yet without success. These include biological control with fungi, bacteria and entomopathogenic nematodes, leaving fallow and using nematicides (CASASSA *et al*., 1996; GUEYE *et al*., 1997; DUPONNOIS *et al*., 1998; MOREIRA *et al*., 2001; BRITO *et al*., 2004; ROCHA *et al*., 2004; SOUZA *et al*., 2006; CARNEIRO *et al*., 2007; CHARCHAR *et al*., 2007; LOPES *et al*., 2009; OKA, 2010; ALMEIDA *et al*., 2011). BURLA *et al*. (2010) and MIRANDA (2012), among others, have identified genotypes or accessions of *Psidium* spp. that are resistant to *M. enterolobii*. Nonetheless, guava producers are unlikely to have commercially available resistant cultivars or rootstocks for some years to come.

GOMES *et al*. (2010) managed a commercial guava plantation affected by guava decline using applications of organic soil amendments, obtaining major

yield gains in comparison to untreated plants. The use of cow manure and poultry compost provided better results than sugarcane filter cake; this agrees with previous reports on other plant-parasitic nematodes, which indicate that the nature of the organic matter determines its nematicidal efficiency, along with biota and chemical properties of the soil (AKHTAR and ALAM, 1993; AKHTAR and MALIK, 2000; FERRAZ *et al*., 2010). Several organic soil amendments have been used with success to manage plant-parasitic nematodes, such as neem (A*zadirachta indica* A. Juss) cake (SILVA and PEREIRA, 2008), chitin-rich products (GALPER *et al*., 1990), meat and bone meal (MBM) and several kinds of waste products (AKHTAR and ALAM, 1993). However, the recommendation for use of any of these amendments depends on the pathosystem: whether it is an annual or perennial crop, the nematode genus and/or species involved, and whether the nematode is associated (or not) with another soil pathogen. The availability of the organic matter source, its cost for purchase and application at the recommended dosage and crop profitability are also aspects that need consideration.

Hence, the present study reports efforts to assess in greenhouse the effect of neem cake, shrimp shell, chitosan and MBM applied as soil amendment on *M. enterolobii* and on the vegetative development of guava seedlings. Since there are no reports on the amount of ammonium released through microbial degradation of the particular MBM used in this study, nor its effect on *M. enterolobii*, a preliminary dose-response assessment was conducted for this product. Promising results were obtained in greenhouse for MBM (see results), so this product was further tested in a commercial guava plantation affected by guava decline to assess its effect on root and/or soil density of bacterivore, mycophagus, predatory and plant-parasitic nematodes (including the abundant *Helicotylenchus*sp. and *M. enterolobii*), and on the soil density of colony forming units (CFUs) of bacteria and fungi. The influence of MBM on soil chemistry and plant nutrition was also investigated.

2. MATERIAL AND METHODS

Greenhouse assessment of different organic soil amendments

Seedling production and inoculation with *M. enterolobii*

Guava seedlings of cultivar Paluma were produced from true seeds in plastic bags filled with Plantmax® substrate for plant growth. At the stage of four leaves, they were transplanted to 2 L plastic pots filled with washed riverbed sand homogenized with 2000 eggs and second-stage juveniles (J_2) of *M. enterolobii*. The seedlings were maintained in greenhouse with mean high and mean low temperatures of 36.6 °C and 21 °C, respectively, and they were watered and fertilized as necessary.

The nematode inoculum used was obtained from guava roots that were washed in tap water and put in 3 L glass vials containing 1.5 L of an aqueous solution at 6% of QBoa® commercial bleach (sodium hypochlorite concentration at 2.5%). The vials were shaken for 4 minutes at 130 cycles per minute using the TE-240 Tecnal®shaker. The suspension was poured through layered sieves with 60 and 500 mesh, and the nematode concentration was obtained counting on a Peters' slide in three aliquots of 1 mL/plant.

Assessment of dosage for MBM use

Thirty days after nematode inoculation, the MBM (produced by Respa Ltda, Campos dos Goytacazes, Brazil), was incorporated at 0-3 cm depth in the sand, at 1, 2, 3, 4 or 5% v/v relative to the volume of 2 L of sand. Plants inoculated with the nematode that received no MBM served as control. These treatments were arranged in an entirely randomized pattern, with six replicates (one plant/pot) per treatment. The average composition of the MBM is dry matter (94%), crude protein (42%), crude fat (12%), mineral content (38%), calcium (14%), phosphorus (6%), chlorine (0.5%) and sodium (0.7%).

Ninety days after MBM application plant height was measured, and the root systems were individually washed in tap water. The root system volume (cm^3) was measured through water displacement in a laboratory graduated cylinder. The plant shoot and roots were fresh weighed. For nematode extraction and counting,

the roots were processed as described before. The following nematode variables were assessed: final nematode population (Fp) = (number of eggs + J_2)/root system, Fp/g of root, and reproduction factor (RF) = Fp/2000. All data were submitted to ANOVA and to regression analysis through SAEG® software (RIBEIRO JÚNIOR, 2001).

Assessment of different organic soil amendments

Guava 'Paluma' seedlings were produced and inoculated as described before. Thirty days after nematode inoculation the following amendments were incorporated at 0-3 cm depth in the sand: chitosan at 0.05% v/v, or shrimp shell at 2% v/v, or neem cake at 0.1% v/v, or MBM at 3% v/v (as recommended by the first experiment - see results). Inoculated, untreated plants served as control. The five treatments were arranged in an entirely randomized pattern, with six replicates (one plant/pot) per treatment. Ninety days after amendment application the same variables described before were analyzed by ANOVA and compared through Tukey's test at 5% of probability.

Effect of MBM on soil bacteria, fungi, and nematofauna in a commercial orchard

The guava 'Paluma' orchard was five years old, planted with 7x7 meter spacing, and located in the municipality of São João da Barra (lat. 21°41'22"S; long. 41°3'20"W). The orchard was irrigated by sprinklers as needed, and the management of pests and diseases [mainly psilids (*Triozoida* sp.) and rust (caused by *Puccinia psiddi* Winter)] was carried out with pesticides at the recommended rates. Organic fertilization was conducted with 60 kg of mature bovine manure per tree twice a year, and chemical fertilization with 300 g per tree of 20-5-20 formulation every three weeks in the period between plant trimming and the beginning of harvest. The orchard was infested by *M. enterolobii* (mean density at 60 J_2 100 cm^{-3} of soil) and some trees showed the typical symptoms of guava decline: chlorosis, scorching of edges, leaf wilt and fall, abundant root galling and root rot. As regards plant-parasitic nematodes, the orchard soil

harbored also *Criconema* sp., *Mesocriconema* sp., *Pratylenchus* sp., *Hemicycliophora* sp., and abundant *Helicotylenchus* sp..

In greenhouse, the best nematode control was obtained with MBM at 3% v/v. For the field assessment, this rate was converted to 1.2 kg of MBM per square meter under the tree canopy (equivalent in this orchard to 25 kg per tree). For application, plant debris was removed from soil surface and the MBM was evenly spread by hand under the canopy. The product was superficially incorporated with a rake and the plant debris was returned under the canopy. Through-irrigation was then applied.

