ROOT-KNOT AND LESION NEMATODES IN COFFEE SEEDLINGS PRODUCED IN THE STATE OF MINAS GERAIS, BRAZIL

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(Received: May 26, 2017; accepted: October 16, 2017)

ABSTRACT: Understanding the mechanisms of plant-parasitic nematodes (PPN) dispersion is vital to improve control strategies aiming to restrict dissemination of these plant parasites. In the present work, we evaluated the presence of PPN in Arabic coffee (*Coffea arabica*) seedlings produced in commercial nurseries in Minas Gerais, state, Brazil. A total of 2830 samples obtained from 318 coffee nurseries, in 84 counties within the South and Zona da Mata regions in Minas Gerais, Brazil and representing more than 62 million coffee seedlings, were analyzed. *Meloidogyne* spp. was identified in 11 samples from four counties. *Pratylenchus* spp. and *Rotylenchulus reniformis* were detected in 281 and 47 samples, respectively. According to the Regulatory Instruction N° 35 from the Ministry of Agriculture, Livestock and Food Supply (MAPA), in Brazil, coffee seedlings infected by *Meloidogyne* spp. are prohibited for commercialization and/or planting. However, such restrictions do not apply to other PPN. Therefore, seedlings sold in Minas Gerais may constitute sources of dissemination for root-lesion nematodes *(Pratylenchus* spp.*)* and the reniform nematode *(R. reniformis)*.

Index terms: *Coffea arabica, Meloidogyne* spp.*, Pratylenchus* spp.*,* Seedlings.

NEMATOIDE DE GALHAS E DAS LESÕES RADICULARES EM MUDAS DE CAFÉ PRODUZIDAS NO ESTADO DE MINAS GERAIS

RESUMO: O entendimento dos mecanismos de dispersão dos fitonematoides é fundamental para o desenvolvimento de estratégias visando restringir a sua disseminação. No presente trabalho foi avaliada a presença de fitonematoides em mudas de café arábica (*Coffea arabica*) produzidas em viveiros comerciais no estado de Minas Gerais. Foram analisadas 2830 amostras, enviadas de 318 viveiros, localizados em 84 municípios, das regiões Sul e Zona da Mata, representando um total de mais 62 milhões de mudas. *Meloidogyne* spp. foi identificado em 11 amostras, enviadas de quatro municípios. A presença *Pratylenchus* spp. e *Rotylenchulus reniformes* foi constatada em, 281 e 47 amostras, respectivamente. Segundo a instrução normativa N° 35 do Ministério de Agricultura, Pecuária e Abastecimento (MAPA), mudas de café infectadas com *Meloidogyne* spp. são impedidas de comercialização e plantio. No entanto, tais restrições não se aplicam a outros fitonematoides. Portanto, as mudas comercializadas em Minas Gerais podem atuar como em agentes disseminadores dos nematoides das lesões (*Pratylenchus* spp.) e do nematoide reniforme (*R. reniformes*).

Termos para indexação: *Coffea arabica, Meloidogyne* spp.*, Pratylenchus* spp.*,* mudas.

1 INTRODUCTION

In 2016, the gross revenue of Brazilian coffee production was estimated in US$ 7.62 billion (CONSÓRCIO PESQUISA CAFÉ 2016). In the state of Minas Gerais, accountable for more than 70% of the nations´ production of Arabic coffee (CONAB, 2016), coffee plantations generates jobs and preserves workforce in rural areas (SANTOS et al., 2009).

Plant-parasitic nematodes (PPN) are considered a limiting factor for coffee production (CAMPOS; VILLAIN, 2005; CARNEIRO et al., 2008). In Brazil, coffee producers have coexisted with these parasites since the end of the 19th century (GOELDI, 1887). Since then, the devastating effects of diseases caused by them have been reported in all production areas of the country (CASTRO et al., 2008; REZENDE et al., 2013; SALGADO et al., 2015).

