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PLANT PARASITIC NEMATODES ASSOCIATED WITH GUAVA PLANT IN THE SOUTH, GOIAS STATE, BRAZIL

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Abstract: In order to study the occurrence of phytonematodes in areas of cultivation with guava in the southern region of Goiás, a phytonematological survey was carried out in 30 properties, in a total area of 80 hectares, between January and March 2016. Soil samples and roots were collected in 14 municipalities: Água Limpa, Bom Jesus de Goiás, Buriti Alegre, Caldas Novas, Corumbaíba, Edealina, Goiatuba, Hidrolândia, Itumbiara, Joviânia, Morrinhos, Piracanjuba, Pontalina and Rio Quente. The main genera/species of phytonematodes found were: *Helicotylenchus*; *Xiphinema*; *Rotylenchus*; *Criconemoides* e *Tylenchus*; *Ditylenchus*; *Scutellonema* and *Tubixaba*; *and Meloidogyne javanica* and *Hemicycliophora*. It is worth mentioning the wide dissemination of the first two genres, in addition to the first report of *Tubixaba* spp. associated with guava in the southern region of Goiás. The present study contributed to the knowledge about the occurrence and dissemination of the main genera/species of phytonematodes in areas cultivated with guava in southern Goiás. In addition to subsidizing new studies, it will serve as a warning to producers and make it possible to define more specific managements aimed at reducing damage by nematodes in infested areas.
Keywords: *Psidium guajava*, *Tubixaba* spp., *Helicotylenchus* spp., *Xiphinema* spp, research.

INTRODUCTION

The guava tree, *Psidium guajava* L., is a fruit-bearing species of the Myrtaceae family, with a probable center of origin in tropical America. Currently, it is widespread in other tropical and subtropical regions of the world (MURPHY, 2017). In a tropical climate, the plant can flower and bear fruit throughout the year, as long as the soil has good water availability. It is a crop tolerant to drought and high temperatures of up to 46 ºC, but its production is limited at temperatures below 12 ºC, and it does not tolerate frost. Temperatures between 25 and 28 ºC are considered optimal for the commercial exploitation of guava (BARBOSA and LIMA, 2010). The fruit is rich in vitamin C, phenolic compounds and lycopene, with high antioxidant activity (OLIVEIRA et al., 2011; FLORES et al., 2014).

Guava production is intended for fresh consumption and industrial processing. In Brazil, commercial guava orchards are found in all regions of its territory. The commercial cultivated area is approximately 22 thousand ha, with production of 566.2 thousand tons year-1 and productivity of 26 thousand kg ha-1. The Northeast region is the largest producer with 50.2% of production, followed by the Southeast region with 41%, the South region with 5.5%, the Midwest region with 2.2% and the North region with 1.1% (IBGE, 2020).

The southern region of Goiás presents growth in the fruit growing sector, mainly in the municipality of Morrinhos, currently the largest producer of guava in the state. Approximately 105 ha of fruit are cultivated in the municipality, with a production of 2,600 tons year-1 and productivity of 24,500 kg ha-1, with the main destination being the processing industry (IBGE, 2020). The increase in guava production is a positive and important point for the region's economy, generally because it raises the level of jobs, keeps people in the countryside, distributes regional income and generates products of commercial value. However, for better productivity and crop quality, the producer must adopt several management techniques, as several factors can lead to lower fruit production in the production area (SANTANA, 2019).

Among the main harmful factors for the cultivation of guava, those of a biotic nature, such as invasive plants, insect pests and diseases can be mentioned (BARBOSA and LIMA, 2010). Regarding the various etiological agents that cause diseases in guava, phytonematodes cause a significant drop in productivity in the culture, and as a consequence, damage to the fruit grower (BARBOSA, 2015). Plant parasitic nematodes cause estimated losses of 12% in world agricultural production, with approximately 9% in developed countries and 15% in developing countries (SASSER and FRECKMAN, 1987). The phytonematodes of the genus:

Meloidogyne, also known as root-knot nematodes, they are considered the most important in the world, as they infect practically all types of agricultural crops (JONES et al., 2013).

