Crop Protection 55 (2014) 74–79

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Root-knot nematodes, a growing problem for Conilon coffee in Espírito Santo state, Brazil

A.F. Barros [a], R.D.L. Oliveira [a,*], I.M. Lima [b], R.R. Coutinho [a], A.O. Ferreira [a], A. Costa [b]

[a] Universidade Federal de Viçosa, Departmento de Fitopatologia, Av. P.H. Rolfs, s.n., 36570-000, Viçosa (MG), Brazil
[b] Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, Cx. Postal 62, 29900-970, Linhares (ES), Brazil

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form
2 October 2013
Accepted 4 October 2013

Keywords:
Meloidogyne spp.
Coffea canephora
C. arabica
Esterase isoenzyme
dissemination
Regulation
Race

ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are non-quarantine pests that are subject to regulations in Brazil because they are limiting to coffee production and easily spread by planting infected seedlings. Containing their dissemination requires knowledge of their distribution in coffee-producing areas in order to establish phyto-sanitary measures. The object of this work is to evaluate the distribution of *Meloidogyne* spp. in *Coffea* spp. in Espírito Santo state, where coffee growing is expanding. Soil and root samples were collected in *Coffea arabica* and *Coffea canephora* Conilon. Identification of *Meloidogyne* spp. was carried out by esterase isoenzyme phenotype and by the host range. In Espírito Santo, *Meloidogyneincognita*, *Meloidogyne exigua*, and *Meloidogyne paranaensis* were identified. Phenotypes I1 and I2 of *M. incognita* were present in 21% of all the sampled properties. This species was found mainly in the Serrana region and north of the state, and principally in plantations of *C. canephora*. Only phenotype E1 of *M. exigua* was detected, distributed in 23.8% of the properties and only in plantations of *C. arabica*. *M. exigua* was found in all the municipalities sampled in the southern region of the state and in 66.6% of the municipalities of the Serrana region. *M. paranaensis*, phenotype P1, was detected only in the northern region, and in 100% of the properties sampled in Baixo Guandu municipality. The study was complemented by sampling in the Zona da Mata region of Minas Gerais state, where only *M. exigua*, phenotype E1, was found in *C. arabica* plantations. *M. exigua* was detected in all the sampled municipalities except Paula Cândido. The populations were subjected to differential host plant tests to determine the physiological races. Races 1 and 2 of *M. incognita* and *M. exigua* were found in Espírito Santo state, and race 2 of *M. exigua* in Zona da Mata in Minas Gerais state. The presence of the three most important root-knot nematodes in coffee plants in Espírito Santo state indicates the need to establish measures that will contain their dissemination.

© 2013 Published by Elsevier Ltd.

1. Introduction

Commercial coffee plantations in Brazil consist primarily of *Coffea arabica* and *Coffea canephora*, which represent approximately 74.5 and 25.5% of Brazilian coffee production, respectively. The estimated harvest amounted to 50.6 million bags for the year 2012 (Conab, 2012). Two Brazilian states stand out as coffee producers: Minas Gerais, which leads national production and is responsible for 97.7% of Arabica coffee (*C. arabica*), and Espírito Santo, which is the biggest producer of Conilon coffee (*C. canephora*).

Diseases caused by plant nematodes limit coffee production, and root-knot nematodes (*Meloidogyne* spp.), especially *Meloidogyne exigua*, *Meloidogyne paranaensis* and *Meloidogyne incognita*, are particularly notable due to their destructive potential (Campos and Villain, 2005). Various studies on the occurrence of root-knot nematodes in coffee plantations have been carried out in a number of states (Barbosa et al., 2004; Castro et al., 2008; Dias et al., 1996; Oliveira et al., 2005a), concentrating on the big coffee producers and on Arabica coffee. It is rare to find information about the occurrence of these nematodes in Conilon plantations, and in the state of Espírito Santo, the largest producer of this type of coffee, there are few reports on their distribution.

In 1971, Lordello and Hashizume found *M. incognita* on Conilon coffee plants in Baixo Guandu municipality (ES), and this nematode was later reported on Arabica coffee in Colatina (Sharma, 1976). The most recent of these studies was carried out by Dias et al. (1996) covering the Serrana and southern regions of the state, where *C. arabica* is cultivated on a large scale, but *M. incognita* was not found. It is important to note that in these studies identification of

* Corresponding author.
E-mail address: rdlima@ufv.br (R.D.L. Oliveira).

0261-2194/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.cropro.2013.10.004

the *Meloidogyne* species was carried out based on female perineal pattern, which is now considered to be an insufficiently precise technique.

Another explanation for the lack of studies on occurrence and distribution of plant nematodes in this region is that it was believed that all materials from *C. canephora* were resistant to root-knot nematodes. It is now known, however, that some clones from the Conilon group (*C. canephora*) are susceptibility to a number of species of *Meloidogyne* (Carneiro et al., 2009; Contarato et al., 2009).