Root-knot nematodes (*Meloidogyne* spp.), particularly *Meloidogyne exigua, Meloidogyne paranaensis* and *Meloidogyne incognita,* are the most important species for coffee crops in Brazil due to their destructive potential. Coffee plants infested by *M. exigua* are usually able to sustain a reasonable production; however, up to 45% yield losses may occur (BARBOSA et al., 2004a 2004b; BARBOSA; SOUZA; VIEIRA, 2010; MUNIZ et al., 2OO8). Parasitism by *M. incognita* and *M. paranaensis* produces more drastic symptoms, causing death of severe infested plantation and making planting of susceptible crops on infested areas an anti-economic activity (BOISSEAU et al., 2009; SHIGUEOKA et al., 2016).

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Another group of nematodes frequently found in coffee areas are the root-lesion nematodes (CASTRO et al., 2008; KUBO; EULALIO; OLIVEIRA, 2015) from the genus *Pratylenchus* spp. In India and Indonesia, *Pratylenchus coffeae* is the most destructive PPN in coffee crops (WIRYADIPUTRA; TRAN, 2008). In Central America this species is also harmful (VILLAIN; HERNÁNDEZ; ANZUETO, 2008), while in Brazil reports of coffee crops being anti-economic due to the incidence of this species are unusual (MOURA, PEDROSA, PRADO, 2002). The reniform nematode, *Rotylenchulus reniformis*, is also a frequently reported species in Brazilian coffee crops (BARROS et al., 2014; CASTRO et al., 2008), however, without any references about its effect.

In the southern region of Minas Gerais state, accountable for more than 54% of the coffee production in the state (CONAB, 2016), the presence of *M. paranaensis* and *M. incognita* in coffee crops was not verified after a broad survey performed in 2008 (CASTRO et al., 2008). However, during the last nine years, *M. paranaensis* was identified in plantations in five counties within this region (Alpinópolis, Coqueiral, Três Pontas) (SALGADO et al., 2015), Carmo do Rio Claro and Carmo de Cachoeira. In such plantations, the presence of this nematode caused severe damages. Thus, it urges to know and understand the factors that have contributed to disseminate this nematode in coffee crops within the main producing region in the country.

Use of coffee seedlings infected with nematodes is the main and more efficient form of dissemination for these microorganisms. During the 70´s the employment of coffee seedlings infected with *M. incognita* produced in the state of Parana, was responsible for the introduction of this nematode in several non-infested areas in the state of Sao Paulo (Ferraz, 2008). In 1976/1977, approximately 3.3 million infected coffee seedlings were discarded only in the state of Sao Paulo (FERRAZ, 2008).

Planting seedlings free from pathogens is imperative to stablish perennial crops, thus, the definition of phytosanitary standards for nematodes in coffee seedlings is primordial. Policies and standards for production and commercialization of coffee (*Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner) propagation's material were established by the Ministry of Agriculture, Livestock and Supply (MAPA), through the Regulatory Instruction (IN 35) in November 29th 2012.

Despite the improvements occurred in the last decades concerning the awareness of growers and extension workers about the importance of PPN; dissemination of extremely harmful species for coffee crops such as *M. paranaensis* is still increasing in Brazil. Besides that, until now, there is no broad survey of nematodes in coffee seedlings traded in the state of Minas Gerais. Thus, the present work had the objective to verify the phytosanitary status of coffee seedlings regarding the most harmful phytonematodes in coffee crops.

2 MATERIALS AND METHODS

2.1 Analysis of samples

According to the Regulatory Instruction IN 35 from MAPA, in order to perform nematological analyses the laboratory must be registered in the National Network of Agricultural Laboratories of the Unified System of Agricultural Health Service, affiliated to the National Registry of Seeds and Seedlings (RENASEM).

