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SHORT COMMUNICATION

Meloidogyne incognita parasitizing coffee plants in southern Minas Gerais, Brazil

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Abstract Root-knot nematodes (RKNs), *Meloidogyne* spp., represent a serious threat to coffee production in Brazil. Although *Meloidogyne exigua* is widely spread in southern Minas Gerais, a major region of coffee production, no major yield losses have been reported for this nematode. Due to the risk of introducing other more aggressive nematode species into coffee fields, such as *M. incognita* and *M. paranaensis*, a survey for *Meloidogyne* spp. in coffee fields was carried out in this region. Based on esterase phenotypes and SCAR markers, RKNs were detected in 37.7% of samples, of which *M. exigua* and *M. incognita* were present in 31.1% and 2.2% of samples, respectively. Mixed populations were observed in 4.4% of samples, i.e. *M. exigua* + *M. incognita* + *M. paranaensis*, or *M. exigua* + *M. incognita*. *Meloidogyne exigua* was the most prevalent species and occurred in majority of counties. Using SCAR markers, *M. incognita* is reported for the first time in coffee fields located in three counties (Três Pontas, Coqueiral and Aguanil) in southern Minas Gerais. Nematode containment strategies are recommended for this region.

Keywords *Coffea arabica* · *Meloidogyne* spp. · Detection · Esterase phenotype · SCAR markers

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Root-knot nematodes (RKNs), *Meloidogyne* spp., occur in several coffee-producing regions in Brazil, being a threat to crop yield. According to Campos and Villain (2005), *M. exigua* Göldi, 1887 is widely distributed and adapted to several regions, including southern Minas Gerais (MG). This is a major region of coffee production and is at risk of introduction of more aggressive RKN species, such as *M. paranaensis* (Carneiro et al. 1996) and *M. incognita* (Kofoide and White) Chitwood, which could lead to substantial yield loss (Ferraz 2008).

Recently, *M. paranaensis* and *M. exigua* were detected in Alpinópolis and Coqueiral counties, southern Minas Gerais (Salgado et al. 2015). *Meloidogyne incognita* is a nematode frequently found parasitizing coffee in São Paulo (SP) state (Carneiro et al. 2005) and has not been detected in Minas Gerais in this crop. Oliveira et al. (2011) reported *M. incognita* associated with other crops in Minas Gerais state, however, the incompatibility between the MG-population and coffee seedlings was evident at the penetration phase, which was also followed by post-penetration resistance factors impeding nematode establishment. The post-infective development in susceptible coffee seedling occurred only in the *M. incognita* SP-population.

In order to prevent dissemination of these nematodes into new areas, it is necessary to know the current distribution of these RKN species in this region and promptly adopt measures to contain the disease foci. The objective of this study was to investigate the occurrence and distribution of *Meloidogyne* spp. in coffee fields located in southern Minas Gerais using PCR SCAR markers and esterase (Est) phenotyping.

Root samples were collected in 45 coffee fields. When possible, females were extracted from roots and characterized with esterase phenotypes (Carneiro et al. 2000; Carneiro and Almeida 2001). The remaining of roots were used for egg extraction using a blender with 0.5% NaOCl according to Bonetti and Ferraz (1981). Nematode suspension was placed into a modified Baermann funnel for second-stage juvenile

(J2) hatching according to Whitehead and Hemming (1965). Juveniles were used to extract DNA using the Quick-gDNA extraction kit (Zymo Research) according to the manufacturer's instructions or according to Randig et al. (2002) and Carneiro et al. (2014). SCAR makers and multiplex PCR reactions were carried out according to Randig et al. (2002).

Coffee cultivar Mundo Novo was grown in 3 L pots filled with a mixture (1:1) of autoclaved soil and Bioplant compost under greenhouse conditions. Seedlings with six pairs of leaves were inoculated with 10,000 eggs of *M. incognita* (isolate Aguanil) extracted from infected tomato roots (*Solanum lycopersicum* L. cv. Santa Clara) using 0.5% NaOCl according to Bonetti and Ferraz 1981. Plants were maintained under greenhouse conditions at 25–30 °C, with watering and fertilization as needed. Six months after inoculation, the root system was rinsed with tap water and weighed. Eggs were extracted as mentioned

Table 1 Survey for *Meloidogyne* spp. associated with coffee in Minas Gerais state