The dosage of 25 kg of MBM per tree was assessed in three application regimes: monthly, bimonthly and trimonthly. For the monthly treatment, the MBM was applied in July/2009 through January/2010. For the bimonthly treatment, the applications were done in July, September and November/2009, and January/2010. For the trimonthly treatment, the applications were in July and October/2009, and January/2010. Untreated plants served as control. These four treatments were arranged in randomized blocks, with six replicates (trees) per treatment. One planting line was kept as a buffer between blocks, and within planting lines two buffer trees were kept between the trees assigned for data collection.

Soil and/or root nematode density was assessed in July, August and October/2009, and January/2010 for all treatments, just prior to MBM applications. Soil and root samples were collected under the trees' canopy, at 0-15 cm depth, with a 15 cm high, 7 cm diameter auger (~ 500 cm^3). Two subsamples were collected on opposite sides of each of the 24 experimental trees, and taken to the laboratory. For each composite sample, the roots were separated and weighed, and the root mass was expressed as g per sampling. The composite samples were individually homogenized and an aliquot of 100 cm^3 of soil was processed for nematode extraction according to JENKINS (1964). The nematode density was calculated from three counts of 1 mL aliquots of the nematode suspension obtained. The densities evaluated per 100 cm^3 of soil were J_2 of *M. enterolobii*, specimens of *Helicotylenchus* sp., and total specimens of the following trophic groups: plant-parasitic, mycophagus, bacterivorous and predatory. In the roots, the

variables evaluated were *M. enterolobii-induced* root galls per g of roots and per sampling. The data were submitted to ANOVA and the treatments were compared through Tukey's test at 5% confidence.

Soil density of CFUs of bacteria and fungi were assessed in October/2009 for all treatments. Soil samples were collected as described before, from which aliquots of 10 g were suspended in 100 mL of sterile 0.85% saline solution. Before sedimentation of the soil particles, an aliquot of 1 mL was pipetted out of the suspension and serially diluted to 10^{-4} through 10^{-6} for fungus isolation, and to 10^{-5} through 10^{-7} for bacterial isolation. For isolation of *Fusarium* sp., the diluted suspensions were incubated in Petri dishes with the *Fusarium sp.-selective*medium proposed by MARTIN (1950), supplemented with 60 mg mL^{-1} of streptomycin sulphate and 70 mg mL^{-1} of stain Rose Bengal. For bacteria isolation, the nutrient agar medium was supplemented with 10 µg mL^{-1} of cyclohexamide. The Petri dishes were turned upside down for incubation at 28 °C in a 12/12 photoperiod, during 3-7 days. The resulting CFUs were counted under magnifying lens.

In July, November and December/2009 foliar and soil samples were collected for assessment of plant nutrition (macro- and micronutrients) and soil chemistry (pH, organic matter content, macro- and micronutrients). The results were compared to recommendations for guava cultivation (SALVADOR *et al*., 2000).

3. RESULTS AND DISCUSSION

In greenhouse, the variables related to *M. enterolobii* and plant development decreased ($p<0.05$) with the increase of concentration of MBM (1-4% v/v) (Figure 1). A phytotoxic effect may have occurred at 4%, since most plants presented the least root (Figure 2) and shoot development, and all plants at 5%

died two weeks after MBM application. Therefore, this experiment suggested a potential benefit of MBM at 3% v/v, although it did not clearly demonstrate a nematicidal effect of this product, since the decrease in nematode-related variables may have been secondary to lesser plant development or to toxicity.

FIGURE LEGENDS

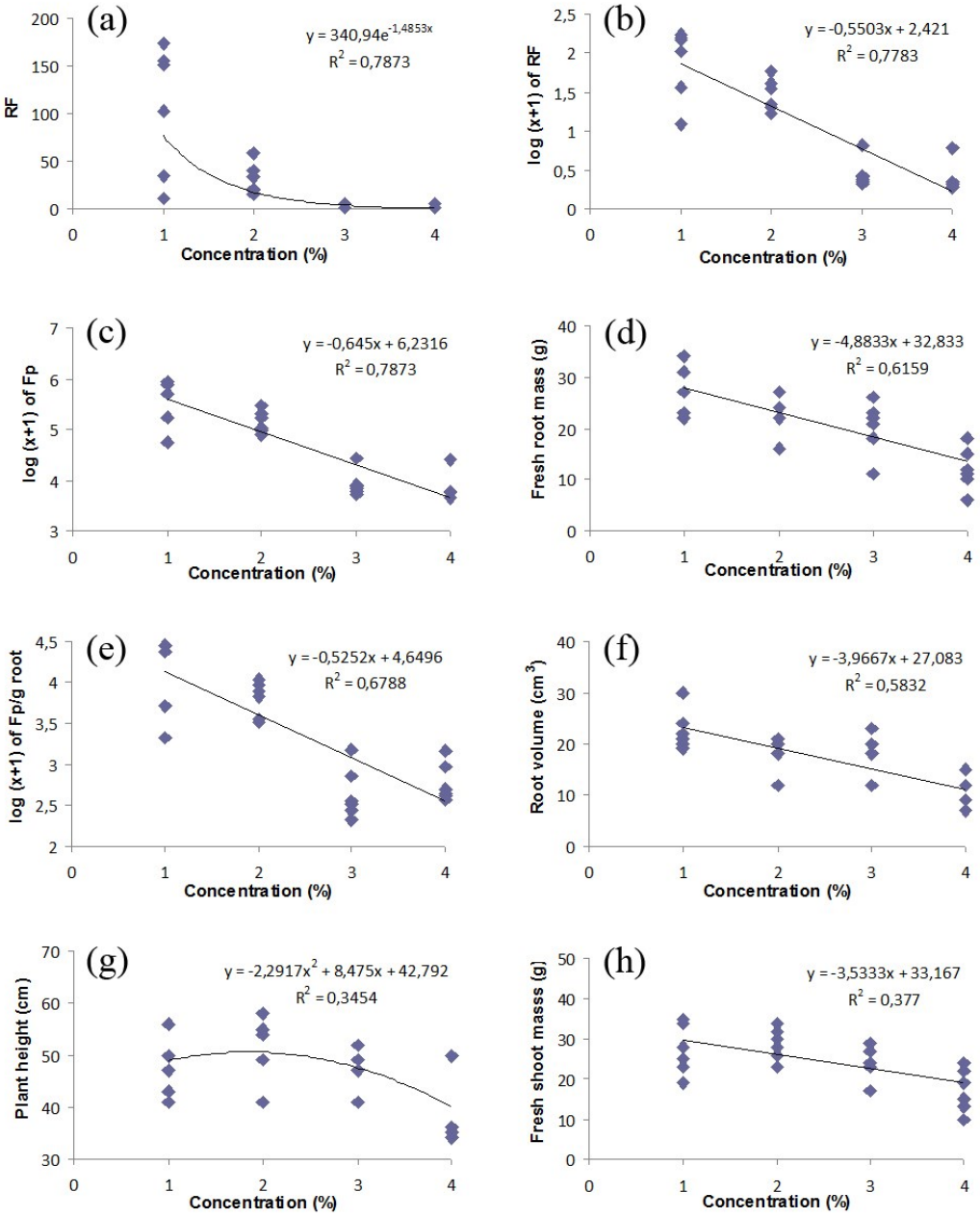

Fig. 1 – Regression analysis of variables related to *Meloidogyne enterolobii* and growth of guava plants inoculated with 2000 eggs and second-stage juveniles of the nematode in greenhouse and 30 days later treated with different rates (% v/v, relative to volume of the substrate soil) of meat and bone meal (MBM). The MBM was incorporated superficially

into the soil, and the evaluations occurred 90 days later. Fp = final nematode population; Reproduction factor (RF) = Fp/2000.