Improvements in production and productivity of Conilon coffee and progress in planting the cultivar in new areas may be compromised by the dearth of information on nematode distribution in Espírito Santo. Besides the cultivation of coffee in the field is done by the use of transplanted seedlings produced in nurseries. Once the nursery is infested, seedlings turn into a very efficient way to disseminate root-knot nematodes. To ensure this crop's economic contribution to the state's economy it is vital to establish preventive measures to curb dissemination of nematodes, so easily moved about on seedlings. This study aimed to clarify the current geographic distribution of the species and races of *Meloidogyne* in Espírito Santo and complementally on Zona da Mata in Minas Gerais.

2. Materials and methods

2.1. Sampling and multiplication of Meloidogyne spp. populations

Three hundred and eighteen soil and root samples were collected, randomly, from coffee-producing areas planted with *C. arabica* and *C. canephora*. Of these, 295 came from 172 properties in 25 municipalities in Espírito Santo, focusing in those one with the greatest production. These have represented 32% of the municipalities coffee producers and about 50% of the cultivated area. The other 23 were collected on 18 coffee farms of *C. arabica* in five municipalities in the Zona da Mata region of Minas Gerais.

About 5–10 sub-samples were collected, depending on the size of the cultivated area, and these were mixed to obtain a compound sample (about 1 kg of soil + 200 g of root). The samples were placed in plastic bags, closed and identified, together with an information form, and sent in a styrofoam box to the Nematology laboratory of the Federal University of Viçosa (UFV), where the samples were processed.

Coffee roots containing galls and/or cracks were immediately dissected with tweezers and fine scalpels under a stereoscopic microscope to release the milky-white females. Females were transferred to microtubes containing protein extraction buffer (Ornstein, 1964; Davis, 1964).

Sample roots that were in an advanced state of degradation were chopped into approximately 2-cm pieces, mixed with the soil sample and placed in plastic pots of 1 L capacity. A seedling of coffee cv. Catuaí was transplanted into each pot, together with either tomato (*Solanum lycopersicum* cv. Santa Cruz Kada) or sweet pepper (*Capsicum annum* cv. Early California Wonder). As the multiplication of *Meloidogyne* spp. is slow on coffee plants, a sweet pepper seedling was also transplanted when the sample roots presented symptoms characteristic of *M. exigua*. When the roots presented possible symptoms of other species, a tomato seedling was transplanted in the pot. Plants were maintained in greenhouse conditions for nematode multiplication and subsequent biochemical and physiological characterization. After 60 days, the root systems were removed from the pots, washed in running water, and nematode species identified.

2.2. Extraction of soil nematodes

In the laboratory, the 295 soil samples from Espírito Santo were subjected to centrifugal flotation (Jenkins, 1964). Identification of genera of plant nematodes was carried out with the help of a dichotomous key (Mai et al., 1996). Identification of the *Pratylenchus* and *Rotylenchulus* species followed Handoo and Golden (1989), and Robinson et al. (1997), respectively.

2.3. Isoenzymatic characterization of populations of Meloidogyne spp.

To identify *Meloidogyne* species, discontinuous vertical polyacrylamide gel electrophoresis was used (stacking 4% and separation 8%), according to the methodology of Ornstein (1964) and Davis (1964). In electrophoresis, one female per gel cavity was used when the plants presented symptoms of *M. incognita* or *M. paranaensis*. When the plants presented galls typical of *M. exigua* 25 females were used per cavity. In all gels, the protein extract of *Meloidogyne javanica* was applied to three cavities as a standard for comparison.

After completing the run, the gel was removed, placed in visualization solution and fixed in alcohol solution (Alfenas and Brune, 2006). Next, the gels were washed and dried for 24 h. For the populations reported for the first time in the state, other enzymatic standard tests were carried out, including malate dehydrogenase (MDH), superoxide dismutase (SOD) and glutamate-oxaloacetate transaminase (GOT). Also for these populations, at least 13 milky-white females were removed from infected roots and used in the preparation of the perineal patterns (Taylor and Netscher, 1974).

2.4. Physiological characterization of the Meloidogyne spp. populations

Populations of *Meloidogyne* obtained were evaluated for intraspecific variability, being submitted to the differential host tests proposed by the State University of North Carolina (Hartman and Sasser, 1985). The populations of *M. incognita* were evaluated in tobacco (*Nicotina tabacum* cv. NC 95), cotton (*Gossypium hirsutum* cv. Deltapine 61) and tomato cv. Rutgers and the populations of *M. exigua* were multiplied on tomato cv. Rutgers and on coffee cv. Catuaí Vermelho IAC 44.

