Greenhouse and field assessment of rhizobacteria to control guava decline

Alexandre Macedo Almeida; Vicente Martins Gomes; Ricardo Moreira Souza (*)

Universidade Estadual do Norte Fluminense Darcy Ribeiro, Laboratório de Entomologia e Fitopatologia, Av. Alberto Lamego, 2000, 28013-620 Campos dos Goytacazes (RJ), Brasil.
(*) Corresponding author: ricmsouza@censanet.com.br

Received: *November 23, 2010;* ***Accepted:*** *March 10, 2011.*

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*.

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

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; Rozane 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; Sousa 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 Macacu (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.

Four months after nematode 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 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°39'21"S and 41°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 all the fruits of each plant individually 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 Júnior, 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*.

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.

Rhizobacteria applications seemed unable to significantly antagonize the parasitism of *M. enterolobii* on guava plants, and even less to reduce the extensive root

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)	Fp/gram of root (× 100)	RF
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

Fp= total eggs + J_2 extracted from the root system. 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 $p < 0.05$.

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 per 100 cm^3 of soil	Number of root galls per sampling	Number of root galls per g of root	Root mass per sampling	Yield (kg $plant^{-1}$)
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 $p < 0.05$.

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.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Fábio Olivares (UENF) for the taxonomic identification of the rhizobacteria isolates.

REFERENCES

CARNEIRO, R.M.D.G.; TIGANO, M.S.; JORGE, C.L.; TEIXEIRA, A.C.O.; CORDEIRO, M.C., Selection and polymorphism of *Pasteuria penetrans* isolates in relation to *Meloidogyne* spp. from coffee. Nematology, v.6, p.37-47, 2004.

CHANWAY, C.P.; TURKINGTON, R.; HOLL. F.B. Ecological implications of specificity between plants and rhizosphere microorganisms. Advances in Ecological Research, v.21, p.121-169, 1991.

CHEN, C.; BÉLANGER, R.R.; BENHAMOU, N.; PAULITZ, T.C. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology, v.56, p.13-23, 2000.

DIAS-ARIEIRA, C.R.; FERRAZ, S.; FREITAS, L.G.; DEMUNER, A.J. Efeito de lixiviados de raízes de gramíneas forrageiras na eclosão de juvenis de *Meloidogyne incognita*, *M. javanica* e *Heterodera glycines*. Nematologia Brasileira, v.27, p.23-28, 2003.

FREITAS, L.G.; NEVES, W.S.; FABRY, C.F.S.; MARRA, B.M.; COUTINHO, M.M.; ROMEIRO, R.S.; FERRAZ, S. Isolamento e seleção de rizobactérias para controle de nematoides formadores de galhas (*Meloidogyne* spp.) na cultura do tomateiro. Nematologia Brasileira, v.29, p.215-220, 2005.

GOMES, V.M.; SOUZA, R.M.; CORRÊA, F.M.; DOLINSKI, C. Management of *Meloidogyne mayaguensis* in commercial guava orchards with chemical fertilization and organic amendments. Nematologia Brasileira, v.34, p.23-30, 2010.

GOMES, V.M.; SOUZA, R.M; MUSSI-DIAS, V.; SILVEIRA, S.F.; DOLINSKI, C. Guava Decline: A Complex Disease Involving *Meloidogyne mayaguensis* and *Fusarium solani*. Journal of Phytopathology, v.159, p.45-50, 2011.

GONZAGA NETO, L.; SOARES, J.M. Goiaba para exportação: aspectos técnicos da producão. Brasília: Embrapa-SPI. 1994. 49p. (Serie Publicações Técnicas FRUPEX, 5)

IBGE-INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Produção Agrícola Municipal. Rio de Janeiro, 2006. Available from: http://www.sidra.ibge.gov.br /bda/agric/default.asp?t=3&z=t&o=11&u1=1&u2=1&u3=1&u4=1&u5=1&u6=1. [cited 2010 Sep 2].

JENKINS, W.R. A rapid centifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter, v.48, p.692, 1964.

KADO, C.I.; HESKETT M.G. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology, v.60, p.969-979, 1970.