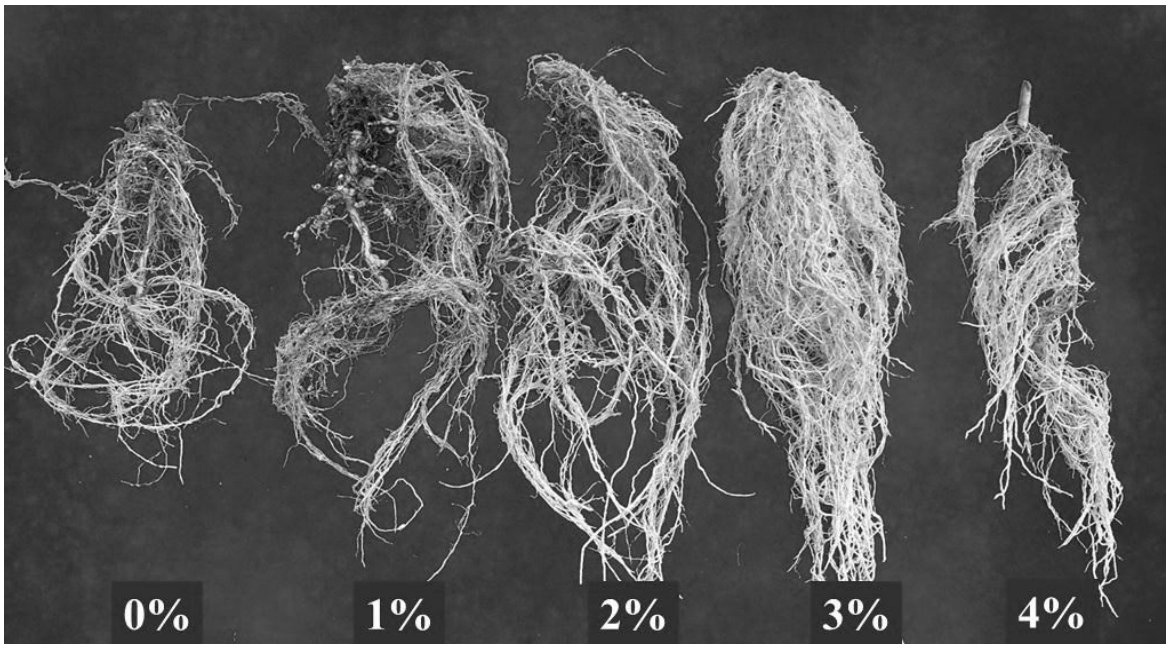

Fig. 2 – Root system of guava plants inoculated with 2000 eggs and second-stage juveniles of *Meloidogyne enterolobii* in greenhouse and 30 days later treated with different rates (% v/v, relative to volume of the substrate soil) of meat and bone meal (MBM). The MBM was incorporated superficially into the soil, and the evaluations occurred 90 days later. From left to right: untreated control, 1, 2, 3, and 4%.

Upon comparison with other soil amendments in a second greenhouse experiment, MBM at 3% v/v was better in reducing ($p<0.05$) all variables related to nematode reproduction (Table 1), and it promoted gains ($p<0.05$) in plant development, particularly for the roots (Figure 3). This nematicidal effect could be related to release of nitrogen-rich compounds in the soil, such as urea and ammonium nitrate, following the microbial degradation of the MBM (RODRIGUEZ-KABANA, 1986). According to ENO *et al*. (1955), ammonium nitrate causes nematode cell plasmolysis when at a concentration above 300 mg kg^{-1} of soil. Neem cake was as good at promoting plant development, but it presented no nematicidal effect, since Fp and RF actually increased in comparison with the control check. Therefore, although the neem cake was used at the dosage recommended by the manufacturer, the results do not line up with several reports on its nematicidal effect (FERRAZ *et al*., 2010). Despite reports on the nematicidal

properties of chitosan and shrimp shell, in this experiment they faired the worst in reducing nematode reproduction.

Table 1. Variables related to *Meloidogyne enterolobii* and growth of guava plants inoculated with 2000 eggs and second-stage juveniles (J_2) of the nematode, and 30 days later treated with different organic soil amendments, at different rates (% v/v) relative to volume of substrate. The amendments were incorporated superficially to the soil, and the evaluations were performed 90 days later.

Treatments	Fp[1] (× 1000)	RF[2]	Fresh root system mass (g)	Fp.gram roots^{-1} (x1000)	Fresh root system volume (cm^3)	Fresh shoot mass (g)	Plant height (cm)
Untreated control	63.4 b	31.7 b	7.1 c	10.20 bc	5.3 b	5.2c	25.8 c
Chitosan (0.05%)	255.7 a	127.8 a	8.8 c	29.60 a	7.3 b	6.7c	26.6 c
Shrimp shell (2%)	65.6 b	32.8 b	18.1 b	3.70 cd	17.5 a	14.6 b	39.5 b
Neem cake (0.1%)	305.4 a	152.7 a	27.1 a	11.80 b	20.6 a	24.0 a	43.6 ab
Meat and bone meal (3%)	10.3 c	5.1 c	20.1 b	0.65 d	17.5 a	24.0 a	47.1 a
CV (%)	2.8	7.7	22.3	38.7	25.1	20.4	9.5

[1] Fp (final nematode population) = eggs + J_2.root system^{-1}.
[2] RF (reproduction factor) = Fp.2000^{-1}.
Values are average of six replicates (one plant/pot) per treatment.
Values followed by different letters in the column are different according to Tukey test at $p< 0.05$.