Samples were received at the Laboratory of Nematology from the Federal University of Lavras and were initially evaluated regarding to the number of seedlings sampled per plot. Samples in divergence with IN 35 standards were not analyzed. Correctly sampled trials were visually checked for root-knots. Once root-knots were identified they were detached to be dissected under stereo microscope where females of *Meloidogyne* spp. were evaluated. The remaining roots were placed in a blender with 200 ml water and milled for 20 seconds. Then, the resulting material was sieved with a 425 µm sieve attached over a 38 µm sieve. The material retained in the 38 µm sieve was then collected and centrifuged. Then, the supernatant was observed under light microscope in order to evaluate the presence of juveniles of *Meloidogyne* spp., adults of *Pratylenchus* spp. and females of *R. reniformis*.

During a four months period (October 2016 to January 2017) 2830 samples were analyzed. All samples were tested for presence of *Meloidogyne* spp., however, the presence of *R. reniformes* and *Pratylenchus* spp. was evaluated in 2414 samples. Samples were received form 84 counties, being 83 counties from the state of Minas Gerais (59 - regions South and Central West, 23 - Zona da Mata and Vale do Rio Doce and one from the Triângulo Mineiro) and one from the state of São Paulo. In relation to nurseries, 318 sent samples (251 - South and Central West, 65 - Zona da Mata and Vale do Rio Doce, one from the Triângulo Mineiro and one from the state of São Paulo) (Figure 1). Samples received represented a total of 62.781.899 seedlings from 31 cultivars of *C. arabica*.

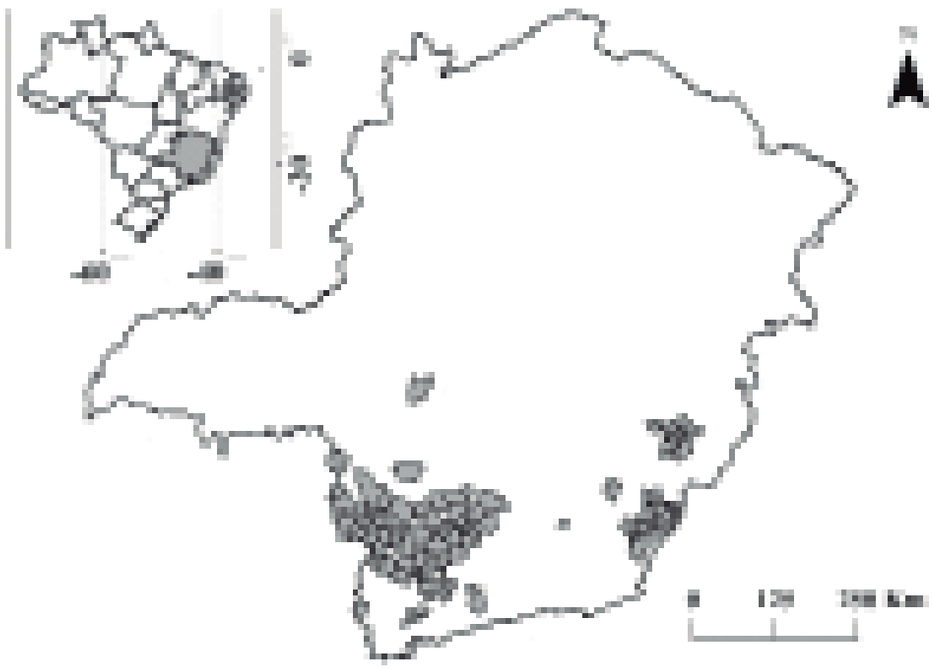

FIGURE 1 - Map of the Minas Gerais state. Highlighted counties that sent samples

3 RESULTS AND DISCUSSION

The presence of *Meloidogyne* spp. was observed in 11 samples obtained from four nurseries in the county of Rosário da Limeira, Muriaé, Carangola and Cabo Verde (Table 1). In these nurseries plants from the cultivars Catuaí Vermelho IAC 44 and Paraíso MG 2 were infected. Unfortunately, due to the great number of samples analyzed and the availability of a short period of time to release the technical report, it was not possible to identify *Meloidogyne* and *Pratylenchus* to the species level. Presence of root-knot nematodes (*Meloidogyne* spp.) in coffee plantations results in low productivity, ineffectiveness of soil fertilization, predisposition to parasitism by soil fungi, higher sensitivity to water stress and reduction of prices of agricultural lands (CAMPOS; VILLAIN, 2005; PEREIRA et al., 2012; SALGADO; REZENDE; CAMPOS 2005). Nevertheless, production of coffee seedlings infected by root-knot nematodes has being going on for decades in Brazil (FERRAZ, 2008). In this study, the presence of *Meloidogyne* spp., in 11 samples, proves the risk of dissemination of these PPN through seedlings is still imminent. Thus, surveillance of state agencies over coffee seedlings' trade avoid dissemination of root-knot nematodes.

