Nematological survey in coffee nursery in Espirito Santo state, Brazil

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ABSTRACT

Despite the important role of coffee production in the economy of Espirito Santo, the second largest coffee producing state in Brazil, productivity is still below the Brazilian average. One of the factors that explain this low productivity is the presence of nematodes of the genus *Meloidogyne*. Contaminated seedlings are an important and efficient agent for disseminating nematodes. According to normative instruction no. 35 (IN 35), of 11/29/2012 of the Ministry of Agriculture, Livestock and Supply (MAPA), the presence of *Meloidogyne* spp. in a single plant among a field-lot of seedlings condemns it, and the plants that compose that lot should be destroyed. In Espirito Santo, no evaluation has been carried out in nurseries covering the entire State for phytonematode detection. Therefore, the objective of this work was to carry out a nematological survey in nurseries to guide the nurserymen in relation to fulfilling IN 35 of MAPA, in addition to guiding them regarding the phytosanitary care during the production of their seedlings. The nurseries were evaluated in 19 municipalities located in both the north and the south of Espírito Santo. There were *Meloidogyne* spp. in evaluated samples.

Keywords: nematodes; *Coffea* spp.; seedlings.

INTRODUCTION

Coffee farming plays a socioeconomic role of great importance for the State of Espírito Santo. According to data collected by the Capixaba Institute for Research, Technical Assistance and Rural Extension (Incaper), Espírito Santo harvested about 9 million sacks of coffee in 2016. Although the state continues to be the country's second largest coffee producer, productivity in Espírito Santo has historically always been below the Brazilian average, which is around 24 sc/ha (CONAB, 2017).

Many factors may be related to low coffee productivity, such as nematodes. The main species of nematodes associated with coffee tree belong to the genus *Meloidogyne*, with emphasis on *M. incognita*, *M. paranaensis*, *M. exigua*, and *M. coffeicola* (Barbosa, 2004; Contarato *et al*., 2014; Sera *et al*., 2017).

Barros *et al*. (2014) made a nematological survey in coffee fields in the State of Espírito Santo regarding the presence of *Meloidogyne* spp. in *Coffea* spp. and reported the presence of *M. exigua*, *M. incognita* and *M. paranaensis*, these latter two species being very aggressive to the coffee tree.

One of the most efficient agents to disseminate nematodes is contaminated seedlings, which makes it necessary to raise awareness about the preventive measures that should be taken by coffee nurseries regarding phytosanitary care during the production of their seedlings (Gonçalves & Silvarola, 2001; Lordello *et al*., 2001).

Normative instruction no. 35 (IN 35) of 11/29/2012 of the Ministry of Agriculture, Livestock and Supply (MAPA) states that if, under laboratory analysis of coffee plants, the presence of *Meloidogyne* spp. is proven in a

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field-lot of seedlings, the sample will be condemned and the seedlings will be destroyed.

Whereas no evaluation has been performed in nurseries covering the entire state of Espirito Santo regarding the presence of phytonematodes, this study aimed to carry out a nematological survey in nurseries, with the following goals: i) to ascertain the possible presence of phytonematodes in nurseries; ii) to guide the nurserymen about fulfilling IN 35 of MAPA; iii) to alert the nurserymen about the phytosanitary care they should employ during the production of their seedlings and, in this way, prevent the dissemination of phytonematodes in coffee farming areas in the State.

MATERIAL AND METHODS

The present project was performed between the years 2011 and 2013. During this period, nematological surveys were carried out in coffee farms implanted in the State of Espírito Santo and registered with the Agricultural Inspection Service - SEFAG/DT.

Samples from 85 nurseries were collected in 21 municipalities located in the north and south of Espírito Santo state, namely: Alegre, Anchieta, Brejetuba, Cachoeiro de Itepemirim, Castelo, Colatina, Conceição do Castelo, Guaçuí, Ibatiba, Ibitirama, Iconha, Iúna, Jaguaré, Linhares, Muniz Freire, Rio Bananal, Santa Teresa, São Gabriel da Palha, São Roque do Canaã, Sooretama and Venda Nova do Imigrante.

For collection of seedlings, sampling was performed following the recommendations of Salgado *et al*. (2007), as follows: a) the nurseries were divided into lots of a maximum of 200,000 seedlings; b) each lot was divided into four quadrants; c) in each quadrant, alternated seedbeds were selected for sampling and 0.1% of the seedlings were evaluated, with a minimum of 30 seedlings; d) each seedbed was divided into five sectors numbered from 1 to 5, from the extremities. In the center of sectors 1, 2, 4 and 5, two seedlings were removed. At the center of sector 3, four seedlings were removed; e) a composite sample was formed with all the plants from each quadrant, which were packed in plastic bags and labeled.