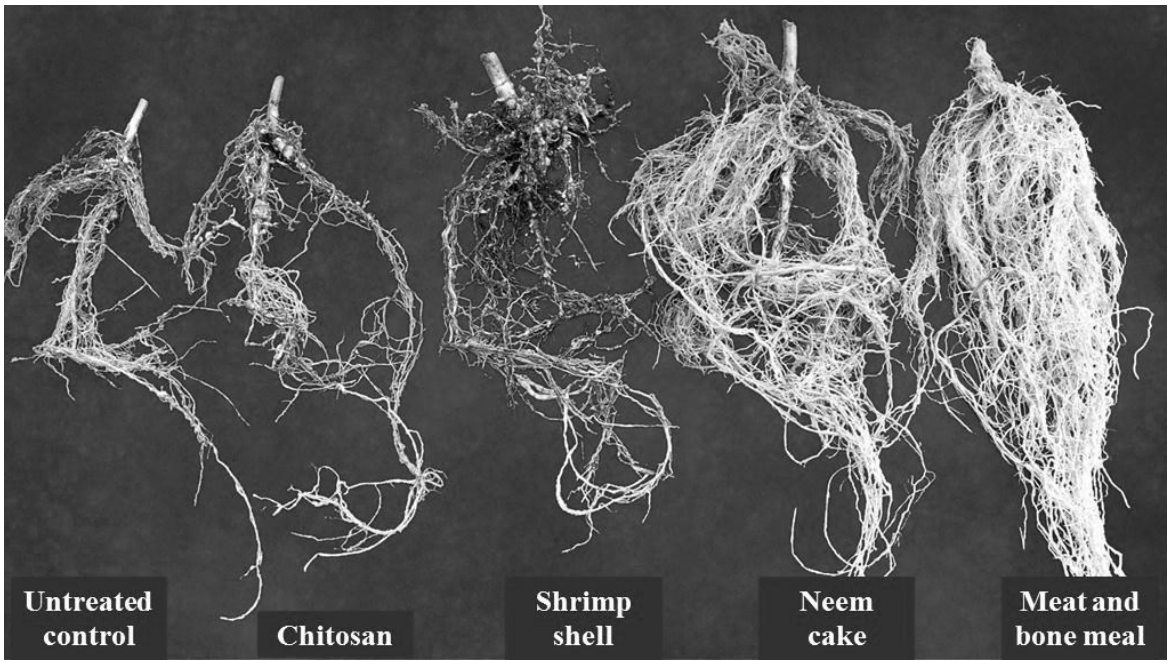

Fig. 3 – Root system of guava plants inoculated with 2000 eggs and second-stage juveniles of *Meloidogyne enterolobii* in greenhouse and 30 days later treated with different organic soil amendments at different rates (% v/v) relative to volume of the substrate soil. The amendments were incorporated superficially into the soil, and the evaluations occurred 90 days later. From left to right: untreated control, chitosan (0.05%), shrimp shell (2%), neem cake (0.1%) and meat and bone meal (3%).

Guava decline can be seen as a complex disease involving two pathogens with distinct interactions with plant root. As a biotrophic, obligatory parasite, *M. enterolobii* is favored by an abundant root system, while the invasion of *F. solani* results in root rot, plant and yield decline. Different variables related to nematode reproduction, abundance of roots, root rot and productivity have been assessed for this disease (BURLA *et al*., 2010; GOMES *et al*., 2010; 2011; MIRANDA *et al*., 2011). In this work, as previously observed by BURLA *et al*. (2010), the variable Fp/g of root was less informative than Fp and RF.

In the six-month field experiment, MBM promoted a reduction ($p<0.05$) in the soil density of *M. enterolobii* J_2 in all three regime applications tested (Table 2). This reduction resulted in no decrease in root galling density, possibly because a substantial J_2 population remained in the soil to infect the guava roots. Also, a reduction ($p<0.05$) occurred in the density of plant-parasitic nematodes, and of *Helicotylenchus* sp. in

particular (Table 3). In addition to releasing urea and ammonium nitrate, the degradation of MBM could also increase the soil biota antagonistic to plant-parasitic nematodes. Indeed, MBM promoted an increase in the soil density of bacteria and bacterivorous nematodes, a phenomenon often associated with biological control of soil-dwelling plant-parasitic nematodes through amendment with organic matter (MCSORLEY and FREDERICK, 1999; OKA, 2010).

Table 2. Variables related to *Meloidogyne enterolobii* in a commercial guava orchard affected by guava decline and treated for six months with 25 kg tree^{-1} of meat and bone meal as a soil amendment under different application regimes, in São João da Barra, Brazil

Treatments	Density of J_2 Per 100 cm^3 of soil	Density of root galls per sampling core[a]	Fresh root mass per sampling core	Density of root galls per g of root
Untreated control	81.94 a	204.5 ^{ns}	111.76 ^{ns}	11.45 ^{ns}
Monthly applications	35.83 b	133.2	98.93	13.05
Bimonthly applications	36.44 b	172.4	108.79	13.32
Trimonthly applications	30.94 b	232.1	104.60	15.89
CV (%)	63.45	24.26	34.66	139.9

[a] Sampling performed with an auger of about 500 cm^3 capacity.
Values are average of six trees per treatment in four evaluations (July, August, October/2009, and January/2010) for each treatment.
In the columns, "ns" indicates that values are not different according to Tukey's test ($p< 0.05$). Values followed by different letters in the column are different according to Tukey test at $p< 0.05$.

Table 3. Soil density of different groups of nematodes (*per* 100 cm^3) and of bacteria and fungi (*per* cm^3) in a commercial guava orchard affected by guava decline and treated for six months with 25 kg $tree^{-1}$ of meat and bone meal as a soil amendment under different application regimes, in São João da Barra, Brazil

Treatments	Fauna of plant-parasitic nematodes[1]	*Helicotylenchus* sp.	Bacterivorous nematodes	CFU of bacteria	Mycophagous nematodes	CFU of fungus	Predatory nematodes
Untreated control	483.3 a	206.7 a	187.8 b	$1.25x10^{10}$c	38.9 ^{ns}	$2.45x10^{8}$a	11.1 ^{ns}
Monthly applications	44.4 b	11.1 b	1290.0 a	$1.24x10^{10}$c	12.2	$4.96x10^{6}$b	0.0
Bimonthly applications	72.2 b	27.8 b	1381.1 a	$2.48x10^{10}$b	12.3	$7.36x10^{6}$b	4.4
Trimonthly applications	67.8 b	33.3 b	676.7 ab	$3.16x10^{10}$a	20.0	$6.11x10^{6}$b	7.8
CV (%)	118.1	146.0	54.2	10.1	109.8	33.8	155.1

[1] Fauna composed of *Criconema* sp., *Mesocriconema* sp., *Pratylenchus* sp., *Hemicycliophora* sp., *Helicotylenchus* sp. and *Meloidogyne enterolobii.*

Values are average of six trees per treatment in four evaluations (July, August, October/2009, and January/2010) for each treatment.

In the columns, "ns" indicates that values are not different according to Tukey's test ($p< 0.05$). Values followed by different letters in the column are different according to Tukey test at $p< 0.05$.

An aspect that prompted an on-going investigation was the reduction ($p<0.05$) in the soil density of fungi in all application regimes of MBM. Although this quantification was performed using *Fusarium*-selective medium, other fungi grew in the Petri dishes. It is conceivable that this product may have an antagonistic effect on *F. solani*, a property that could offer a reduction in the severity of guava decline.

In addition to its potential role in controlling *M. enterolobii*, the use of MBM could have additional benefits for the chemistry and structure of the soil, and plant fertilization (MALAVOLTA *et al*., 2000). In this field experiment, the three analyses conducted on soil chemistry and plant nutrition revealed values (data not shown) that were within the ranges recommended for guava cultivation.