The presence of *Pratylenchus* spp. was verified in 281 samples, representing 11.6% of the total evaluated samples. The root-lesion nematode was found in samples sent from 39 counties in ten coffee cultivars (Table 1 and Figure 2), however, with a low population density in the samples, mostly lower than 10 specimens per sample. The higher value found was of 38 specimens in a sample.

In India and Indonesia, *P. coffeae* is the most destructive PPN in coffee crops (WIRYADIPUTRA; TRAN, 2008). In Central America lesion nematodes are widely distributed and cause severe losses (VILLAIN; HERNANDEZ; ANZUETO, 2008). In Brazil, despite being a common group of PPN, their influence on coffee plantations has yet to be determined, even though there are reports of the damaging potential of this group of nematodes in coffee seedlings (KUBO et al., 2003; TOMAZINI et al., 2005). Inomoto et al. (2007), suggested that some populations of *Pratylenchus* spp. are highly pathogenic to coffee plants in Brazil, causing damages even when in low population densities. Nevertheless, there are few records of unproductive coffee plantations due to the incidence of these nematodes (MOURA, PEDROSA, PRADO, 2002).

TABLE 1 - Counties, number of samples and nurseries investigated as incidence of *Meloidogyne* spp., *Pratylenchus* spp. e *Rotylenchulus reniformes* .

Counties	*N of samples*	*N of nurseries*	*Meloidogyne* spp	*Pratylenchus* spp	*R. reniformis*
Alfenas	8	1			
Alpinópolis	8	2		X	
Alterosa	26	4		x	X
Andradas	20	1		X	
Areado	32	1			
Baependi	12	1		X	
Boa Esperança	84	12		x	X
Bom Jesus da Penha	4	1			
Bom Sucesso	32	2		X	
Botelhos	60	7		X	
Cabo Verde	44	6	x	X	
Cachoeira de Minas	12	1			
Caiana	8	1		X	
Camacho	8	1			
Cambuquira	24	2		X	
Campanha	8	1			
Campestre	32	7		x	X
Campo Belo	28	2			X
Campos Gerais	64	10			
Candeias	56	7		x	X
Caparaó	8	1			
Capela Nova	4	1			
Carangola	20	2	x		
Carantiga	16	2			
Carmo da Cachoeira	20	2		x	X
Carmo do Rio Claro	16	4		x	
Carvalhópolis	24	4			X
Conceição da Aparecida	20	3		x	X
Coqueiral	56	5		x	
Cordislândia	12	1		X	
Cristais	32	4		x	X
Divino	140	15		x	X
Elói Mendes	12	2			
Ervalia	12	2			
Espera Feliz	72	7			
Espírito Santo do Pinhal (SP)	20	1			
Fervedouro	64	10		X	
Guaranésia	4	1			
Guaxupé	128	11		X	X
Heliodora	12	3		X	
Ibiraci	72	6		X	
Ilicínea	28	3		x	X
Imbé de Minas	4	1			
Inhapim	4	1			

