Major Guava Nematodes and Control Prospects Using Resistance on *Psidium* spp. and Non-Host Crops

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Abstract

The worst nematode problem affecting guava is that created by root-knot nematode, which is a recognized limiting factor in commercial guava production in Central and South America. Considering the difficulty of identifying *Meloidogyne enterolobii* (=*M. mayaguensis*) only by the perineal pattern, this species has been misidentified in different regions around the world and identified frequently as *M. incognita* or *Meloidogyne* spp. The species' identification is possible using esterase phenotype and molecular markers. Using these techniques, only *M. enterolobii* was detected on guava in Brazil, confirming the incorrect identification. The intraspecific genetic variability of 16 *M. enterolobii* isolates from different geographical regions and hosts were analysed with different neutral molecular markers (RAPD, ISSR and AFLP) and showed a high level of homogeneity among the populations. Considering the low variability among *M. enterolobii* isolates, genetic resistance could be considered the most effective method of control, but only one accession of *P. friedrichstalianium* (Costa Rican wild guava) was resistant and compatible as rootstock with *P. guajava* 'Paluma', in field conditions. Although this root-knot nematode displays a very wide host range, studies showed that crop rotation is possible for cleaning areas infested with the nematode, using 35 antagonistic plants. Some cultivars of corn are also very promising for use in reducing populations of *M. enterolobii* in infested fields. Fourteen fruit trees are non-host to *M. enterolobii* and only four fruit trees are good hosts. Considering the impossibility of cultivating guava in fields infested by *M. enterolobii*, crops presented as non-hosts or poor hosts could be used by the growers, but more studies should be done in the field, in infested areas, to support the results obtained in greenhouse conditions.

INTRODUCTION

The common guava (*Psidium guajava* L.) is indigenous to tropical America. The root-knot nematode (*Meloidogyne* spp.), is a recognized limiting factor in commercial guava production in Central and South American countries. In Cuba, Puerto Rico, Mexico, Venezuela and Brazil guava production has declined during the past quarter century due to increasing pressure of *Meloidogyne* spp. (El Borai and Duncan, 2005).

The root-knot nematode (RKN) *Meloidogyne enterolobii* Yang & Eisenback, 1983 was described from a population sampled in China and isolated from a tree species (*Enterolobium contortisiliquum* Vell). This nematode was also reported from other regions in China, but mainly isolated from guava (*Psidium guajava*) (Xu et al., 2004). More recently, it has been suggested that *M. enterolobii* was a senior synonym of *Meloidogyne mayaguensis* Rammah and Hirschmann, 1988 (Hunt and Handoo, 2009), a species which had been originally described in Puerto Rico, isolated from aubergine (*Solanum melongena* L.) roots. The presence of this parasite has increasingly been detected in different geographical regions from a wide range of hosts, including crops carrying genes of resistance to the main *Meloidogyne* spp. (Blok et al., 2002; Brito et al., 2007; Carneiro et al., 2006a; Fargette et al., 1996).

Recently, *M. enterolobii* was detected in two commercial greenhouses in Switzerland on tomato rootstock (*Solanum lycopersicum* L. 'Maxifort') resistant to *Meloidogyne* spp. (Kiewnick et al., 2008). Considering the risk of introduction and dissemination of this pest in the European region, *M. enterolobii* was recently added to the EPPO Alert List (EPPO, 2008).

In Brazil, *M. enterolobii* was originally detected in guava orchards in 2001 in Pernambuco and Bahia states (Carneiro et al., 2001). Since then, this nematode has been a matter of grave concern in the country because it has been spreading rapidly with infected seedlings, making the cultivation of guava unviable in the heavily infested areas (Carneiro et al., 2007; Siqueira et al., 2009). Unfortunately, the Ministry of Agriculture (MAPA) has not yet established legislative measures to prevent the spread of this pest in Brazil.

Morphological identification demands considerable skill and can be unreliable due to significant intra-specific morphological variation in *Meloidogyne* spp. Because of its morphological resemblance to *M. incognita* and *M. arenaria*, considering only the perineal patterns (Brito et al., 2004; Carneiro et al., 2001), *M. enterolobii* might have been misidentified in a number of surveys. It is possible that the severe root-knot problem on guava in the Americas and the isolated cases in Africa and Asia involve only this particularly virulent species.

The objective of this paper is to clarify the difficulties with identification of *M. enterolobii*, the guava root-knot nematode, and discuss possibilities for resistance on *Psidium* spp. rootstocks and crop rotation with other plants.