LOPES, E.A.; FERRAZ, S.; FERREIRA, P.A.; FREITAS, L.G.; DHINGRA, O.D.; GARDIANO, C.G.; CARVALHO, S.L. Potencial de isolados de fungos nematófagos no controle de *Meloidogyne javanica*. Nematologia Brasileira, v.31, p.78-84, 2007.

MAFIA, R.G.; ALFENAS A.C.; FEREIRA, E.M.; BINOTI, H.B.; MAFIA, G.M.V.; MOUNTEER, A.H. Root colonization and interaction among growth promoting rhizobacteria isolates and eucalypts species. Revista Árvore, v.33, p.1-9, 2009.

MEDEIROS, J.E.; MARIANO, R.L.R.; PEDROSA, E.M.R.; SILVEIRA, E.B. Inconsistency of the biological control of *Meloidogyne incognita* race 2 in melon by endophytic bacteria. Horticultura Brasileira, v.27, p.319-324, 2009.

MENDOZA, A.R.; SIKORA, R.A. Biological control of *Radopholus similis* in banana by combined application of the mutualistic endophyte *Fusarium oxysporum* strain 162, the egg pathogen *Paecilomyces lilacinus* strain 251 and the antagonistic bacteria *Bacillus firmus*. Biological Control, v.54, p.263-272, 2009.

MOLINA, J.P.; DOLINSKI, C.; SOUZA, R.M.; LEWIS, E.E. Effect of entomopathogenic nematodos (Rhabditida:Steinernematidae and Heterorhabditidae) on *Meloidogyne mayaguensis* Rammah & Hirschmann (Tylenchida: Meloidogynidae) infection on tomato plants. Journal of Nematology, v.39, p.338-342, 2007.

NATALE, W.; COUTINHO, E.L.M.; BOARETTO, A.E.; PEREIRA, F.M. Goiabeira: calagem e adubação. Jaboticabal: FUNEP, 1996. 22p.

PEREIRA, F.O.M., SOUZA, R.M., SOUZA, P.M., DOLINSKI, C.; SANTOS, G.K. Estimativa do impacto econômico e social direto de *Meloidogyne mayaguensis* na cultura da goiaba no Brasil. Nematologia Brasileira, v.33, p.176-181, 2009.

PIRES, A.M.M.; MATTIAZZO, M.E. Avaliação da Viabilidade do Uso de Resíduos na Agricultura, 2008. 8p. (Circular técnica 19)

RIBEIRO JÚNIOR, J.I. Análises Estatísticas no SAEG. Viçosa: UFV, 2001. 301p.

ROCHA, F.S.; CAMPOS, V.P.; SOUZA, R.M. Efeito de exsudatos radiculares em endósporos de *Pasteuria penetrans* e em juvenis do segundo estádio de *Meloidogyne incognita*. Fitopatologia Brasileira, v.29, p.644-650, 2004.

ROCHA, M.; CASTRO, R.; PINA, R.; MARTINI, A. Efeito do acibenzolar-s-methyl (benzothiadiazole) como indutor de resistência sistêmica em soja (*Glycine max* cv. FT-Cristalina) sobre *Heterodera glycines*. Pesquisa Agropecuária Tropical, v.30, p.35-38, 2000.

RODRIGUES, A.K.; FREITAS, L.G.; AZEVEDO, A.A.; FERRAZ, S. Desenvolvimento de *Pasteuria penetrans* em *Meloidogyne* spp. parasitando diferentes espécies vegetais. Fitopatologia Brasileira, v.28, p.267-272, 2003.

ROZANE, D.E.; COUTO, F.A.A. Cultura da goiabeira: tecnologia e mercado. 1 ed. Visconde do Rio Branco: Suprema Gráfica e Editora, 2003.

SOUSA, C.S.; SOARES, A.C.F.; GARRIDO, M.S.; ALMEIDA, G.M.C.O. Estreptomicetos no controle da meloidoginose em mudas de tomateiro. Pesquisa Agropecuária Brasileira, v.41, p.1759-1766, 2006.