Meloidogyne enterolobii Yang and Eisenback (1983) (sin. *Meloidogyne mayaguensis* Rammah and Hirschmann) is one of the most destructive species of root-knot nematodes. It is the main phytonematode of the guava tree, which has already decimated several orchards located in the São Francisco Valley (CARNEIRO et al., 2001). It is present in the soil of practically all producing regions in Brazil, and its dissemination may have occurred systematically through infested seedlings. Symptoms on the plant are strong yellowing of the leaves, intensive plant decline, unproductivity, and even early death (ALMEIDA et al., 2010). Other fruit trees can also be hosts of*M. enterolobii*, such as the acerola, banana, fig and vine (FREITAS et al., 2017). In addition, the phytonematode parasitizes vegetables such as lettuce, beetroot, coriander, jurubeba, melon, strawberry, pepper, bell pepper, radish and tomato (FREITAS et al., 2017; ROSA et al., 2015; SILVA and SANTOS, 2017) ; large crops, such as cotton and sunflower (GALBIERI et al., 2020; ROSA et al., 2015); green manure plants, such as jack bean and pigeon pea (ROSA et al., 2015); and invasive plants such as: buva (*Coniza canadensis*), capim-colchão (*Digitaria horizontalis*), caruru (*Amaranthus retroflexus*), erva-de-santa-luzia (*Chamaesyce hirta*), falsa-serralha (*Emilia fosbergii*), maria-pretinha (*Solanum americanum*), picão-preto (*Bidens pilosa*) and sojinha (*Cleome affinis*) (ALMEIDA et al., 2011; SILVA e SANTOS, 2017).

Guava is subject to environmental variations throughout the year in the most diverse regions where it is cultivated. Thus, knowledge about the population dynamics of phytonematodes is essential, since it allows establishing more suitable periods for phytonematological surveys. Consequently, the identification of the phytonematode population in the cultivation area favors the adoption of more efficient management, minimizing economic losses (DINARDO-MIRANDA et al., 1997).

It is worth mentioning that there are few or almost null studies aimed at determining the association and identification of phytonematodes in guava in the Cerrado region. In the state of Goiás, these studies are even scarcer. Therefore, this study aimed to obtain information on the association and identification of the main genera/species of phytonematodes in guava cultivation areas in municipalities in the southern region of Goiás.

MATERIAL AND METHODS

The study was carried out between January and March 2016, in which 30 samples were collected in 30 different areas with guava in 14 municipalities in the southern region of Goiás, distributed in: Morrinhos (10), Piracanjuba (5), Pontalina (3), Joviânia (2), Caldas Novas (1), Buriti Alegre (1), Água Limpa (1), Hidrolândia (1), Rio Quente (1), Corumbaíba (1), Bom Jesus de Goiás (1), Goiatuba (1), Edealina (1) and Itumbiara (1). The samples consisted of 500 g of soil and 100 g of roots. Sampling followed the pattern of soil collection for analysis, in addition to including points where plants had possible symptoms caused by phytonematodes. For each composite sample, subsamples of soil and roots were collected at a depth of 20-25 cm in the region of projection of the canopy of the plants, discarding the surface organic matter. In each collection location, the geographic coordinates were recorded with the aid of a device with a Global Positioning System (GPS) (GARMIN 60 CSx). Samples were collected both in commercial orchards and in home orchards. Those below 0.5 ha were considered homemade (26), and those measuring 0.5 ha or more were considered commercial (4). The samples were collected, placed in plastic bags, identified and packed in a styrofoam box. Subsequently, they were stored in a refrigerator at 8 °C until they were sent for analysis at the Agricultural Nematology Laboratory of the Instituto Federal Goiano - Campus Morrinhos, Morrinhos-GO, Brazil.

For the extraction of nematodes from soil, samples of 150 cm³ of soil were analyzed according to the flotation and centrifugation method in sucrose solution proposed by Jenkins (1964). For the extraction of nematodes in roots, samples of 10 g of roots were processed according to the method of Boneti and Ferraz (1981) and clarified according to Coolen and D'Herde (1972). After extraction, the solution of each sample was decanted, concentrated and taken to a water bath at 55 °C for 5 minutes to kill the nematodes. In order to preserve it, TAF fixative was added to each sample (7 parts of 40% formalin + 2 parts of triethanolamine + 91 parts of distilled water). Afterwards, they were placed in 15 mL test tubes for later identification. Temporary slides were made for the identification and quantification of nematodes in a photonic microscope at 100, 400 and 1000X magnifications. The identification of the genera was based on the identification key of plant nematodes by Mai and Mullin (1996). For roots with eventual root galls, these were dissected with the aid of a stylet under a stereoscopic microscope, and milky-white females typical of the genus:

Meloidogyne were collected and analyzed for verification at the species level. Analysis was used through the study of the perineal pattern (TAYLOR and NETSCHER, 1974).