SYMPTOMS AND DAMAGE

Severely infected trees decline rapidly, culminating in the death of the plants. Moderate infestations are associated with general chlorosis, nutrient deficiency symptoms and reduced flowering and fruiting. Roots of infected trees show multiple galls and root necrosis, causing a drastic decrease in fine roots. The root-knot nematode infects the whole root system, from small rhizoids to more lignified pivotants roots. The decline and death of guava trees parasitized by *Meloidogyne enterolobii* is a complex disease involving other micro-organisms like *Fusarium solani* (Gomes et al., 2010)**.** Other fungi were also related to disease syndrome: *Verticillium dahliae*, *Pythium aphanidermatum*, *Trichoderma* sp. (Avelar-Mejía et al., 2001). This syndrome is making cultivation of guava in the infested areas unfeasible, causing serious economic problems to growers and the economy of the region (Carneiro et al., 2007).

In the San Francisco (Pernambuco state of Brazil) region, the area cultivated with guava has decreased from 6,000 to 1,669 ha in seven years (2000-2006), a reduction of more than 70% in guava production (Plantec/Codevasf), a reduction of 10% per year was estimated (Carneiro et al., 2007)**.** Pereira et al. (2009) verified direct damages of R$ 112,7 in guava production in the Brazilian states of RJ, CE, RN, PE and BA. In addition to these damages, 3,703 jobs were lost due to the decline and death of the orchards in these regions. As these regions are also an important seedling producer, this nematode has spread to other Brazilian states (Carneiro et al., 2007; Siqueira et al., 2009). However, this species was detected in the state of Rio de Janeiro in native areas of Atlantic forest (Lima et al., 2005), suggesting that the nematode has not been introduced in Brazil from other

countries, as suggested when it was reported for the first time in the country (Carneiro et al., 2001). In Paraná state it was also detected in guava without introduction from other states (Carneiro et al., 2006b).

SPECIES IDENTIFICATION AND GENETIC VARIABILITY

Isozyme phenotyping has been shown to be a valuable tool for precise female identification of major *Meloidogyne* species (Esbenshade and Triantaphyllou, 1990; Carneiro et al., 1996, 2000). In all recent surveys in Brazil, *M. enterolobii* has been identified using esterase profile (Carneiro et al., 2000, 2001; Siqueira et al., 2009). However, the limitation of this technique is that second-stage juveniles (J2) cannot be reliably diagnosed, which hinders its use in routine examination of soil samples that often contain only J2. Regarding the use of molecular tools, the analyses of a specific mitochondrial DNA region (Block et al., 1997b, 2002; Brito et al., 2004; Jeyaprakash et al., 2006; Tigano et al., 2005; Xu et al., 2004) and the IGS from the ribosomal DNA (Adam et al., 2007) have been demonstrated as efficient in differentiating *M. enterolobii* from the most common *Meloidogyne* species, based on the size of the PCR-amplified fragments. Recently, a new species-specific satellite DNA family and a species-specific DNA fragment obtained from the RAPD analyses (SCAR marker) were identified for *M. enterolobii* and shown to be good molecular markers for use in the diagnostics of this nematode (Randig et al., 2009; Tigano et al., 2010).

Regarding the genetic diversity in *M. enterolobii*, RFLP and RAPD analyses showed a low variability among isolates used in these studies (Fargette et al., 1996; Block et al., 1997a). Recently, the analyses of molecular markers AFLP, ISRR and RAPD were applied to examine the genetic variation of *M. enterolobii* isolates from different Brazilian geographical regions, and the low variability was confirmed (Tigano et al., 2010). Until now, more than 80 samples from guava have been analysed in our Laboratory in Embrapa-Recursos Genéticos e Biotecnologia and only *M. enterolobii* was detected on guava in Brazil.

Considering the difficulty of identifying *M. enterolobii* only by the perineal pattern (Brito et al., 2004; Carneiro et al., 2001) or host races (=*M. incognita* race 2), it is possible that *M. enterolobii* from guava was misidentified in many countries (Mexico, Venezuela, Brazil and Colombia). Since then, the identification of *Meloidogyne* in this plant has been based on morphological data and differential hosts (Avelar-Mejia et al., 2001; Crozzoli and Cassava, 1998; Gallegos-Morales et al., 2009; Moura and Moura, 1989; Villota and Agudelo, 1997). Recently, studying the population from Venezuela using esterase phenotype, Molinari et al. (2005) identified *M. enterolobii* in this country.