Itamogi	52	6			x	
Jacuí	24	3			x	
Jacutinga	100	8			X	
Jesuânia	32	5				
Juruaia	16	2			x	
Lavras	12	2				
Machado	164	17			x	X
Miradouro	4	1				
Monte Belo	12	2			x	
Monte Santo de Minas	36	4				
Muriaé	4	1				
Muzambinho	84	9				
Nepomuceno	88	13				
Nova Resende	72	8				
Oliveira	60	5				
Orizania	8	1				
Paraguaçu	16	2				
Passos	16	2				
Pedra Bonita	24	2			x	
Perdões	16	1				
Piumhi	96	5				
Poço Fundo	28	4				
Ponte Nova	4	1				
Rosário da Limeira	20	4	x		x	
Santa Margarida	8	2				
Santa Rita do Sapucaí	20	2				
Santo Antônio do Amparo	28	2				
São Domingos das Dores	8	2				
São Francisco do Glória	8	1				
São Gonçalo do Sapucaí	12	1				
São Gotardo	4	1			X	
São Pedro da União	12	1				
São Sebastião da Anta	12	1				
São Sebastião do Paraíso	32	3			x	X
São Tomás de Aquino	16	1				
Tombos	4	1				
Três Pontas	260	22			x	
Ubaporanga	16	4				
Varginha	16	2				
Vieiras	16	2				

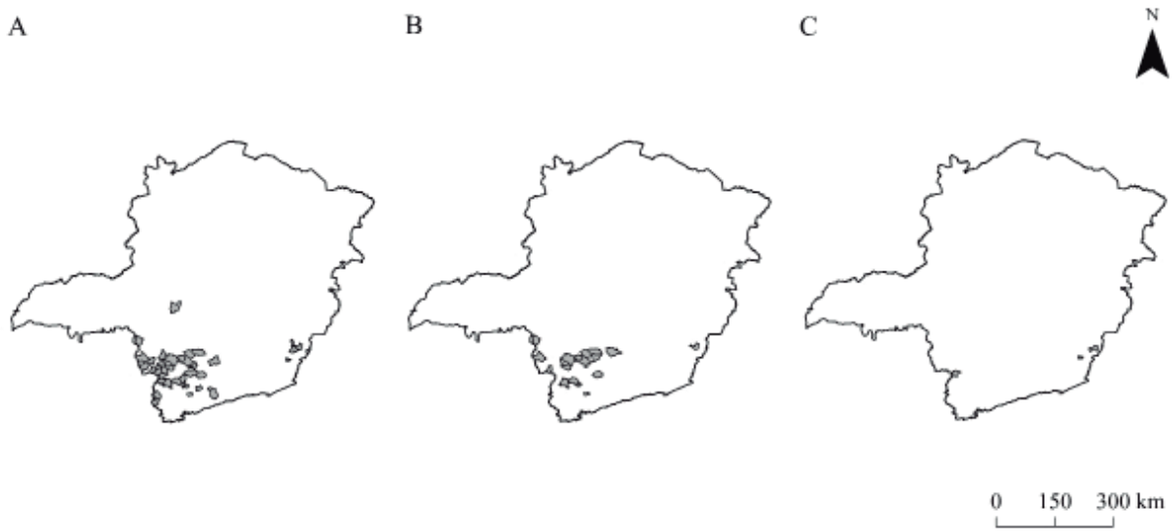

FIGURE 2 - Map of the Minas Gerais state. **A** counties with *Pratylenchus* spp. incidence. **B** counties with *Rotylenchulus reniformis* spp. incidence. **C** counties with *Meloidogyne* spp. incidence.

In the present study, the low population of *Pratylenchus* spp. observed in the samples may suggest the presence of some resistance factor in the cultivars evaluated.

Females of *R. reniformes* were observed in 47 samples, received from 15 counties in six coffee cultivars (Table 2), representing 1.94% of the evaluated samples. Only one sample had a population density higher than 20 females of *R. reniformes*.

However, the presence of *R. reniformes* juveniles above 20 specimens per sample was observed in some cases. The reniform nematode (*R. reniformes*) is frequently associated to coffee roots (CASTRO et al., 2008; BARROS et al., 2014). However, Kubo et al., (2009) demonstrated that eight coffee cultivars inoculated with a population of *R. reniformes* did not perform as suitable hosts for this nematode. However, in the present study a sample from cultivar Oeiras was observed with a high number of *R. reniformes* females. Studies concerning resistance of this cultivar against reniform nematodes are scarce.