Unfortunately, the effect of the MBM on productivity could not be assessed because the orchard's last pruning was not uniform across the experimental plot, which would invariably affect the treatment's productivity. A two-year long experiment has been set up in three different commercial orchards to further evaluate the effect of different rates and regime applications of the MBM on the soil density of *M. enterolobii* and *F. solani*, the incidence and severity of guava decline, and productivity. These orchards have been selected to accommodate different technological levels of guava production and different levels of severity of guava decline.

4. CONCLUSION

In greenhouse, the MBM at 3% v/v showed potential as a organic soil amendment against *M. enterolobii*, ranking first among other amendments tested. In

the field, MBM applied monthly, bimonthly or trimonthly at 25 kg/tree reduced the soil density of fungus CFUs, *M. enterolobii*, *Helicotylenchus* sp. and total plant-parasitic nematodes, while it increased bacteria CFUs and bacterivorous nematodes.

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3.3- Field assessment of meat and bone meal for management of guava orchards affected by guava decline[56]

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ABSTRACT

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Guava decline is a disease complex involving *Meloidogyne enterolobii* and *Fusarium solani*, which causes root rot, defoliation and death of guava trees within months from onset of symptoms. Since no resistant cultivar or rootstock is available nor are there nematicides registered for this crop, management strategies for this disease are needed. Meat and bone meal (MBM) was applied to the soil of a commercial guava orchard affected by guava decline, in three dosage/regime applications: i) quarterly applications of 12.5 Kg/tree, ii) quarterly applications of 25 Kg/tree, and iii) semiannual applications of 50 Kg/tree. During this 24 month-experiment, the following variables were assessed: *M. enterolobii* and other soil plant-parasitic and free-living nematodes presence and density, *Fusarium* sp. density in soil and guava roots, release of ammonia in the soil upon MBM decomposition, and productivity in two harvests. The quarterly application of 25 Kg/tree was further assessed in three different orchards, which were clearly distinct in age and in the levels of disease severity and agronomic care. The low levels of ammonia observed in the soil upon MBM decomposition in all three dosage/regime applications likely explain the modest reduction of second-stage juveniles of *M. enterolobii* in soil and other minor plant-parasitic nematodes. The parasitism by *M. enterolobii*, expressed as density of root galls, and density of *Fusarium* sp. in soil and roots were unaffected by MBM applications, which led to progression of guava decline and reduced the orchard productivity. This work shows how difficult is the management of plant-parasitic nematodes when organic amendments are applied in soil, particularly in the control of disease complexes as guava decline.

Keywords: disease complex, *Fusarium solani*, *Meloidogyne enterolobii*, organic soil amendment, *Psidium guajava*

RESUMO

Almeida, A. M., R. M. Souza, V. M. Gomes, T. F. Ferreira, and V. Mussi-Dias. 2013. Avaliação a campo de farinha de carne e osso para o manejo de pomares de goiaba afetados pelo declínio da goiabeira, Nematropica 43.

O declínio da goiabeira é uma doença complexa que envolve o *Meloidogyne enterolobii* e *Fusarium solani*, causando podridão radicular, desfolhamento e morte das goiabeiras após alguns meses a partir do início dos sintomas. Uma vez que não existe nenhum cultivar ou porta-enxerto resistente, e nem existem nematicidas registrados para esta cultura, estratégias de manejo para esta doença são necessários. Na sequência, a partir de um trabalho anterior, farinha de carne e ossos (FCO) foi aplicada ao solo de um pomar comercial de goiabeira afetada pelo declínio, em três doses/regime de aplicações: i) aplicações trimestrais de 12,5 kg/planta, ii) aplicações trimestrais de 25 kg/planta, e iii) aplicações semestrais de 50 kg/planta. Este experimento durou 24 meses e um conjunto de variáveis foram avaliadas em relação ao *M. enterolobii*, nematoides de vida livre no solo, outros fitoparasitas, densidade do *Fusarium* sp. no solo e nas raízes da goiabeira, liberação de amônia no solo após a decomposição da FCO e produtividade nas duas colheitas. A aplicação trimestral de 25 kg/planta foi ainda avaliada em três pomares diferentes, distintos em idade, em níveis de severidade da doença e em tratos culturais. Os níveis baixos de amônia observadas no solo após a decomposição FCO em todas as três dosagens/regime de aplicações podem explicar a redução modesta da densidade no solo de juvenis de segundo estádios de *M. enterolobii* e outros poucos nematoides fitoparasitas. O parasitismo por *M. enterolobii* - expresso em densidade de galhas radiculares e da densidade no solo e na raiz de *Fusarium* sp. não foram afetados pelas aplicações FCO, o que levou à progressão do declínio da goiabeira e redução da produtividade. Este trabalho demostra como é difícil o manejo de nematoides fitoparasitas através do uso de alterações orgânicas do solo, especialmente em doenças complexas tão agressiva como o declínio da goiabeira.

Palavras-chave: Doença complexa, *Fusarium solani, Meloidogyne enterolobii,* condicionador de solo orgânico, declínio da goiabeira, *Psidium guajava*

1. INTRODUCTION

Guava (*Psidium guajava* L.) (Myrtaceae) is a robust fruit-bearing tree originating from Central America that is cultivated in tropical and subtropical regions worldwide (Gonzaga Neto *et al.*, 2001). Brazil ranks first in red guava production, with an annual output of over 300,000 tons of fruit, and a total cultivated area of about 15,000 hectares (ha) (Anonymous, 2010). In Brazil, the guava market is worth around US$ 38 million/year, which sustains thousands of small growers, family-operated facilities for pulp processing, and sales of pesticides, machinery and fertilizers.

Guava decline is a disease complex caused by the synergistic interaction between *Meloidogyne enterolobii* Yang and Eisenback, 1983 and *Fusarium solani* (Mart.) Sacc. (Gomes *et al.*, 2011). In this disease complex, *F. solani*-immune guava trees suffer extensive root decay caused by this fungus upon parasitism by *M. enterolobii*. Assays performed with root samples from different regions in Brazil confirmed that guava decline is the disease responsible for the extermination of about 5,000 ha of guava orchards nationwide, causing direct damage estimated to cost more than US$ 70 million (Pereira *et al*., 2009; Gomes *et al*., 2012).

Since parasitism by *M. enterolobii* is the predisposing factor for guava decline, the search for control or management alternatives of this disease has focused on the nematode. Unsuccessful attempts involved fallowing, use of nematicides, and biological control using fungi, bacteria and entomopathogenic nematodes (Casassa *et al*., 1996; Gueye *et al*., 1997; Duponnois *et al*., 1998; Rodriguez *et al*., 2003; Carneiro *et al.*, 2004; Souza *et al*., 2007; Molina *et al.*, 2007; Almeida *et al.*, 2011). Procedures for screening of *Psidium* spp. genotypes for resistance to *M. enterolobii* have been proposed (Burla *et al*., 2010; Miranda *et al.*, 2010), and hundreds of accessions have been tested (Maranhão *et al.,* 2001; 2003; Carneiro *et al.,* 2007; Rodriguez *et al.*, 2007; Almeida *et al.*, 2009; Miranda, 2012). However, no resistant guava or rootstock cultivar is likely to be available to growers in the near future.