Eggs from each population of *Meloidogyne* spp. were obtained according to Boneti and Ferraz (1981). Each plant was inoculated with 5000 nematode eggs from the species to be tested, and for each differential plant species six randomly distributed repetitions were used. Sixty days after inoculation, the inoculated roots were treated with Phloxine B (150 mg in 1 L of water), for 15–20 min, and then the number of galls and egg masses per root system were determined (Taylor and Sasser, 1978). Plants that presented an index higher than 2 (number of galls or egg masses > 10) were considered susceptible hosts, and those that presented indices equal to or lower than 2 (number of galls or egg masses ≤ 10) were considered resistant.

3. Results

In Espírito Santo (ES), root-knot nematode was detected in all municipalities that were evaluated, except in Colatina, Governador Linderberg, João Neiva, and Pinheiros (Fig. 1, Table 1). In Ibatiba, Ibitirama and Baixo Guandu, plant nematodes were detected in 100% of the evaluated properties and samples. Approximately 47% of the sampled coffee plantations in the state were infested with nematodes of *Meloidogyne*.

M. incognita, present in 56% of the municipalities sampled in ES, was found especially in the Serrana and northern regions of the state (Table 1), with five and eight municipalities infested, respectively. *M. incognita* was detected in 21% of the sampled properties, of which 92% were *C. canephora*. In the municipality of Brejetuba,

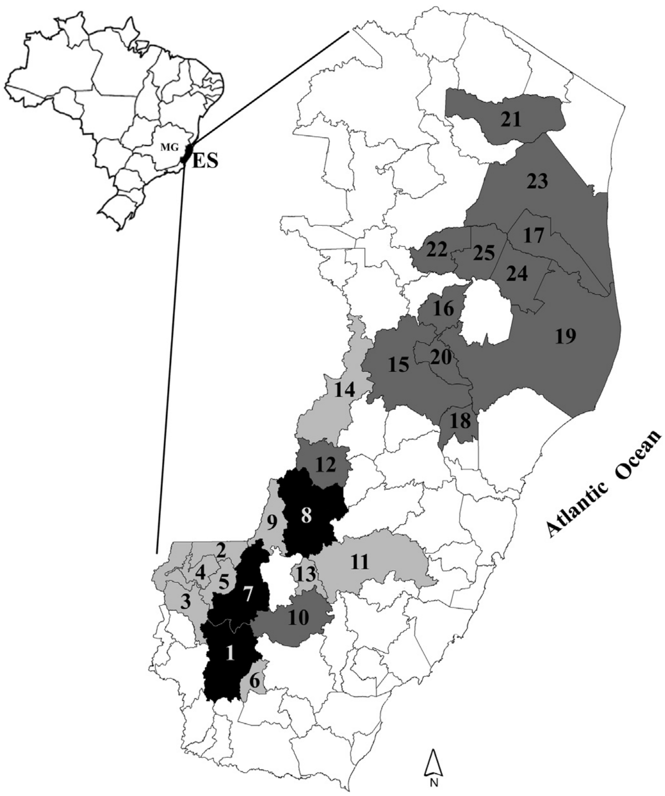

Fig. 1. Zonal distribution of sampling on coffee plantations in 25 municipalities of Espírito Santo state (ES), Brazil. For the name of the municipalities see Table 1.

M. incognita was detected on only one property, where it was found mixed with *M. exigua*. However, in some areas of Sooretama municipality, *M. incognita* was mixed with *M. paranaensis*. In Vila Valério, *M. incognita* was detected in a mixture with *Meloidogyne arenaria* on one property, but the *M. arenaria* population was not present on the coffee plant's roots, appearing instead on the weeds throughout the area. Phenotypes I1 and I2 of *M. incognita* were found on 73% and 27% of properties, respectively.

Most properties infested with *M. incognita* were race 1, which was found in Afonso Cláudio, Alegre, Baixo Guandu, Castelo, Laranja da Terra, São Mateus, Venda Nova do Imigrante and Vila Valério. Race 2, on 25% of the properties, was found in Afonso Cláudio, Linhares, Jaguaré and Sooretama.

M. paranaensis was found in the municipalities of Baixo Guandu, Jaguaré, Linhares, Sooretama and Vila Valério, in a total of 4.7% of the sampled properties (Table 1). It is notable that all these coffee farms are in the northern region of the state, where Conilon coffee is cultivated, except in Baixo Guandu, where plantations of *C. arabica* occur. All the populations of *M. paranaensis* were phenotypes P1 (EST), N1 (MDH), KP2 (SOD) and N1 (GOT), which are typical of *M. paranaensis*. By perineal pattern analysis, it could be seen that the *M. paranaensis* females presented a raised dorsal arch, with a trapezoidal format that was more evident in some cuts than in others, and smooth and wavy striae.