Subsequently, the samples were sent to the phytopathology/nematology laboratory of the Center of Agricultural Sciences and Engineering of UFES (CCAE-UFES), where extractions and evaluation of soil and root samples were carried out for the presence of phytonematodes.

For the extraction of nematodes from the soil, the method of Jenkins (1964) was used, and for the root nematode extraction, the Coolen & D'Herde technique (1972). After the extractions of soil and root nematodes were finished, the evaluations were done in an inverted trinocular biological microscope for identification at the genus level of phytonematodes with the aid of specific identification keys.

When nematodes of the genus *Meloidogyne* were found, the genus was confirmed only by the observation of second-stage juveniles (J2), because one of the main goals of the work was to guide the nurserymen in fulfilling IN 35 of MAPA, in which the presence of a single individual of the genus is sufficient to condemn the subplots, regardless of the species, so that the seedlings therein contained must be destroyed.

RESULTS AND DISCUSSION

Some nurseries that researchers expected to visit were not accessed because no one was found at the time of the visit.

It is very important to make nematological surveys not only in the field but also in nurseries, because despite the growing incentive for the production of quality coffee seedlings, *Meloidogyne* spp. were found in Ibatiba, Santa Teresa, Linhares, Rio Bananal, São Gabriel da Palha, Sooretama and Jaguaré (Table 1, Figures 1a, b, c, e, f, respectively), and *Helicotylenchus* sp. was present in Castelo and Venda Nova do Imigrante. According to Campos (1999), once introduced in the area, it is extremely difficult to manage phytonematodes of economic importance in perennial crops, such as coffee.

Normative instruction (IN) no. 35, of 11/29/2012 of the Ministry of Agriculture, Livestock and Supply (MAPA), states in Article 29 of Chapter III that 'the seedlings will be sampled before commercialization, in order to verify the existence of defective roots and *Meloidogyne* spp. It is further stated in Article 32 that in any subplot where the result of the analysis proves the presence of *Meloidogyne* spp. will be condemned and the seedlings must be destroyed by the producer. Article 34 reaffirms that 'the coffee seedling should be free from *Meloidogyne* spp'. According to this information, if the seedlings from nurseries where *Meloidogyne* spp. were present are submitted to an evaluation before a certificate of phytosanitary origin (CFO) is issued, those seedlings in the sub-plots where the nematodes were detected must be destroyed.

Barros *et al*. (2014) studied the distribution of *Meloidogyne* spp. in *Coffea* spp. in some properties of the main coffee farming regions in the State of Espírito Santo. *M. incognita* was present in 21% of the properties. This species, one of the most aggressive to the coffee tree, was mainly associated with *C. canephora* in fields in the northern and mountainous regions of the State. *M. exigua* was found mainly in *C. arabica* in 23.8% of the properties, and it was present in 66.6% of the municipalities of the mountainous region and in all the southern areas. *M. paranaensis* was present only in the north, and it was found in 100% of the properties of Baixo Guandu.

Table 1: Municipalities of the State of Espirito Santo where evaluations were carried out for the presence of phytonematodes in coffee nurseries between the years 2011 and 2013. The municipalities were divided per region according to Law 11,174 of 09/25/2020

Year	Municipality evaluated per region	Number of nurseries evaluated		Number of samples evaluated per nursery		Number of nurseries where phytonematodes were found			
						C. arabica (CA)		*C. canephora* (CC)	
	Caparaó Region	CA	CC	CA	CC	*Me*	*He*	*Me*	*He*
	1. Alegre	4		1 (120) 2 (120) 3 (060) 4 (240)		0	0	0	0
	2. Guaçuí	1		1 (060)		0	0	0	0
	3. Ibatiba	3		1 (120) 2 (240) 3 (240)		1	0	0	0
	4. Ibitirama		4		1 (060) 2 (060) 3 (120) 4 (060)	0	0	0	0
	5. Iúna	6		1 (120) 2 (240) 3 (240) 4 (060) 5 (060) 6 (240)		0	0	0	0
2011	6. Muniz Freire	2	8	1 (060) 2 (060)	1 (120) 2 (060) 3 (060) 4 (120) 5 (120) 6 (060) 7 (060) 8 (060)	0	0	0	0
	Central South Region								
	7. Cachoeiro de Itapemirim		5	1 (060) 2 (030) 3 (060) 4 (060) 5 (240)		0	0	0	0
	8. Castelo	2	2	1 (240) 2 (120)	1 (240) 2 (060)	0	0	0	1
	Southern Coastal Region								
	9. Anchieta		1	1 (060)		0	0	0	0
	10. Iconha		4		1 (120) 2 (060) 3 (060) 4 (120)	0	0	0	0
	Southwestern Region								
	11. Brejetuba	4		1 (120) 2 (120) 3 (480) 4 (120)		0	0	0	0
	12. Conceição do Castelo	4		1 (060) 2 (240) 3(060) 4 (060)		0	0	0	0
2012	13. Venda Nova do Imigrante	7		1 (360) 2 (180) 3 (060) 4 (120) 5 (060) 6 (060) 7 (060)		0	1	0	0

Continua...