Several kinds of organic soil amendments have been used successfully to manage plant-parasitic nematodes, such as green covers, chitin-rich residues, municipal compost, waste sludge, and animal by-products (Stirling, 1991; Ferraz *et al.*, 2010). Nonetheless, the efficacy of these amendments must be assessed for the pathosystem that one aims to manage. Other aspects to be considered are the availability and the cost of the amendment, the dosage and the application regime, and the profitability of the crop. For instance, Gomes *et al.* (2010) achieved promising results in guava decline-affected orchards upon soil amendment with poultry compost, combined with proper soil and foliar fertilization. Nonetheless, this management strategy was not adopted by local growers due to the relative difficulty in obtaining the compost.

Meat and bone meal (MBM) is a product of the rendering industry. It has been widely used as a source of protein and minerals in diets of production animals (Hendriks *et al.*, 2002), and its high content of organic matter, nitrogen, phosphorus and calcium has incited its assessment as fertilizer (e.g., Jeng *et al.*, 2006). MBM has also been assessed as organic soil amendment to manage verticillium wilt, common scab and root lesion nematodes in potato fields, with modest results (Lazarovits *et al.*, 1999; 2001). In greenhouse, Almeida *et al.* (2012) assessed different amendments against *M. enterolobii*, obtaining promising results for MBM at 3% v/v. For a preliminary field trial, this dosage was converted to 25 Kg/tree and tested in three application regimes (monthly, bimonthly or quarterly). All three regimes significantly ($P < 0.05$) reduced soil density of *M. enterolobii* second-stage juveniles (J_2), but no effect on plant productivity could be evaluated.

This article reports the results of a two-year effort to further evaluate the efficacy of MBM to manage guava decline-affected orchards. To identify the best cost- and labor-effective scheme, different dosages and application regimes of MBM were assessed in one orchard. Furthermore, the most promising scheme (25 Kg/tree, quarterly) was assessed in three different orchards, which were distinct in age, levels of disease severity and agronomic input. A suite of variables was assessed, related to *M. enterolobii* and other soil plant-parasitic and free-living nematodes, *Fusarium* sp. density in soil and guava roots, release of ammonia in the soil upon MBM decomposition, and productivity in two harvests.

2. MATERIAL AND METHODS

Effect of different dosages and application regimes of MBM on guava decline – The experiment was established in a 10-year-old commercial guava 'Paluma' orchard, planted with 6 x 6 m spacing, in São João da Barra, State of Rio de Janeiro, Brazil (lat. 21^{0}41'22"S; long. 41^{0}3'20"W). The orchard was managed with annual prunings and irrigated with microsprinklers as needed. Fertilization was application of 60 Kg of cow manure/tree, under the canopy, twice a year, combined with hand-spread fertilization with nitrogen (N), phosphorus (P) and potassium (K) using the N-P-K formulation 20-5-20, at 300 g/tree, every three weeks. The incidence of rust, caused by *Puccinia psidii* Winter, and thrips was kept low by use of fungicides and insecticides. Previous systematic samplings indicated an average density of 60 *M. enterolobii* J_2/100 cm^3 of soil, and a moderate severity of guava decline, with some trees with chlorosis, scorched edges, wilting and falling of leaves, and root rot.

Three dosage/regime applications of MBM were tested: i) quarterly applications of 12.5 Kg/tree, ii) quarterly applications of 25 Kg/tree, and iii) semiannual applications of 50 Kg/tree. Untreated trees served as control. Concerns that ammonia released from MBM decomposition could drift laterally – despite the flat terrain - led to establishment of a second control plot in a nearby area in the same orchard. The four treatments were arranged in randomized blocks. Seventy-two trees were subdivided into six blocks of 12 trees each. Within each block, each treatment was represented by three plants, with only the middle one being assigned to data collection. Two planting rows were maintained as buffer between blocks. For MBM application, the organic debris below the canopy was removed manually with a rake. The MBM was hand-spread uniformly under the canopy, and superficially incorporated into the soil (0-5 cm) with the rake. The plant debris was returned and the trees were irrigated.

Routine chemical analysis of MBM indicated an average composition of 93.7% of dry matter, 41.5% of crude protein, 12% of crude fat, 38% of ash, 60 g/Kg of N, and 274 g/Kg of carbon.

Every three months, just before MBM applications, nematode density was evaluated under the canopy of each of the 30 trees assigned for data collection. Two soil samples (~ 500 cm^3) were collected on both sides of the canopy with an auger,

at 0-20 cm depth, and homogenized. In the laboratory roots and rootlets of each composite sample were separated and weighed. The number of *M. enterolobii*-induced galls were counted under magnifying lens and expressed as number of root galls/g of root and number of root galls/sample. One hundred cm^3 of soil was processed for nematode extraction according to Jenkins (1964). From the resulting nematode suspension, three aliquots of 0.25 ml were observed under the optical microscope for nematode counting. The following variables were assessed: density of mycophagous, bacterivorous and predaceous nematodes, *M. enterolobii* J_2, *Helicotylenchus dihysteroides* Siddiqi, 1972, and all plant-parasitic nematodes. In addition to high populations of *M. enterolobii* and *H. dihysteroides*, the orchard soil harbored also low populations of *Criconema* sp., *Mesocriconema* sp., *Pratylenchus* sp. and *Hemicycliophora* sp.

Soil density of colony-forming units (CFUs) of *Fusarium* sp. (at 0-20 cm depth) and soil concentration of ammonia (at 0-5 and 5-15 cm depths) were assessed in March 2012 for each of the 30 trees assigned for data collection, 15 days after MBM application. For fungus isolation, soil samples were collected as described before. A soil aliquot of 10 g was suspended in 100 ml of sterile water. Before sedimentation of the soil particles, an aliquot of 1 ml was pipetted out of the suspension and serially diluted from 10^{-4} through 10^{-6}. For each dilution, an aliquot of 0.1 ml was transferred to Petri plates with the medium Martin (1950), supplemented with 60 mg/ml of penicillin and 70 mg/ml of Rose Bengal stain. The Petri plates were incubated upside down for 3-7 days, at 28°C in a 12/12 h photoperiod. The resulting CFUs were counted under magnifying lens.

To assess the concentration of ammonia, soil samples (~50 cm^3) were collected with an auger as described previously. Immediately after sampling, the subsamples were homogenized and an aliquot of 10 g was placed in 50 ml plastic falcon tubes and kept cold in ice chest until processing. In the laboratory, 50 ml of deionized water was added to each tube and agitated. Ten ml of the supernatant was pipetted out and centrifuged at 1509 g for 20 min. After centrifugation, 2 ml of the supernatant was transferred to another glass tube, to which 6 ml of deionized water, 1 ml of disodium tartrate, and 1 ml of Nesler solution were added. After 10 min, the concentration of ammonia was determined through the spectrophometric method, with the aid of a spectrophotometer UV/VIS SPEKOL®, and a standard ammonium

chloride solution (Van Standen and Taljaard, 1997). The concentration of ammonia was expressed in mg/Kg of soil.

For each of the 30 trees used for data collection, average productivity was assessed in two harvests (2009/10 and 2010/2011). Guava fruits were hand-picked daily and productivity was expressed as kilograms of fruits/tree.