Thus, the abundance (A) of phytonematodes in the soil (AS) and in the roots (AR) was calculated, determined by the number of specimens of a given genus/species in the samples. Subsequently, the relative abundance (Ar%) in the soil and roots was calculated, according to the formula Ar% = (A x 100) / N, where 'A' represents the number of specimens of a given genus/species in the sample and 'N' the total number of nematodes in the sample. Frequency (F%) was also calculated using the formula F% = (na x 100) / Na, where 'na' represents the number of samples in which a given genus/species occurred and 'Na' the total number of samples collected (DIAS-ARIEIRA et al., 2010; NORTON, 1978).

RESULTS AND DISCUSSION

In all samples, free-living nematodes that do not parasitize plants were found, and many of them can be bacteriophages, fungivores or mycophages, omnivores and even predators of other nematodes, which contributes to the dynamic population balance of the soil. Phytoparasitic nematodes were found in all samples, and the main genera/species found in order of higher relative frequency were: *Helicotylenchus* (76,6 %); *Xiphinema* (53,3 %); *Rotylenchus* (26,6 %); *Criconemoides* (16,6 %); *Tylenchus* (16,6 %); *Ditylenchus* (10 %); *Scutellonema* (6,6 %); *Tubixaba* (6,6 %); *Meloidogyne javanica* (3,3 %) and *Hemicycliophora* (3,3 %).

Among the phytonematodes found in association with guava, it was observed that *Helicotylenchus* spp. was present in 76.6% of the samples collected, followed by *Xiphinema* spp., present in 53.3%. In addition to higher frequencies, they presented the highest mean population values per 150 cm³ of soil, with 54 specimens of *Helicotylenchus* and 26 species of *Xiphinema* (Table 1).

The highest frequency of *Helicotylenchus* spp. in guava samples in the present study is consistent with those obtained by Dias-Arieira et al. (2010), who in a survey of phytonematodes in fruit trees in the Northwest of Paraná. The authors reported that *Helicotylenchus* was the most frequent genus in samples of 100 cm³ of guava soil, with a frequency of 50%, in addition to 25% in samples of 10 g of roots. The authors also reported that the phytonematode was associated in approximately 50% of the fruit trees in the region. This fact raises the hypothesis that this nematode can cause damage to the guava tree, so that data level studies are still necessary.

In several municipalities in Mato Grosso, Garbin and Costa (2015) reported *Helicotylenchus* spp. in practically all samples of 200 cm³ of soil and 5 g of roots in cropped areas for four consecutive years. The frequency of this ectoparasite in infested areas in the state was high, even in months with low rainfall, thus considering it an opportunistic nematode, which takes advantage of greater plant stress.

Helicotylenchus is a spiral nematode ectoparasite of plant roots and has a wide geographic distribution, being associated with several host plants and, together with other genera of phytonematodes, causes reductions in plant growth and development (MACHADO et al., 2019; OLIVEIRA et al, 2007; PEREIRA et al., 2015; ROSA et al., 2014; SHARMA et al., 1993). Although there is a significant occurrence o *Helicotylenchus* spp. in cultivated soils in Brazilian territory, the damage they can cause to agricultural production is unnoticed and little is known about the losses.

In the present study, nematodes of the genus: *Xiphinema* were found in more than 53% of the samples. population study of nematodes associated with guava in the Vale do Submédio São Francisco, Moreira et al. (2003) reported that *Xiphinema* was the second most frequent genus in samples of 100 g of soil and 10 g of roots, with a frequency of 11.4%, behind only *Meloidogyne enterolobii* with 54.4%. It is worth mentioning that in our work: *Xiphinema* was also the second most frequent gender in the samples, but with a value 4.6 times higher. In a survey in the southern region of Goiás of phytonematodes associated with vegetables, Oliveira (2016) reported the presence of *Xiphinema* spp. in samples of 150 cm³ of soil, but with a frequency of 6%, a value 8.8 times lower. The different frequencies found can be understood by sampling, climate and soil variations, in addition to host preference.