M. exigua, phenotype E1, was detected in 23.8% of the properties and only in *C. arabica* plantations. This species was found in all the municipalities sampled in the southern region and in 66.6% of those in the Serrana region, where Arabica coffee is grown on a large scale (Table 1). Races 1 and 2 of this species were found, but race 1 of *M. exigua*, which does not multiply on tomato, was found on only one property in both Afonso Cláudio and Brejetuba municipalities. The other *M. exigua* populations were race 2 (multiplying on tomato).

In the Zona da Mata region of Minas Gerais, only *M. exigua* race 2 with esterase phenotype E1, was found in sampled municipalities, except for Paula Cândido (Table 2). *M. exigua* was found in 61.1% of the coffee-growing properties.

In coffee plantations where *M. incognita* or *M. paranaensis* were present, plants showing stunting, yellowing and apparent nutritional deficiency were observed. The thickest roots presented irregular thickening, without the characteristic typical galls. It was common to find peeling or stripping on these roots, but peeling and galls were not observed on the radicles. In some *C. canephora* areas with *M. incognita* or *M. paranaensis*, no visible symptom was observed in the aerial part of the coffee plants, but only on the root system. In plants parasitized by *M. exigua*, rounded galls were always seen on the fine roots, and a few deaths of the lateral roots, without specific symptomatology in the aerial part.

In the soils of coffee plantations in Espírito Santo (Table 3), other nematode genera including *Rotylenchulus*, *Tylenchus* and *Helicotylenchus* (35.7; 27.5 and 17.6% of samples, respectively) were found. In the areas with *Rotylenchulus*, the species identified was *R. reniformis*, which occurred in 42.6% of the sampled municipalities. *Tylenchus*, the most widely disseminated genus, was only not present in Jerônimo Monteiro. *Helicotylenchus* was found in 50% of the sampled municipalities. The least frequently found nematodes (1.1% of the samples) were *Ditylenchus*, *Discocriconemella*, *Hemicriconemoides*, *Psilenchus* and *Rotylenchus*. The greatest diversity of nematodes associated with the coffee rhizosphere was found in Venda Nova do Imigrante, where in the 10 samples, all genera were present except *Rotylenchus* and *Hemicriconemoides*.

The root lesion nematode, *Pratylenchus brachyurus*, was found in 7.6% of the samples and in 42.6% of the sampled municipalities. In 71.4% of the infested samples, it was associated with *M. exigua*, *M. incognita* or *M. paranaensis*. In the fields, there was no difference observed between parasitism by *P. brachyurus* in *C. arabica* and in *C. canephora* (57.7% of the infested samples were from *C. arabica*) or any symptom in the plants attacked in the absence of root-knot nematodes.

In *C. arabica* plantations, 12 of the 13 genera were detected, with the exception of *Rotylenchus*. The broadest occurrence was of *Tylenchus* (41.7% of samples). In *C canephora* plantations, *Rotylenchulus*, *Tylenchus*, *Helicotylenchus*, *Pratylenchus*, *Mesocriconema*, *Xiphinema*, and *Aphelenchus* were found and of these, most notably *R. reniformis*, which was present in 57.4% of the samples.

4. Discussion

In Espírito Santo, *M. exigua* occurred more frequently than *M. incognita* and *M. paranaensis*. *M. exigua* has already been reported on *C. arabica* (Dias et al., 1996) and *M. incognita* on *C. canephora* (Lordello and Hashizume, 1971) and *C. arabica* (Sharma, 1976), but it is only recently that *M. paranaensis* has been detected on *C. arabica* (Barros et al., 2011). However, *M. paranaensis* may have been present in the region for as long as the other species. This species was described in 1996 (Carneiro et al., 1996), based mainly on biochemical characters, which were a reliable tool for the taxonomy of root-knot nematodes (Esbenshade and Triantaphyllou, 1985). Traditionally, species identification was based on analyzing female perineal pattern, but it is now known that this character is subjective because of the wide intraspecific variability in *Meloidogyne*, which can lead to doubts even among experienced nematologists. Thus, separating closely related species, such as *M. incognita*, *M. paranaensis* and *Meloidogyne enterolobii*, is imprecise based solely upon perineal patterns. Multiple methods were employed to identify *Meloidogyne* species in ES.

The populations of *M. incognita* found in coffee plantations in ES represented the most common phenotypes known in Brazilian

Table 1
Percentage of properties (samples) infested with *Meloidogyne* spp. in coffee plants (*Coffea arabica* and *C. canephora*) in Espírito Santo state.