Continuação

Year	Municipality evaluated per region	Number of nurseries evaluated		Number of samples evaluated per nursery		Number of nurseries where phytonematodes were found			
						C. arabica (CA)		*C. canephora* (CC)	
	Central South Region	CA	CC	CA	CC	*Me*	*He*	*Me*	*He*
	14. Castelo	4		1 (120) 2 (120) 3 (240) 4 (060)		0	1	0	0
	Central Highlands Region								
	15. Santa Teresa	2	4	1 (060) 2 (060)	1 (240) 2 (060) 3 (060) 4 (060)	0	0	1	0
	Midwest Region								
	16. Colatina	3		1 (060) 2 (120) 3 (240)		0	0	0	0
	17. São Gabriel da Palha	5		1 (120) 2 (120) 3 (060) 4 (120) 5 (060)		0	0	1	0
	18. São Roque do Canaã	1		1 (120)		0	0	0	0
2013	**Rio Doce Region**								
	19. Linhares	3		1 (120) 2 (240) 3 (120)		0	0	1	0
	20. Rio Bananal	4		1 (240) 2 (120) 3 (060) 4 (060)		0	0	2	0
	21. Sooretama	1		1 (060)		0	0	1	0
	Northeast Region								
	22. Jaguaré	1		1 (120)		0	0	1	0

Me: *Meloidogyne* sp.; *He: Helicotylenchus* sp.

This work performed by Barros *et al*. (2014) indicates that some of the most damaging nematodes for coffee cultivation are widely distributed in Espirito Santo and, if they continue to be disseminated in the State, coffee cultivation in Espirito Santo is at risk. Therefore, the crucial point during phytosanitary management in the State is the production of good quality seedlings that are free from phytonematodes.

In a study carried out by Lordello *et al*. (2001), soil samples and coffee roots were collected from 37 municipalities in the State of São Paulo, and breeds 1, 2 and 3 of *M. incognita*, *M. exigua*, *M. paranaensis* and *M. javanica* were found. The authors suggested that nematode dissemination might have occurred through seedlings.

Helicotylenchus sp. was observed in the municipalities of Castelo and Venda Nova do Imigrante. It is important to highlight that, although it is a nematode with a large geographic distribution, it is not damaging to the coffee crop (Tihohod, 1993).

In Espírito Santo, the areas that most present problems with *M. paranaensis* and mainly with *M. incognita* are found in the mountainous region and in the northern part of the State. A larger number of nurseries with the presence of phytonematodes are observed in these localities. The seedlings may be acting as disseminators of nematodes in these regions.

Before we started the visits to the nurseries at the beginning of this work, a questionnaire was produced with several questions that were asked during the sample collections in all the nurseries visited. These questions aimed to evaluate the level of technical knowledge of the nurserymen concerning the conduct of their nurseries. Here are some of the questions that were asked: i) what kind of substrate do you use in your nursery?; ii) if the substrate used contains soil, what is its origin and what kind of treatment do you perform?; iii) is there a technical expert responsible for the monitoring of your nursery during the production of your seedlings?; iv) are you aware of the soil pathogens that can be transmitted