Sample number	Number in the gel	County	Species identified by SCAR marker	Species identified by Esterase phenotypes (Est)
6	-	Três Pontas	Negative	Negative
7	1	Três Pontas	*M. exigua*	Negative
8	2	Três Pontas	*M. exigua, M. incognita* and *M. paranaensis*	*M. exigua* (E1)
9	3	Três Pontas	*M. exigua*	*M. exigua* (E1)
10	4	Três Pontas	*M. exigua*	*M. exigua* (E2)
11	5	Coqueiral	*M. exigua*	*M. exigua* (E1)
12	6	Coqueiral	*M. exigua*	*M. exigua* (E2)
13	-	Coqueiral	Negative	Negative
14	7	Coqueiral	*M. exigua* and *M. incognita*	*M. exigua* (E1)
15	8	Coqueiral	Negative	Negative
16	9	Boa Esperança	*M. exigua*	*M. exigua* (E2)
21	10	Três Pontas	*M. exigua*	*M. exigua* (E2)
22	11	Três Pontas	*M. exigua*	*M. exigua* (E2)
23	12	Aguanil	Negative	Negative
24	13	Aguanil	*M. incognita*	Negative
25	-	Aguanil	Negative	Negative
26	14	Aguanil	*M. exigua*	*M. exigua* (E2)
27	-	Boa Esperança	*M. exigua*	Negative
28	-	Boa Esperança	Negative	Negative
29	-	Boa Esperança	Negative	Negative
30	-	Boa Esperança	*M. exigua*	Negative
31	-	Boa Esperança	Negative	Negative
32	-	Boa Esperança	Negative	Negative
33	-	Boa Esperança	Negative	Negative
34	-	São José da Barra	Negative	Negative
35	-	São José da Barra	Negative	Negative
36	-	São José da Barra	Negative	Negative
37	-	Santo Antônio do Amparo	*M. exigua*	Negative
38	-	Santo Antônio do Amparo	*M. exigua*	Negative
39	-	Santo Antônio do Amparo	Negative	Negative
40	-	Santo Antônio do Amparo	*M. exigua*	Negative
41	-	Santo Antônio do Amparo	Negative	Negative
42	-	Camacho	Negative	Negative
43	-	Camacho	Negative	Negative
44	-	Camacho	Negative	Negative
45	-	Camacho	Negative	Negative

(−) samples not included in Fig. 1

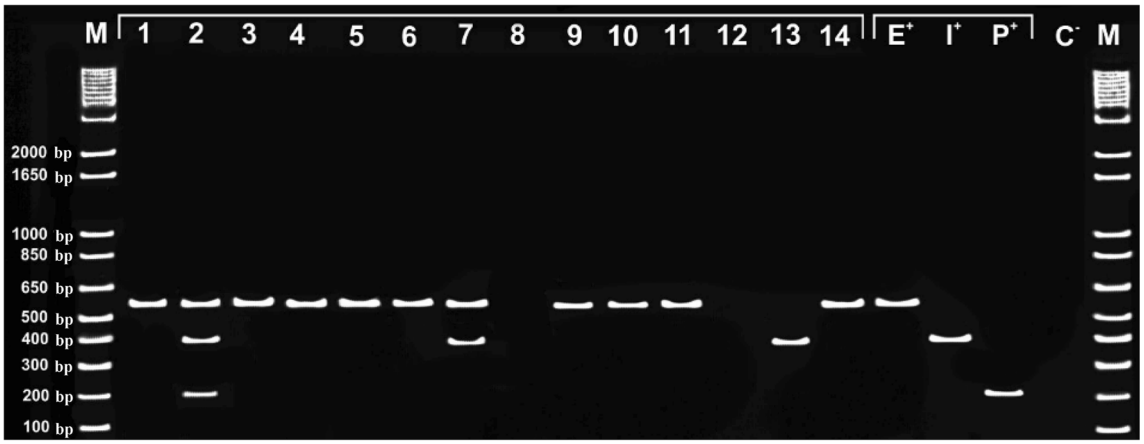

Fig. 1 Nematode identification in samples from different locations in Minas Gerais state using SCAR-PCR. Sample numbers: 1–4 and 10–11, Três Pontas; 5–8, Coqueiral; 9, Boa Esperança; 12, Aguanil. Abbreviations: E^+, *Meloidogyne exigua*; I^+, *M. incognita*, P^+; *M. paranaensis*; C-, Negative control, M, molecular weight marker

above using 1% NaOCl and quantified under a light microscope using Peters' slides. The reproduction factor (RF) was calculated as RF = FP/IP, where FP = final nematode population and IP = initial nematode population (IP = 10,000).

Nematodes in most of the samples were identified either by SCAR markers or by Est profile. RKNs were detected in 37.7% of samples, including *M. exigua* (Est E1, E2, 562 bp fragment) in 80% of positive samples and *M. incognita* (399 bp fragment) in 5% of positive samples. RKNs were also detected in mixtures in 10% of positive samples, including *M. exigua* + *M. incognita* + *M. paranaensis* (208 bp fragment) and *M. exigua* + *M. incognita* (Table 1, Fig. 1). Most of the populations were identified as *M. exigua* with SCAR markers, which was more efficient in its detection compared to Est phenotyping (Table 1, Fig. 1), since *M. exigua* is more difficult to be detected using the isoenzyme phenotype due to the low concentration of esterase in this coffee RKN species (Carneiro et al. 2000). The species *M. incognita* and *M. paranaensis* were not detected in coffee roots through esterase phenotypes due to their low occurrence and bad condition of samples (Table 1), a fact frequently found in coffee roots. *Meloidogyne exigua* was detected in almost every sampled county, except in Camacho, where no nematodes were detected. This survey confirmed *M. exigua* as a major RKN species associated with coffee damage in coffee-producing areas in Minas Gerais (Gonçalves and Silvarolla 2001; Carneiro et al. 2005; Ferraz 2008; Salgado et al. 2015).