We studied, the host suitability of *P. guajava* 'Paluma' to *M. incognita* race 1 and race 2, *M. javanica*, *M. arenaria* race 2 and *M. enterolobii* (control) in Embrapa Recursos Genéticos e Biotecnologia, DF, Brazil, in greenhouse conditions. The plants were inoculated with 10,000 eggs/plant and evaluated after 12 months. The Reproduction Factor (RF) of the control was 182.4 (High Susceptible) and the other inoculated plants presented RF<1.0, showing that *P. guajava* is not a good host (Resistant or Immune) of these four populations of *Meloidogyne* spp. (Table 1). Rossi et al. (2002) confirmed these results, showing resistance in three commercial guava cultivars inoculated for four months with *M. incognita* race 2 and *M. javanica*. These results confirmed the misidentification of *M. enterolobii* using only morphological approaches.

RESISTANCE IN *PSIDIUM* SPP. TO ROOT-KNOT NEMATODES

The susceptibility of 46 and 112 wild *P. guajava* to *M. enterolobii* was observed in our study in Brasília and Petrolina, respectively (data not shown). The same was detected by other authors for *M. enterolobii* (Almeida et al., 2009; Carneiro et al., 2007; Maranhão et al., 2001; Scherer, 2009). In disagreement with this work, Cassava et al. (1997) evaluated 7 accessions of *P. guajava* in relation to *M. enterolobii* (=*M. incognita* race 1). The resistance was observed only in *P. guajava* accession S3.

Milan (2007, 2010) also reported resistance on guava accessions. In the first work

P. guajava "B-12" was classified as resistant to *M. incognita* (FR=0.88) having the potential to control the nematode, so that it could be used as a rootstock for commercial guava clones. A total of more than 2000 F_1 seedlings were produced in the hybridization programme and the F_1 seedlings are in the nursery stages for further evaluation. The results also showed that *P. longipes* and *P. arayan* showed potential resistant characters to the root-knot nematode, but their potential as rootstocks for guava cultivation is still questionable. In the second work, this author observed that three accessions of *P. guajava* (K-10, A-06, J-16) were resistant to *M. incognita*, with IG<2. Unfortunately, the RFs were not measured. These accessions are graft-compatible with local commercial clones and thus could be used as a rootstock. No more information about the reaction of 2000 F_1 seedlings to *M. enterolobii* was available.

P. friedrichsthalianium (Berg) Ned (Costa Rican wild guava) was considered resistant to *Meloidogyne enterolobii* (=*M. mayaguensis*, *M. incognita* race 1 and race, *M. arenaria*, *M. javanica*) in different countries: Venezuela (Casassa et al., 1997, 1998; Molero et al., 2010), Colombia (Vilota and Agudelo, 1997) and Brazil (Carneiro et al., 2007).

The performance of guava (*P. guajava*) grafted onto tolerant *P. guajava* rootstock and onto resistant *P. friedrichsthalianium* was evaluated. In the nursery, 100% successful grafts were obtained with guava and Brazilian wild guava (*P. guineense*) rootstocks, while success with P. *friedrichsthalianium* was 90% ('Arrayán', 'Criollo'). Initial vegetative growth was significantly higher in the guava rootstock treatment. In the field this treatment also showed more vigorous growth. 'Arrayán' rootstock clearly showed a strong dwarfing effect while the 'Brazilian' and 'Criollo' wild guava rootstocks exhibited an intermediate effect on vegetative vigor. Number and weight of fruits was higher when guava was used as a rootstock and no differences were observed with wild guava rootstocks (Bogantes-Arias and Newcomer, 2010). In order to evaluate the susceptibility of the rootstocks to nematodes of adult plants, an additional control treatment was included in the field, which consisted of the application of nematicides to the guava rootstock treatment. The results indicated that the root-knot nematode was able to colonize the roots of the guava rootstocks with and without nematicides, as well as colonizing the roots of Brazilian wild guava. No nematodes were detected in the roots of the 'Criollo' and 'Arrayán' rootstocks, confirming the resistance of *P. friedrichsthalianium* (Bogantes-Arias and Newcomer, 2010).