Municipalities	No of properties (samples) analyzed		% Of properties (samples) positive					
			C. arabica (CA)			*C. canephora* (CC)		
			Esterase phenotypes[a]			Esterase phenotypes[a]		
			Me	Mi	Mp	Mi		Mp
	CA	CC	E1	I1	P1	I1	I2	P1
Southern region								
1. Alegre	1 (1)	2 (2)	100 (100)	–	–	50 (50)	–	–
2. Ibatiba	3 (3)	–	100 (100)	–	–	–	–	–
3. Ibitirama	4 (4)	–	100 (100)	–	–	–	–	–
4. Irupi	5 (5)	–	80 (80)	–	–	–	–	–
5. Iúna	5 (5)	–	20 (20)	–	–	–	–	–
6. Jerônimo Monteiro	2 (2)	–	50 (50)	–	–	–	–	–
7. Muniz Freire	4 (4)	2 (2)	50 (50)	–	–	–	–	–
Serrana region								
8. Afonso Cláudio	3 (4)	12 (37)	33.3 (50)	–	–	8.3 (5)	25 (16)	–
9. Brejetuba	13 (25)	–	92.3 (84)	7.7 (4)	–	–	–	–
10. Castelo	–	4 (9)	–	–	–	25 (11)	–	–
11. Domingos Martins	8 (11)	–	12.5 (9)	–	–	–	–	–
12. Laranja da Terra	–	5 (8)	–	–	–	40 (25)	20 (13)	–
13. Venda Nova Imig.	12 (24)	–	91.6 (71)	8.3 (4)	–	–	–	–
Northern region								
14. Baixo Guandu	2 (3)	–	–	50 (33)	100 (67)	–	–	–
15. Colatina	–	3 (4)	–	–	–	–	–	–
16. Gov. Linderberg	–	5 (5)	–	–	–	–	–	–
17. Jaguaré	–	15 (22)	–	–	–	26.7 (23)	13.3 (9)	6.7 (9)
18. João Neiva	–	5 (5)	–	–	–	–	–	–
19. Linhares	–	8 (11)	–	–	–	37.5 (36)	–	12.5 (9)
20. Marilândia	–	5 (5)	–	–	–	20.0 (20)	–	–
21. Pinheiros	–	2 (2)	–	–	–	–	–	–
22. São Gabriel Palha	–	10 (12)	–	–	–	20.0 (25)	–	–
23. São Mateus	–	7 (10)	–	–	–	28.6 (30)	–	–
24. Sooretama	–	7 (36)	–	–	–	14.3 (8)	28.6 (42)	28.6 (39)
25. Vila Valério	–	18 (34)	–	–	–	33.3 (35)	11.1 (12)	11.1 (8)
Total	62 (91)	110 (204)						

[a] Me = *M. exigua*, Mi = *M. incognita*, Mp = *M. paranaensis*.

coffee. S1 was recently identified by Oliveira et al. (2006) in a plantation in Garça, São Paulo, but had already been reported in India, in studies by Esbenshade and Triantaphyllou (1985).

Only phenotype E1 of *M. exigua* was observed in coffee plantations in Espírito Santo and in the region studied in Zona da Mata, Minas Gerais (MG). It is noteworthy that other phenotypes have already been identified in Brazilian coffee plantations, such as E2 (Oliveira et al., 2005b) and E3 (Muniz et al., 2008). In Zona da Mata, especially, according to Oliveira et al. (2005b) and Muniz et al. (2008), esterase phenotype E2 was most disseminated through the municipalities.

In ES, race 1 of *M. incognita* predominated (75%), and according to countrywide results this predominance is not generalized, because although race 1 also predominates in São Paulo (Monteiro et al., 1995), race 2 is more found in Paraná (Carneiro et al., 1990). As regards *M. exigua*, race 2 was found spread through almost all municipalities, which was new information for the state.

Table 2
Number of properties and samples with their respective percentage of occurrence of *Meloidogyne exigua* (esterase E1) in coffee plantations (*Coffea arabica*) in the Zona da Mata region of Minas Gerais.

Municipalities	No properties (no samples)	% Of properties (samples) positive
Paula Cândido	3 (6)	0
Viçosa	6 (8)	50 (37.5)
Ervália	4 (4)	100 (100)
São Miguel do Anta	2 (2)	50 (50)
Araponga	3 (3)	100 (100)
Total	18 (23)	

In the Minas Gerais municipalities, only race 2 of *M. exigua* was detected. No variability has found in the populations of *M. exigua* in Brazil (Oliveira et al., 2005b; Muniz et al., 2008).

Although *M. paranaensis* has been found in Arabica coffee in Baixo Guandu (ES), this is the first report of its presence in Jaguaré, Linhares, Sooretama and Vila Valério, in Conilon coffee. This is of concern to coffee growers in the state, because of the destructive power of this species. In infestations, *M. paranaensis* can reduce the productivity of susceptible cultivars even in their first crop, and may occur in both sandy and clay soils (Mata et al., 2000).

Other species, such as *M. javanica* and *M. arenaria*, were also observed, but parasitizing weeds present in the area. Although *M. arenaria* is reported in coffee plantations, its destructive effect on the crop is not known and, up to now, *M. javanica* has not been confirmed parasitizing coffee plants in the field. However, the occurrence of these species in the state should be evaluated if the area is replanted by crops that are good hosts.