The SCAR multiplex-PCR method allowed amplification of specific DNA fragments from only a few J2 extracted from roots or soil (Randig et al. 2002, 2004). This species-specific marker allowed detection of *M. exigua*, *M. incognita* and *M. paranaensis* in mixtures from field samples, confirming the results obtained by Carneiro et al. (2005). This result was not possible to obtain using Est phenotyping, showing that this marker is less effective with samples containing mix of species in small numbers and females in bad condition for analyses, which results from the advanced degree of root decomposition occurring when roots are parasitized by *M. paranaensis* and *M. incognita* (Fig. 2).

Meloidogyne exigua was widely distributed in southern Minas Gerais, while *M. incognita* occurred only in three counties (Aguanil, Coqueiral, Três Pontas). *Meloidogyne paranaensis* was detected only in one sample (Três Pontas) (Table 1, Fig. 1). This species was previously detected in Minas Gerais

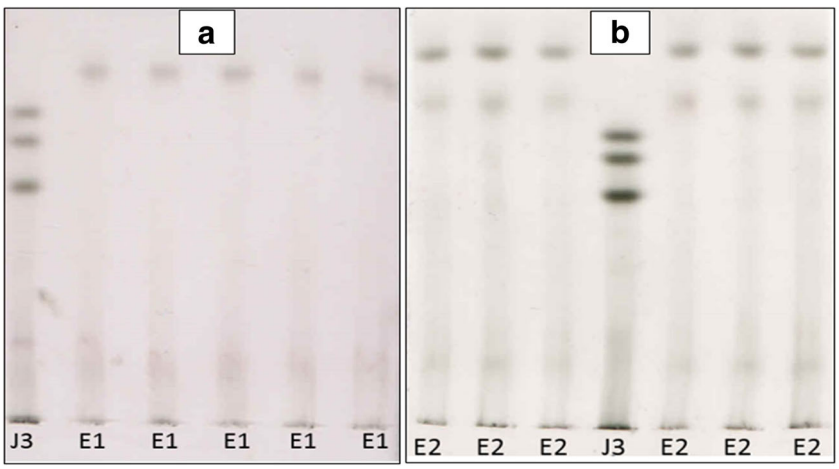

Fig. 2 Esterase (Est) phenotypes of *Meloidogyne exigua* populations found in fields of some coffee-producing areas in southern Minas Gerais. J3, *M. javanica* (Est reference); **a**) Sample numbers: 2, 3, 5 and 7 (Est E1- *M. exigua*, one band); **b**) 4, 6, 9, 10, 11 and 14 (Est E2 - *M. exigua*, two bands)

in the counties of Serra do Salitre and Patrocínio (in Alto Paranaíba region) (Castro et al. 2003), Piumhi (Castro et al. 2008), Alpinópolis and Coqueiral (Salgado et al. 2015).

In greenhouse tests, arabica coffee plants cv. Mundo Novo inoculated with the original *M. incognita* population from Aguanil (maintained in tomato plants cv. Santa Clara for nine months with three successive inoculations) showed typical symptoms of swellings evolving into extensive areas of corky tissues and reduced fresh weight of roots. Symptoms of swelling and cracking were similar to those observed in the field. This population reproduced well in coffee plants as shown by the nematode RF (32.2). These results confirmed the pathogenicity of the *M. incognita* population from Aguanil (MG) on susceptible coffee plants (Koch's postulates), differing from results obtained by Oliveira et al. 2011, who reported that the *M. incognita* MG-population was unable to infect susceptible coffee plants (Oliveira et al. 2011). The genetic variability of *M. incognita* isolates was studied by Santos et al. 2012. Overall, they showed a low genetic variability in this species. In addition, no association was observed between the genetic variability of four *M. incognita* races studied and clustering in phylogenetic analyzes (Santos et al. 2012). These findings suggest that *M. incognita* races and pathotypes are not determined by overall genetic differences (Carneiro and Cofcewicz 2008). Thus, the physiological variability of *M. incognita* isolates needs to be further studied.

The actual distribution of *M. incognita* in Minas Gerais state is not known because of the small number of samples collected in this preliminary study. This study showed only a restricted occurrence of this species on coffee plants and its ability to parasitize the susceptible coffee cultivar Mundo Novo.

This is the first report of *M. incognita* parasitizing coffee plants in three counties of southern Minas Gerais. This finding has great importance for the agriculture in Minas Gerais, considering this nematode may damage coffee plants and become an additional problem for this crop. Although the occurrence of *M. incognita* and *M. paranaensis* is still restricted in southern Minas Gerais, these results show the importance to adopt measures for their containment in this region, where coffee production is socioeconomically important for the state.

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