For all variables, data were transformed [log (x+1)] prior to analysis using ANOVA, and the treatment means were compared through Tukey test at 5%, using the software SAEG® (Ribeiro Júnior, 2001).

Effect of MBM on orchards with different ages and levels of disease severity and agronomic input - The experiment was established in three commercial 'Paluma' orchards located in two different properties in São João da Barra (lat. 21°39'21"S, long. 41°02'07"W; lat. 21°39'18"S, long. 41°02'02"W; lat 21°41'25"S, long $41^0$3'23"W). Orchards 1, 2 and 3 were ten, five and seven years old, respectively, and they were planted with 6 x 6 m spacing. The orchards were pruned, fertilized with N-P-K, and protected against rust and thrips as described previously.

Previous samplings indicated an average soil density of 57, 97 and 72 *M. enterolobii* J_2/100 cm^3 of soil in orchards 1, 2 and 3 respectively, and the trees had abundant root galls. At the beginning of the experiment, in orchard 1 the trees had only little root rot and no symptoms in the shoot. In orchard 2, a few trees had leaf chlorosis and scorched edges. In orchard 3, several trees had abundant root rot, chlorosis, scorched edges, wilting and falling of leaves.

Local growers tend to reduce agronomical inputs when shoot symptoms are detected. In line with prevalent practices, orchard 1 was irrigated with sprinklers as needed, and fertilized with cow manure at 60 Kg/tree, under the canopy, twice a year. Orchard 2 was irrigated with microsprinklers daily for two hours, and fertilized with cow manure at 40 Kg/tree, twice a year. Orchard 3 received no organic fertilization, and was irrigated lightly with a hose, every other day.

In all orchards, two treatments were assessed: i) treatment with MBM at 25 Kg/tree quarterly and ii) no application of MBM (control). These treatments were arranged in randomized blocks. In each orchard, 36 trees were subdivided into six blocks of six trees each. Within each block, each treatment was represented by three trees, with only the middle one being assigned to data collection. Two planting rows were maintained as buffer between blocks. In all orchards, MBM application was performed as described previously.

For the variables assessed - soil and root density of *M. enterolobii*, soil density of nematode trophic groups, and productivity – data were collected and analyzed as described previously.

3. RESULTS

Table 1 shows the soil density of *H. dihysteroides* and four nematode trophic groups in relation to different dosages and application regimes of MBM, and the soil concentration of ammonia at two different soil depths. There was a reduction ($P < 0.05$) in the soil density of all plant-parasitic nematodes upon the quarterly application of 25 Kg of MBM/tree and the semiannual application of 50 Kg of MBM/tree, although there was no effect of MBM on *H. dihysteroides* in particular. The soil concentration of ammonia was low in all dosages and application regimes of MBM, with the exception of 50 Kg semiannualy at 0-5 cm depth.

Table 2 shows the variables related to guava decline proper, i.e., the density of *M. enterolobii* and *F. solani*, and the average productivity/tree in two harvests. For *M. enterolobii*, only the dosage of 25 Kg of MBM/tree reduced ($P < 0.05$) the soil density of J_2, although all dosages of MBM showed a tendency to reduce the J_2 density. No significant difference was observed among the treatments for the other variables.

Table 1 – Nematode density (in 100 cm^3 of soil) and concentration of ammonia (in mg/Kg of soil) upon soil application of meat and bone meal (MBM) at different doses and application regimes, in a commercial guava orchard affected by guava decline, in São João da Barra, Brazil.

Treatments	*Helicotylenchus dihysteroides*	Plant-parasitic nematodes	Bacterivorous nematodes	Mycophagous nematodes	Predaceous nematodes	Ammonia concentration[x]	
						0-5 cm depth	5-15 cm depth
Untreated control[y]	28.3[aw]	98.1[a]	147.5[b]	20.0[a]	28.3[a]	2.7[c]	2.2[b]
Untreated control[z]	28.3[a]	92.2[a]	241.7[b]	15.5[a]	28.3[a]	2.9[c]	1.6[b]
12.5 Kg/tree, quarterly	23.1[a]	46.7[a]	461.1[a]	26.7[a]	23.1[a]	1.9[c]	0.2[c]
25 Kg/tree, quarterly	23.3[a]	37.2[b]	466.7[a]	21.1[a]	23.3[a]	38.7[b]	3.2[b]
50 Kg/tree six-monthly	26.7[a]	47.2[b]	508.9[a]	20.0[a]	26.7[a]	221.2[a]	17.8[a]
CV (%)	68.8	45.4	15.7	77.1	56.8	16.8	34.9

[x]Determined once in March 2012. Values are average of six guava trees/treatment. Soil samples were taken 15 days after MBM application.

[y]Guava decline-affected trees located within the experimental plot.

[w]Values are average of six guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Values followed by same letters in the column are not different at $P < 0.05$

[z]Guava decline-affected trees located outside the experimental plot, in the same orchard.

Table 2 – Variables related to *M. enterolobii*, incidence of *Fusarium* sp., and productivity upon soil application of meat and bone meal (MBM) at different doses and application regimes, in a commercial guava orchard affected by guava decline, in São João da Barra, Brazil.

Treatments	Density of J_2/ 100 cm^3 of soil	Density of root galls/sampling	Density of root galls/g of root	CFUs[x] of *Fusarium* sp./g of soil	Root fragments positive for *Fusarium* sp. (%)	Productivity (Kg of fruit/tree)
Untreated control[y]	56.4[abw]	102.4[a]	30.9[a]	397.8[a]	31.1[a]	126.4[a]
Untreated control[z]	71.7[a]	105.5[a]	38.9[a]	818.0[a]	31.1[a]	Not assessed
12.5 Kg/tree, quarterly	25.5[bc]	179.7[a]	54.0[a]	713.6[a]	51.1[a]	109.8[a]
25 Kg/tree, quarterly	17.2[c]	73.2[a]	44.5[a]	11,839[a]	35.5[a]	159.0[a]
50 Kg/tree six-monthly	35.5[bc]	79.6[a]	32.6[a]	5,764[a]	33.3[a]	119.2[a]
CV (%)	60.7	33.7	32.3	140.9	67.8	36.4

[x]Colony forming units.

[y]Guava decline-affected trees located within the experimental plot.

[w]Values are average of six guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Productivity is the average of two harvests. Values followed by same letters in the column are not different at $P< 0.05$

[z]Guava decline-affected trees located outside the experimental plot, in the same orchard.

For the experiment that evaluated the quarterly application of 25 Kg/tree of MBM in three distinct orchards, Table 3 shows the results of a factorial analysis highlighting the effect of MBM on *M. enterolobii*, and the average productivity/tree in two harvests. In each of three orchards, the quarterly application of 25 Kg/tree of MBM had no effect on nematode variables and productivity, in comparison with the untreated controls (results not shown). For all three orchards combined, the factorial analysis indicated no differences between MBM-treated and non-treated trees except for the variable density of *M. enterolobii* J_2/100 cm^3 of soil. For the same experiment, Table 4 shows the results of the factorial analysis highlighting the comparative productivity of the three orchards.