In our studies only Costa Rican wild guava *P. friedrichsthalianium*, *P. cattleyanum* (yellow wild guava), *A. sellowiana* and *P. rufum* presented resistance or immunity to *M. enterolobii*. *P. acutangulum*, *P. guineense* (Brazilian guava) were susceptible (data not shown). In disagreement with our results, Lee et al. (1998) classified the only *P. guineense* accession tested as immune. The same results were observed by Costa et al. (2012). Almeida et al. (2009) classified *P. friedrichsthalianum* (probably an incorrect identification) as susceptible to this pathogen (FR=13.03). Additionally, these authors verified that three non-identified *Psidium* sp. accessions and *Eugenia stipitata* were resistant to *M. enterolobii*, with FRs<1. Dr. Carolyn Elinore Barnes Proença (pers. commun.), explained that *P. friedrichsthalianum* is not originally from Brazil, and this Brazilian rootstock is probably another *Psidium* species. Ten plants of *P. friedrichsthalianum* and nine of *P. cattleyanum* were compatible with 'Paluma' guava when grafted by budding technique in greenhouse conditions. These grafted plants were maintained in field conditions. One year later, five Costa Rican plants were still alive and producing buds, flowers and fruits, while the plants grafted on *P. cattleyaum* presented an incompatibility reaction (enlargement in grafting region); consequently all nine plants died during the first year (data not shown).

HOST STATUS OF COVER CROPS, CORN AND FRUIT PLANTS TO *MELOIDOGYNE ENTEROLOBII*

An effective alternative control of this nematode is the use of non-host cover crops. This study aimed to know the reactions of 38 plant species and genotypes to

M. enterolobii under greenhouse conditions. Tomato 'Rutgers' was used as control of the inoculum viability. Among 38 cover crops assessed, 26 were resistant: peanut (*Arachis hypogaea*) 'Cavalo vermelho', white oat (*Avena sativa*) 'IAPAR 126', black oat (*Avena strigosa*) 'IAPAR 61', rapeseed (*Brassica napus*) 'CAN 420', 'CAN 401', foxtail millet (*Setaria italica*), millet (*Eleusine coracana*), rattlepod (*Crotalaria anguroides*, *C. apiclolice*, *C. grantiana*, *C. juncea*, *C. okraeluka*), cowpea (*Vigna unguiculatta*), hyacinth bean (*Dolichos lablab*), castor bean (*Ricinus communnis*) 'IAC 80', grey velvet bean (*Mucuna cinerea*), green velvet bean (*Mucuna* sp.), black mucuna (*Mucuna aterrima*), forage radish (*Raphanus sativus* var. *oleiferus*) 'AL 1006', 'Seletina Nova', 'N4', 'Jesuíta', white tephrosia (*Tephrosia candida*), timbó (*Ateleia glazioveana*), triticale (*Triticum aestivum* × *Secale cereale*). Nine species/genotypes of plants were immune: peanut 'IAC Oirã', 'IAC Poitã', 'IAC Tatuí', Italian ryegrass (*Lollium multiflorum*), rye (*Secale cereale*) 'IPR 89', butterfly pea (*Clitoria ternatea*), mungo bean (*Vigna radiata*), cooper (*Glycine wightii*). Only three species of plants were susceptible: hairy vetch (*Vicia villosa)* 'Ostssat', ricebean (*Vigna unbellata*) and jack bean (*Canavalia ensiformis*) (Table 1). Some crop systems alternating non-host plants in summer and winter or only planting in the summer may work to control *M. enterolobii*, taking into account different climatic regions where this nematode causes damage (Table 2).

Six genotypes of corn ('NB-7361', 'SHS-5080', 'GNX-1020', 'GNX-3010', 'BRS-1031' and 'BM-1115') were highly resistant (FR<1.0) to *M. enterolobii* and can be used in crop rotation systems to reduce or eliminate nematode populations (Dias et al., 2010). Field experiments are necessary to validate these greenhouse results.

Fourteen fruit trees are non-hosts to *M. enterolobii* (atemoya, avocado, cashew, citrus rootstocks, coconut and mango) or poor hosts (assai palm, common mulberry, jaboticaba, grape rootstocks, passion fruit, sapodilla and soursop); only four fruit trees are good hosts (fig, banana, 'Chardonnay' and 'Solferino' grapes and melon). More studies should be done in infested fields to confirm the poor host status of these fruit trees tested in greenhouse conditions (data not shown).

PROSPECTS AND FUTURE CHALLENGES

1) Improve techniques of *Meloidogyne* identification in different countries, using isozymes (esterase phenotypes) or molecular markers.
2) Develop nematode screenings with more *Psidium* spp. with correct rootstock botanical classification.
3) Improve nematode screening methodology, using the Reproduction Factor (RF) as an obligatory parameter.
4) Improving grafting techniques in *Psidium* spp.
5) Develop interspecific hybridization with *Psidium* spp. to obtain nematode-resistant rootstocks.
6) Study in vitro production of resistant plants by somatic embryogenesis.
7) Study the mechanisms and molecular characterization of resistance in *Psidium* spp.
8) Develop molecular markers related to resistant genes in *Psidium* spp.

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Tables

Table 1. Reaction of 'Paluma' guava to *Meloidogyne* spp.