In ES, the nematode species that are most aggressive on coffee, *M. incognita* and *M. paranaensis*, were mainly found in plantations of *C. canephora* Conilon. *C. canephora* was believed to be resistant to root-knot nematodes, but this was erroneous as most studies referred to *M. exigua* (Fazuoli et al., 1977; Ribeiro et al., 2005). This could be the reason why *M. exigua* is found widely spread in the southern region of the state, where susceptible Arabica coffee is the

Table 3
Frequency, in percentage, of the genera or species of plant nematodes in soil samples from coffee plantations in Espírito Santo state.

Municipalities	Rch[a]	Tyl	Hel	Pra	Cri	Aph	Xip	Ach	Dit	Dis	Psi	Rot	Hem
Ibitirama		33.3	33.3										
Ibatiba		33.3											
Irupi	40	20	40	20									
Iúna		60	60										
Muniz Freire	25	100		25								25	
Alegre		100											
Jerônimo Monteiro						50							
Venda Nova Imig.	20	70	50	10	10	10	10	20	10	10	10		
Brejetuba		14.3	14.3										
Domingos Martins		33.3	33.3			33.3		33.3					
Laranja da Terra	87.5	25		12.5	12.5								
Afonso Cláudio	54	8.1	8.1	2.7	5.4		2.7						
Baixo Guandu	33.3	33.3		66.7			33.3						33.3
Linhares		100						100					
% Of samples	35.7	27.5	17.6	7.6	4.5	3.7	3.3	2.2	1.1	1.1	1.1	1.1	1.1

[a] Rch = *Rotylenchulus reniformis*, Tyl = *Tylenchus* sp., Hel = *Helicotylenchus* sp., Pra = *Pratylenchus brachyurus*, Cri = *Mesocriconema* sp., Aph = *Aphelenchus* sp., Xip = *Xiphinema* sp., Ach = *Aphelenchoides* sp., Dit = *Ditylenchus* sp., Dis = *Discocriconemella* sp., Psi = *Psilenchus* sp., Rot = *Rotylenchus* sp., Hem = *Hemicriconemoides* sp.

most widely planted, and it would also explain why there are so few prospecting works on nematodes in coffee plantations. Furthermore, recent studies have shown the susceptibility of various clones of the *C. canephora* Conilon variety Vitória – Incaper 8142 to the three main species of root-knot nematode that parasitize coffee (Carneiro et al., 2009; Contarato et al., 2009).

P. brachyurus is a species that damages coffee crops, and it is more widespread in the state of São Paulo (Kubo et al., 2004). In 71.4% of the samples infested with *P. brachyurus*, populations of *M. exigua*, *M. incognita* or *M. paranaensis* were also found, as has already been observed by Souza et al. (2000) in the state of Bahia. Campos and Villain (2005) emphasized that *Pratylenchus* sp. mixed with *Meloidogyne* sp. make management more complex, because it is difficult to select non-host plants for use in the area before starting new plantations or indeed in consortium with coffee. In this work, no difference was observed between their parasitism in *C. arabica* (57.7%) or *C. canephora* (42.6% of samples infested) plants, probably because they present a similar level of susceptibility to the pathogen.

Anyway, the actual knowledge on root-knot nematodes distribution and species occurrence on the regions producing coffee on Espírito Santo reveals a condition that requires precaution to ensure the productivity of the State's crops on next decades. With the presence of these root-knot species, measures to contain their dissemination should be implemented to prevent greater damage. Dissemination can occur through the use of seedlings infected with the nematode, use of agricultural machines and implements in the infested area and through rainwater or irrigation. Therefore, producers should acquire seedlings free of plant nematodes, avoid planting new crops below infested clumps or where rainwater and irrigation can trickle down, also cleaning machinery to prevent rapid spread of the nematode.

The use of infected seedlings is certainly a possible explanation for the wide dissemination of root-knot nematodes on Arabica and Conilon coffee plants in Espírito Santo. In previous surveys (Sharma, 1976; Dias et al., 1996) the occurrence of different species and their distribution in the areas of crop growing were restricted, indicating that some form of effective dissemination of nematodes, as contaminated seedlings, has been liable for the current situation.

Uncertified seedlings should not be transported from one coffee-growing region to another, especially seedlings coming from regions where species that are aggressive to coffee predominate. As this is an extremely polyphagous soil pathogen, with the capacity to attack thousands of species of host plants, additional care should be taken with seedlings of other plant species that may contribute to transporting the nematode to another farm.