The genders: *Rotylenchus, Criconemoides and Tylenchus* presented less expressive frequencies in the present study, 26.6, 16.6 and 16.6%, respectively. Other genres associated in the samples such as: *Ditylenchus*, *Scutellonema* and *Hemicycliophora* were even less frequent, 10, 6.6 and 3.3%, respectively. In the southern region of Goiás, but in vegetable growing areas, Oliveira (2016) reported the presence of *Tylenchus* spp. in samples of 150 cm³ of vegetable soil, with a similar frequency of 13%. In the study carried out by Moreira et al. (2003), *Ditylenchus* spp. and *Hemicycliophora* spp. were also less frequent in relation to the other genera, with a frequency of 1 and 10%, respectively, in samples of 100 g of soil and 10 g of guava roots. However, Dias-Arieira et al. (2010) reported tha *Hemicycliophora* was the most abundant genus in samples of 100 cm³ of guava soil, frequent in 25% of the samples. These differences found can also be

Number	City	Area (ha)	Geographic Coordinates	Gender (s) / species	AS	AR	Ar%S	Ar%R
1	Piracanjuba	0,1	Lat.: 17° 27' 17,51" S Long.: 49° 10' 23,18" W	*Helicotylenchus* *Tylenchus Xiphinema*	56 14 38	25 2 0	51,8 13 35,2	92,6 7,4 0
2	Morrinhos	0,5	Lat.: 17° 49' 21,20" S Long.: 49° 12' 09,06" W	*Helicotylenchus* *Tylecnhus Xiphinema* *M. javanica*	124 15 51 14	43 7 0 260	60,8 7,4 25 6,8	13,8 2,2 0 83,9
3	Piracanjuba	0,1	Lat.: 17° 27' 14,61" S Long.: 49° 09' 57,88" W	*Helicotylenchus* *Xiphinema*	43 15	14 0	74,1 25,9	100 0
4	Piracanjuba	0,1	Lat.: 17° 27' 14,84" S Long.: 49° 09' 47,27" W	*Ditylenchus* *Helicotylenchus*	13 39	0 13	25 75	0 100
5	Piracanjuba	0,1	Lat.: 17° 27' 11,59" S Long.: 49° 09' 30,53" W	*Helicotylenchus* *Rotylenchus*	52 21	18 0	71,2 28,8	100 0
6	Morrinhos	0,1	Lat.: 17° 43' 42" S Long.: 49° 05' 46" W	*Helicotylenchus* *Tylenchus* *Rotylenchus*	14 6 2	1 2 0	63,6 27,3 9,1	33,3 66,7 0
7	Piracanjuba	0,1	Lat.: 17° 21' 26,6" S Long.: 49° 03' 43" W	*Criconemoides* *Helicotylenchus* *Xiphinema*	16 22 23	0 12 0	26,2 36,1 37,7	0 100 0
8	Pontalina	0,1	Lat.: 17° 30' 51,7" S Long.: 49° 33' 31,01" W	*Xiphinema*	27	0	100	0
9	Morrinhos	0,1	Lat.: 17° 44' 12,18" S Long.: 49° 05' 54,67" W	*Hemicycliophora*	24	0	100	0
10	Morrinhos	16,8	Lat.: 17° 44' 21,96" S Long.: 49° 04' 06,13" W	*Helicotylenchus* *Rotylenchus*	168 23	66 0	88 12	100 0
11	Morrinhos	36	Lat.: 17° 44' 03,54" S Long.: 49° 03' 00,94" W	*Criconemoides* *Helicotylenchus*	24 198	0 52	10,81 89,19	0 100
12	Morrinhos	24	Lat.: 17° 51' 38,68" S Long.: 48° 58' 05,60" W	*Helicotylenchus*	183	71	100	100
13	Joviânia	0,1	Lat.: 17° 47' 56,58" S Long.: 49° 36' 50,46" W	*Tylenchus*	18	5	100	100
14	Rio Quente	0,1	Lat.: 17° 48' 45,90" S Long.: 48° 46' 23,70" W	*Helicotylenchus* *Rotylenchus* *Xiphinema*	21 14 23	0 0 0	36,2 24,1 39,7	0 0 0
15	Buriti Alegre	0,1	Lat.: 18° 08' 20,30" S Long.: 49° 02' 05,80" W	*Ditylenchus* *Helicotylenchus* *Xiphinema*	3 32 9	0 15 0	6,8 72,7 20,5	0 100 0
16	Hidrolândia	0,1	Lat.: 16° 58' 19,68" S Long.: 49° 13' 56,90" W	*Helicotylenchus*	27	13	100	100