Populations	RF[1]	Status
M. incognita race 1	0.03	R
M. incognita race 2	0.05	R
M. arenaria race 2	0.0	I
M. javanica	0.0	I
M. enterolobii	182.4	HS

[1] RF = final population/initial population.
[2] Status: I = immune (RF=0), R = resistant (RF<1.0) and HS = high susceptible (RF>1.0).

Table 2. Reaction of different summer and winter cover crops to *Meloidogyne enterolobii*.

Scientific name	Common name/cultivar	Ne/g of RFW[1]	RF[2]	Status[3]
Arachis hypogaea L.	Peanut 'Cavalo Vermelho'	133 e	0.03	R
Arachis hypogaea L.	Peanut 'IAC Poitã'	0 f	0.00	I
Arachis hypogaea L.	Peanut 'IAC Tatuí'	0 f	0.00	I
Arachis hypogaea L.	Peanut 'IAC Oirã'	0 f	0.00	I
Ateleia glazioveana Baill	Timbó	80 e	0.02	R
Avena sativa L.*	White oat 'IAPAR126'	120 e	0.02	R
Avena strigosa Screb.*	Black oat 'IAPAR 61'	200 e	0.04	R
Brassica napus L.*	Rapeseed 'CAN 420'	1.820 d	0.36	R
Brassica napus L.*	Rapeseed 'CAN 401'	1.800 d	0.36	R
Canavalia ensiformes (L) DC	Jack bean	15460 d	3.09	S
Clitoria ternatea L.	Butterfly pea	20 f	0.00	I
Crotalaria anagyroides Kunth	Rattlepod	114 e	0.02	R
Crotalaria apioclice L.	Rattlepod	742 e	0.15	R
Crotalaria grantiana (Harvey) Polh.	Rattlepod	140 e	0.03	R
Crotalaria juncea L.	Rattlepod	2.520 d	0.50	R
Crotalaria ochroleuca L.	Rattlepod	85 e	0.02	R
Dolichus lablab L.	Hyacinth bean	140 e	0.03	R
Eleusine coracana (L.) Gaertn	Millet	240 e	0.05	R
Glycine wightii (Wight & Arn.)	Cooper	0 f	0.00	I
Lollium multiflorum Lam.*	Italian ryegrass	0 f	0.00	I
Solanum lycopersicum L.	Tomato 'Rutgers'	85.476a	17.10	S
Mucuna aterrima (Piper & Tracy) Holland	black velvet bean	1.355 d	0.27	R
Mucuna sp.	green velvet bean	320 e	0.06	R
Mucuna cinerea Piper & Tracy	grey velvet bean	60 e	0.01	R
Raphanus sativus L. var. *oleiferus**	Forage radish 'AL 1006'	4.000 d	0.80	R
Raphanus sativus L. var. oleiferus*	Forage radish 'Seletina Nova'	200 e	0.04	R
Raphanus sativus L. var. *oleiferus**	Forage radish 'Jesuíta'	50 e	0.01	R
Raphanus sativus L. var. *oleiferus**	Forage radish 'IPR 116'	25 f	0.01	R
Raphanus sativus L. var. *oleiferus**	Forage radish 'N 4'	0 f	0.00	I
Ricinus communis L.	Castor bean 'IAC 80'	640 e	0.13	R
Secale cereale L.*	Rye 'IPR 89'	0 f	0.00	I
Setaria italica (L.) Beauv.*	Foxtail millet	155 e	0.03	R
Tephrosia candida DC	White tephrosia	1.000 d	0.20	R
Triticum aestivum L. × *Secale cereale* L.*	Triticale 'IPR 111'	333 e	0.07	R
Vicia villosa Roth*	Hairy vetch .'Ostssat'	28.850 b	5.77	S
Vigna radiata (L.)Wilczed	Mung vean	0 f	0.00	I
Vigna unbellata (Thunb) Ohwi hashi	Rice vean	9.800 d	1.96	S
Vigna unguiculatta (L) Walp.	Cowpea 'Australian'	180 e	0.04	R
Vigna unguiculatta (L.) Walp	Cowpea	2.980 d	0.60	R
CV %	63,35			

Averages of 10 repetitions: the data were transformed in $\sqrt{x + 0.5}$ for statistical analysis. Means followed by same letter in column do not differ from each other by Scott-Knot test at 5% of probability.
[1] Number of eggs/gram of root fresh weight.
[2] RF = final population/initial population
[3] Status: I = immune (RF=0), R = resistance (RF<1.0) and S = susceptibility (RF≥1.0).
* Winter plants; those not marked are summer plants.