In these regions, most seedling producers are still not aware of the importance of preventive measures. The result may be broad dissemination of these nematodes, especially *Meloidogyne* spp., to areas that have been clean and where coffee production is expanding. It is of vital importance to continue surveys for nematodes in coffee-producing areas and to manage infested areas correctly; prior knowledge of nematode diversity will define breeding program targets to achieve resistance.

In this study, nematode distribution in coffee plantations in Espírito Santo state has been determined and complemented in the Zona da Mata area of Minas Gerais state. With the knowledge of the wide distribution of *M. paranaensis* in the Baixo Guandu, Jaguaré, Linhares, Sooretama and Vila Valério, comes recognition of the need to maintain *Meloidogyne* spp., on the list of 'regulated non-quarantine pests' and to intensify monitoring of seedlings in nurseries to contain dissemination.

Acknowledgments

The authors would like to thank Programa Nacional de Pesquisa e Desenvolvimento do Café (PNP & D-Café) for financial support.

References

Alfenas, A.C., Brune, W., 2006. Eletroforese em gel de poliacrilamida. In: Alfenas, A.C. (Ed.), Eletroforese de Isoenzimas e Proteínas Afins: Fundamentos e Aplicações em Plantas e Microrganismos. Editora UFV, Viçosa, Brasil, pp. 151–182.

Barbosa, D.H.S.G., Vieira, H.S., Souza, R.M., Silva, C.P., 2004. Survey of root-knot nematode (*Meloidogyne* spp.) in coffee plantations in the State of Rio de Janeiro. Nematologia Brasileira 28, 43–47.

Barros, A.F., Oliveira, R.D.L., Zambolim, L., Ferreira, A.O., Coutinho, R.R., 2011. *Meloidogyne paranaensis* attacking coffee trees in Espírito Santo State, Brazil. Australas. Plant Dis. Notes 6, 43–45.

Boneti, J.I.S., Ferraz, S., 1981. Modificação do método de Hussey and Barker para extração de ovos de *Meloidogyne exigua* de raízes de cafeeiro. Fitopatologia Brasileira 6, 553.

Campos, V.P., Villain, L., 2005. Nematodes parasites of coffee and cocoa. In: Luc, M., Sikora, A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB. International, Wallingford, pp. 529–579.

Carneiro, R.G., Antonio, H., Brito, J.A., Alteia, A.A.K., 1990. Identificação de espécies e raças de *Meloidogyne* na região noroeste do Estado de São Paulo: resultados premilinares. In: XIV Congresso Brasileiro de Nematologia, Londrina (Resumos), p. 4.

Carneiro, R.M.D.G., Carneiro, R.G., Abrantes, M.O., Santos, M.S.N.A., Almeida, M.R., 1996. *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. J. Nematol. 28, 177–189.

Carneiro, R.M.D.G., Costa, S.B., Sousa, F.R., Santos, D.F., Almeida, M.R.A., Santos, M.F.A., Siqueira, K.M.S., Tigano, M.S., Fonseca, A.F.A., 2009. Reação de cafeeiros 'Conilon' a diferentes populações de *Meloidogyne* spp.. In: VI Simpósio de Pesquisa dos Cafés do Brasil, Vitória, ES (Resumos).

Castro, J.M.C., Campos, V.P., Pozza, E.A., Naves, R.L., Júnior, W.C.A., Dutra, M.R., Coimbra, J.L., Maximiniano, C., Silva, J.R.C., 2008. Levantamento de Fitonematóides em Cafezais do Sul de Minas Gerais. Nematologia Brasileira 32, 56–64.

Conab: Convênio Ministério da Agricultura – Secretaria da Produção e Comercialização, 2012. Acompanhamento da safra brasileira de café, safra 2012, setembro/2012. Terceira estimativa. Disponível em: <http://www.conab.gov.br/OlalaCMS/uploads/arquivos/12_09_06_10_10_21_boletim_cafe_-_setembro_2012.pdf>. Acesso 11 de setembro de 2012.

Contarato, C.C., Tomaz, M.A., Sobreira, F.M., Alves, F.R., Jesus Jr., W.C., Rodrigues, A.A., Ferrão, M.A.G., Ferrão, R.G., 2009. Resistência da variedade "Vitória Incaper 8142" de café conilon a Meloidogyne exigua. In: VI Simpósio de Pesquisa dos Cafés do Brasil, Vitória, ES (Resumos).

Davis, B.J.B., 1964. Disk electrophoresis. II. Method and application to human serum proteins. Ann. N Y Acad. Sci. 121, 404–427.

Dias, W.P., Liberato, J.R., Fonseca, A.F.A., 1996. Nematóide associados ao cafeeiro no estado do Espírito Santo. Revista Ceres 43, 808–812.

Esbenshade, P.R., Triantaphyllou, A.C., 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. J. Nematol. 17, 6–20.

Fazuoli, L.C., Mônaco, L.C., Carvalho, A., Reis, A.J., 1977. Resistência do cafeeiro a nematoides. I – Testes em progênies e híbridos, para *Meloidogyne exigua*. Bragantia 36, 297–307.