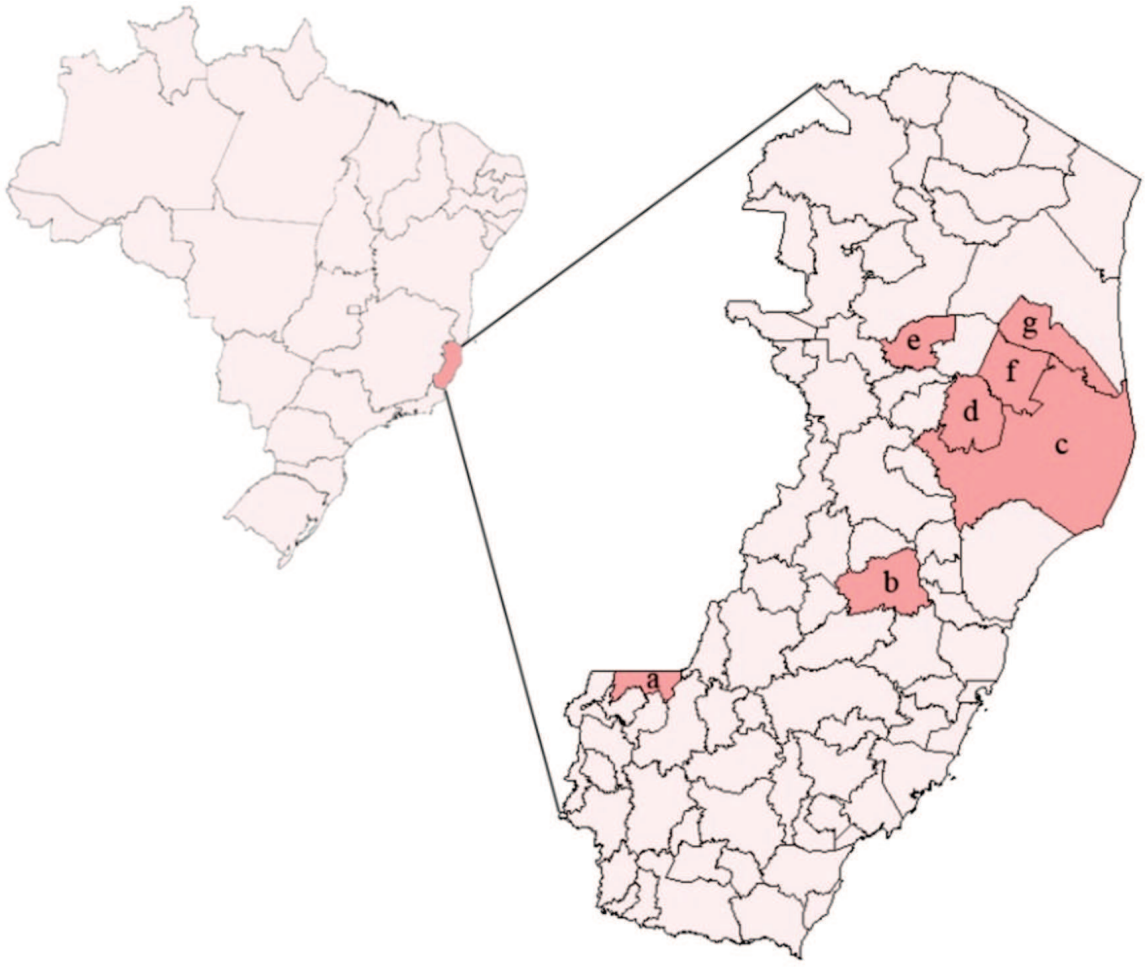

Figure 1: Municipalities of the State of Espirito Santo where second-stage juveniles (J2) of *Meloidogyne* spp. were present in coffee nurseries in a nematological survey carried out between the years 2011 and 2013. *Meloidogyne* spp. were present in Ibatiba (a), Santa Teresa (b), Linhares (c), Rio Bananal (d), São Gabriel da Palha (e), Sooretama (f) and Jaguaré (g).

through seedlings and the difficulties of controlling them after they are established in crop fields?

All nurseries answered that they use ravine or virgin forest soil without any type of treatment. All of them also stated that they have a technical expert responsible for their nursery and that they know nematodes can be transmitted by the seedlings and their management is difficult in the field. The seedlings of most nurseries evaluated in this study did not present phytoparasitic nematodes, probably because they receive adequate orientation regarding the phytosanitary aspects of a technical manager.

The use of virgin forest soil, common in nurseries in Espirito Santo, does not guarantee that such soils do not contain *Meloidogyne* spp. since there are reports of these pathogens in native areas in the Atlantic Forest (Lima *et al*., 2005, Carneiro *et al*., 2006a, 2006b). Thus, it is suggested that nurserymen who use soil from the Atlantic Forest of Espírito Santo to produce their seedlings should treat this substrate.

It is concluded with this work that although phytonematodes have been found in only a few nurseries, it is necessary to observe the choice of the substrate, irrigation water and other phytosanitary care. Most nurserymen in Espírito Santo produce their seedlings in direct contact with the soil. This is a situation that needs to be changed, and it is necessary for nursery owners to be aware that to be safer regarding the dissemination of nematodes and other soil pathogens, these seedlings need to be produced suspended on benches.

Finally, it is important to note that during the visits to the State nurseries, a small number of clandestine nurseries were registered with the SEFAG/DT. These nurseries are a risk to coffee cultivation because they are managed without technical criteria that take into consideration all essential phytopathological care to avoid the dissemination of phytonematodes in Espírito Santo State nurseries should not purchase seedlings from these nurseries.

CONCLUSIONS

We have detected the presence of *Meloidogyne* spp. in nurseries located in the north and south of Espírito Santo, which is very worrying information, since coffee growing is of great socio-economic importance for Espirito Santo and the main species, ie *M. exigua*, *M. incognita* e *M. paranaensis* may be being spread by seedlings and reducing the productive potential of crops in this state.

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