Table 3 – Factorial analysis of variables related to *M. enterolobii* and productivity in three commercial guava orchards affected by guava decline, upon quarterly soil application of 25 Kg/tree of meat and bone meal, in São João da Barra, Brazil.

Treatments	Density of J_2/ 100 cm^3 of soil	Density of root galls/ sampling	Density of root galls/ g of root	Productivity (Kg of fruit/tree)
Treated	41.9[bx]	47.9[a]	37.2[a]	84.8[a]
Untreated (control)	77.1[a]	54.9[a]	45.1[a]	65.5[a]
CV (%)	61.5	36.8	38.5	54.6

[x]Values are average of 18 guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Productivity is the average of two harvests. Values followed by same letters in the column are not different at $P< 0.05$.

Table 4 – Factorial analysis of productivity in guava decline-affected orchards in São João da Barra, Brazil. Orchards 1-3 presented different ages and levels of disease severity and agronomic input upon start of the experiment.

Treatments	Productivity (Kg of fruit/tree)	Productivity increase (in %) in relation to orchard 3
Orchard 1	153.5[ax]	+634.3
Orchard 2	47.5[b]	+127.5
Orchard 3	20.9[b]	-
CV (%)	54.6	-

[x]Values are average of 12 guava trees/treatment, in two harvests. Values followed by same letters in the column are not different at $P< 0.05$

4. DISCUSSION

The unsatisfactory results observed in the present work for management of guava decline likely stem from the relatively low concentration of ammonia observed in the soil upon MBM decomposition, with the exception of the 50 Kg/tree dosage at 0-5 cm depth. Indeed, ammonia was reported to have a nematicidal effect when present in soil at 300 mg/Kg of soil (Eno *et al.*, 1955; Rodriguez-Kabana *et al*., 1981; 1982). As reported by these and many other authors, in the present work the input of organic matter resulted in an increase ($P< 0.05$) in the soil density of bacterivorous nematodes. This likely occurred following an increase in the soil density of bacterial CFUs upon application of MBM, as observed by Almeida *et al.* (2012).

Contrary to the observation reported by Gomes *at al.* (2010), the variables involving density of *M. enterolobii*-induced root galls did not relate to J_2 density in the soil i.e., in all treatments the guava trees were heavily parasitized with as many as 54 galls/g of root. Consequently, the conspicuous presence of *Fusarium* sp. in the roots and soil led to an increasing severity of guava decline during the

experiment, with several trees advancing the sequence of symptoms described before. Therefore, the low and statistically similar productivity of all treatments comes as no surprise. The use of MBM in three different orchards showed that age and the level of disease severity at the beginning of the experiment, and the corresponding level of agronomic input during the 24-month period of the experiment, were the key factors determining the differences ($P< 0.05$) observed in the orchards' productivity, which were in excess of 600%.

In conclusion, soil application of as much as 50 Kg/tree of MBM resulted in only marginal reduction of *M. enterolobii* population. This could stem from the relatively low concentration of ammonia in the soil upon MBM decomposition. Most likely ammonia was lost to the atmosphere and/or lixiviated by irrigation water because of the sandy nature of the soil (98% quartz sand). MBM holds potential, nonetheless, for non-complex diseases involving plant-parasitic nematodes, either in perennial or annual horticultural crops. In the São João da Barra area, MBM is presently sold to growers at US$ 0.15/Kg, which is a reasonable cost considering its nematicidal potencial and high content of organic matter, nitrogen, phosphorus and calcium.

Most importantly, the present work shows how intractable plant-parasitic nematodes can be when involved in a disease complex, in which case management through the use of soil organic amendments may not be advisable. Indeed, any effective control or management strategy would need to significantly reduce parasitism - viz, root galls and nematode feeding females in the case of *Meloidogyne* spp. - to minimize the alterations in plant physiology that allow the synergistic interaction to occur between the nematode and the fungus or bacterium. For instance, in guava decline the parasitic capability of *F. solani* towards guava roots seems to be mediated by chemical alteration(s) in exudate released from gall-laden roots (Gomes, 2011).

Since no nematicide is registered for use in guava orchards to promote significant decrease in *M. enterolobii*-parasitism, nematode resistance seems the only feasible strategy to control guava decline (Miranda *et al.*, 2010). So far, a major obstacle towards this goal has been the non-existence of *M. enterolobii*-resistant guavas, and the grafting incompatibility between guava cultivars and a few *M. enterolobii*-resistant *Psidium* sp. genotypes, which hampers the release of resistant rootstocks.

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4. CONCLUSÕES

- ✓ Várias rizobactérias reduziram a população final do nematoide *M. enterolobii* (Fp), Fp/grama de raiz e fator de reprodução, embora não em níveis satisfatórios;
- ✓ As aplicações de rizobactérias parecem incapazes de reduzir o parasitismo de plantas de goiaba por *M. enterolobii* no campo, e menos ainda reduzir a extensa necrose radicular ou aliviar o estresse fisiológico sofrido por árvores afetadas pelo declínio da goiabeira;
- ✓ Os melhores resultados relativos ao controle do nematoide e ao desenvolvimento das mudas foram obtidos com a concentração de 3% v/v de FCO;
- ✓ Quitosana, casca de camarão, torta de nim não proporcionaram controle do nematoide na casa de vegetação;
- ✓ A aplicação de 12 Ton.ha^{-1} FCO em condições de campo implicou em redução do número de nematoides fitoparasitas no solo em todos os intervalos de tempo testados;

- ✓ Houve redução na população de *Helicotylenchus* sp. e aumento da população de nematoides bacteriófagos, nas três doses de FCO utilizadas;
- ✓ Não houve efeito da FCO sobre as variáveis peso de raiz, número de galhas/amostragem e número de galhas/g de raiz em nenhum dos experimentos;
- ✓ Os baixos níveis de amônia observados no solo após a decomposição FCO em todas as três dosagens/regime aplicações neste trabalho podem explicar a redução modesta da densidade de juvenis de segundo estádio de *M. enterolobii* no solo e outros nematoides fitoparasitas;
- ✓ Mesmo a FCO reduzindo o parasitismo por *M. enterolobii*, a densidade no solo e na raiz de *Fusarium* sp. não foram afetadas pelas aplicações FCO não controlando ou impedindo a progressão do declínio da goiabeira e consequentemente a redução da produtividade.

5. CONSIDERAÇÕES FINAIS

Este trabalho demonstra como é difícil o manejo de *M. enterolobii* utilizando compostos orgânicos no solo, especialmente em doença complexa tão agressiva como declínio de goiabeira.

A forma de manejo recomendado desta doença complexa é a utilização de áreas livres, plantio de mudas sadias de goiabeiras, utilização de máquinas e implementos desinfestados, proibição do trânsito dessas máquinas em áreas infestadas e monitoramento periódico do pomar.

Necessidades de se desenvolver variedades imunes ao *M. enterolobii* e ao *Fusarium* sp. é de extrema urgência para que se possa continuar com o cultivo da goiabeira e para que se mantenham no campo milhares de trabalhadores que dependem desta cultura para sobreviver.

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