Handoo, Z.H., Golden, A.M., 1989. A key and diagnostic compendium to the species of the *Pratylenchus* Filipjev, 1936 (lesion nematodes). J. Nematol. 21, 202–218.

Hartman, K.M., Sasser, J.N., 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker, K.R., Carter, C.C., Sasser, J.N. (Eds.), An Advanced Treatise on *Meloidogyne*. North Carolina State University, North Carolina, pp. 69–77.

Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48, 629.

Kubo, R.K., Oliveira, C.M.G., Antedomênico, S.R., Monteiro, A.R., Ferraz, L.C.C.B., Inomoto, M.M., 2004. Ocorrência de nematóides do gênero *Pratylenchus* em cafezais do Estado de São Paulo. Nematologia Brasileira 28, 159–165.

Lordello, L.G.E., Hashizume, H., 1971. Suscetibilidade da variedade Kouillou de *C. canephora* a um nematóide. Revista de Agricultura 46, 157–158.

Mai, W.F., Mullin, P.G., Lyon, H.H., Loeffler, K., 1996. Plant-parasitic Nematodes: A Pictorial Key to Genera, fifth ed. Cornell University Press, USA.

Mata, J.S., Sera, T., Azevedo, J.A., Altéia, M.Z., Colombo, L.A., Sanches, R.S., Petek, M.R., Fadelli, S., 2000. Seleção para resistência ao nematóide *Meloidogyne paranaensis* EMN-95001: IAPARLN 94066 de "Catuaí x Icatu" em área altamente infestada. In: I Simpósio de Pesquisa dos Cafés do Brasil, Poços de Caldas MG (Resumos), pp. 515–518.

Monteiro, A.R., Oliveira, C.M.G., Ferraz, L.C.C.B., Gonçalves, W., 1995. Identificação morfológica de populações de *Meloidogyne* de cafezais paulistas. In: Congresso Internacional de Nematologia Tropical, Rio Quente (Resumos), p. 82.

Muniz, M.D.S., Campos, V.P., Castagnone-Sereno, P., Castro, J.M.D.E., Almeida, M.R.A., Carneiro, R.M.D.G., 2008. Diversity of *Meloidogyne exigua* (Tylenchida: Meloidogynidae) populations from coffee and rubber tree. Nematology 10, 897–910.

Oliveira, D.S., Oliveira, R.D.L., Freitas, L.G., Silva, R.V., 2005a. Variability of *Meloidogyne exigua* on coffee in the Zona da Mata of Minas Gerais State, Brazil. J. Nematol. 37, 323–327.

Oliveira, D.S., Oliveira, R.D.L., Gonçalves, W., 2006. Fenótipo S1 de esterase em *Meloidogyne incognita* no Brasil. Fitopatologia Brasileira 31, 207.

Oliveira, D.S., Oliveira, R.D.L., Silva, R.V., 2005b. Caracterização fisiológica de populações de *Meloidogyne exigua* associadas a cafeeiros na Zona da Mata de Minas Gerais. Nematologia Brasileira 29, 279–283.

Ornstein, L., 1964. Disc electrophoreses. I. Background and theory. Ann. N Y Acad. Sci. 121, 321–349.

Ribeiro, R.C.F., Pereira, A.A., Oliveira, C.H., Lima, R.D., 2005. Resistência de progênies de híbridos interespecíficos de *Coffea arabica* e *Coffea canephora* a *Meloidogyne exigua*. Nematologia Brasileira 29, 11–16.

Robinson, A.F., Inserra, R.N., Caswell-Chen, E.P., Vovlas, N., Troccoli, A., 1997. *Rotylenchulus* species: identification, distribution, host ranges, and crop plant resistance. Nematropica 27, 127–180.

Sharma, R.D., 1976. Nematodes of the cocoa region of the state of Espírito Santo, Brazil. II. Nematodes associated with field crops and forest trees. Revista Theobroma 6, 109–117.

Souza, S.E., Santos, J.M., Matos, R.V., Ramos, J.A., Santos, F.S., Ferraz, R.C.N., Carvalho, G.S., Oliveira, C.A., 2000. Levantamento preliminar de *Meloidogyne* em cafeeiros no Estado da Bahia – Planalto de Vitória da Conquista e Chapada Diamantina. In: Simpósio de Pesquisa dos Cafés do Brasil. Poços de Caldas MG (Resumos), pp. 167–170.

Taylor, A.L., Sasser, J.N., 1978. Biology, Identification and Control of Rootknot Nematodes (*Meloidogyne* spp.). North Carolina State University Graphics Raleigh: Coop. Publ. Dep. Plant. Pathol.

Taylor, D.P., Netscher, C., 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica 20, 268–269.