The infested nurseries by *Pratylenchus* spp. and *R. reniformis* observed in this study were allowed to sell seedling to the coffee farmers. Dispersion of PPN in coffee areas occurs via propagation materials, agricultural tools and floods. However, the most efficient way for dissemination occurs via plant roots, especially seedlings of coffee or arboreal species used in the plantation. Therefore, is important that nurseries and public control agencies constantly inspect the phytosanitary quality of seedlings produced, especially concerning to the presence of PPN (CAMPOS; VILLAIN, 2005)

Regulatory Instruction IN 35 from MAPA determines the technical manager of the nursery as responsible for collecting seedlings samples. However, the number of incorrectly obtained samples received in the laboratories is substantial; in the majority of cases were samples with insufficient number of seedlings. This fact illustrates that care should be taken by the people responsible for sampling to fulfill the standards established by MAPA.

Samples analyzed represent more than 62 million coffee seedlings, 84% of them are traditional *C.* cultivars Catuaí and Mundo Novo (Table 3), which are susceptible to root-knot nematodes. Thus, proper care during sampling in the nurseries and commitment of surveillance institutions to avoid planting of infested seedlings are guaranties to avoid dissemination of PPN. Nevertheless, other PPN may be disseminated, once they are not included in the system of inspection by the surveillance institutions.

TABLE 2 - Coffee cultivars associated with nematodes *Meloidogyne* spp., *Pratylenchus* spp. e *Rotylenchulus reniformes.*

Meloidogyne spp	*Pratylenchus* spp	*R. reniformis*
Catuai vermelho IAC 44	Catuai vermelho IAC 144	Catuai vermelho IAC 144
Paraíso MGS 2	Catuai vermelho IAC 44	Catuai amarelo IAC 62
	Catuai vermelho IAC 99	Mundo novo IAC 379-19
	Catuai amarelo IAC 62	Mundo novo IAC 376-4
	Catuai amarelo IAC 2SL	Paraiso MG H 491-1
	Mundo novo IAC 379-19	Acauã
	Mundo novo IAC 376-4	
	Catucai 785-15	
	Topázio	
	Bourbon IAC J10	

TABLE 3 - Total number of seedlings analyzed by cultivar

Cultivars	Total seedlings
Catuaí Vermelho IAC 144	17,757,302
Catuaí Vermelho IAC 44	3,111,582
Catuaí Vermelho IAC 99	7,309,370
Catuaí amarelo IAC 62	9,666,624
Catuaí amarelo IAC 39	95,200
Catuaí amarelo 24/137	283,500
Catuaí amarelo MULTILINEA F5	280,000
Catuaí amarelo 2015	90,000
Mundo Novo IAC 376-4	5,974,127
Mundo Novo IAC 379-19	8,341,502
Mundo Novo ACAIA	25,000
Catucai 785-15	2,237,966
Catucai amarelo 2SL	2,430,951
Catucai 24-137	142,179
Catucai vermelho MFS	120,000
Oeiras MG6851	628,884
Bourbon amarelo IAC J9	167,791
Bourbon amarelo IAC J10	415,993
Acaia IAC 474/19	1,216,070
Acaia do cerrado 1479	52,200
Topazio MG 1190	1,499,000
Arara	136,000
Icatu amarelo	20,000
Acaua	114,000
Acaua Novo	40,000
MGS Paraiso 2	50,000
Paraiso MG H 419-1	19,457
Obatã IAC 1669-20	355,962
Catigua MG2	63,282
MGS ARANAS	17,957
Catucaiam 24137	120,000
Total seedlings analyzed	62,781,899

4 CONCLUSION

Coffee seedlings produced in Minas Gerais may disseminate PPN such as *Pratylenchus* spp. and *Rotylenchulus reniformes*, which are not targeted by the seedlings' surveillance system.

5 ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Brazilian financial support provided by FAPEMIG, CAPES, CNPq, Instituto Nacional de Ciência e Tecnologia do Café INCT- Café, and Consórcio Pesquisa Café.

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