17	Pontalina	0,1	Lat.: 17° 32’ 26,9” S Long.: 49° 29’ 42,23” W	*Helicotylenchus* *Scutellonema* *Xiphinema*	33 17 21	0 0 0	46,5 24 29,5	0 0 0
18	Pontalina	0,1	Lat.: 17° 30’ 9,12” S Long.: 49° 30’ 7,04” W	*Helicotylenchus* *Xiphinema*	18 24	6 0	42,9 57,1	100 0
19	Água Limpa	0,1	Lat.: 18° 08’ 10,40” S Long.: 48° 47’ 40,80” W	*Criconemoides* *Xiphinema*	14 29	0 0	32,6 67,4	0 0
20	Morrinhos	0,1	Lat.: 17° 42’ 36,6” S Long.: 48° 56’ 54,8” W	*Tubixaba* *Xiphinema*	3 46	2 0	6,1 93,9	100 0
21	Morrinhos	0,1	Lat.: 17° 41’ 0,82” South Long.: 48° 57’ 34,5” West	*Helicotylenchus* *Criconemoides* *Xiphinema*	23 8 31	9 0 0	37,1 12,9 50	100 0 0
22	Itumbiara	0,1	Lat.: 18° 24’ 48,86” S Long.: 49° 14’ 30,46” W	*Criconemoides* *Rotylenchus* *Tylenchus Xiphinema*	13 11 7 18	0 0 2 0	26,5 22,5 14,3 36,7	0 0 100 0
23	Joviânia	0,1	Lat.: 17° 48’ 28,76” S Long.: 49° 37’ 26,58” W	*Helicotylenchus* *Rotylenchus*	29 14	5 0	67,4 32,6	100 0
24	Morrinhos	0,1	Lat.: 17° 38’ 43,60” S Long.: 49° 09’ 07,20” W	*Helicotylenchus* *Xiphinema*	32 15	13 0	68,1 31,9	100 0
25	Morrinhos	0,1	Lat.: 17° 44’ 36,80” S Long.: 49° 08’ 07,40” W	*Tubixaba*	4	2	100	100
26	Caldas Novas	0,1	Lat.: 17° 47’ 23,50” S Long.: 48° 36’ 56,10” W	*Helicotylenchus* *Xiphinema*	16 29	0 0	35,6 64,4	0 0
27	Edealina	0,1	Lat.: 17° 25’ 07,57” S Long.: 49° 40’ 31,93” W	*Helicotylenchus* *Xiphinema*	29 17	13 0	63 37	100 0
28	Corumbaíba	0,1	Lat.: 18° 08’ 13,00” S Long.: 48° 33’ 30,00” W	*Helicotylenchus* *Rotylenchus*	31 14	0 0	68,9 31,1	0 0
29	Bom Jesus de Goiás	0,1	Lat.: 18° 12’ 29,50” S Long.: 49° 43’ 59,20” W	*Helicotylenchus* *Scutellonema* *Rotylenchus*	21 11 9	6 0 0	51,2 26,8 22	100 0 0
30	Goiatuba	0,2	Lat.: 18° 00’ 24,90” S Long.: 49° 21’ 02,80” W	*Ditylenchus* *Helicotylenchus*	11 43	0 17	20,4 79,6	0 100 0

AS and AR = abundance of phytonematodes in the soil (AS) and in the roots (AR), determined by the number of specimens of a given genus/species in the samples;

Ar% = relative abundance in soil (Ar%S) and roots (Ar%R), Ar% = (A x 100) / N, where A represents the number of specimens of a given genus/species in the sample, and N the total number of nematodes in the sample

Table 1. Phytonematodes associated with guava in southern Goiás, expressed by abundance in 150 cm^3 of soil (AS) or 10 g of roots (AR), and relative abundance in soil (Ar%S) and roots (Ar%R).

understood by the type of sampling, climate and soil.

This is the first report of *Tubixaba* spp. associated with guava in the Cerrado region of Goiás. Gender was frequent in 6.6% of the analyzed samples. Therefore, more surveys and phytonematological studies must be carried out in this and other regions to observe its association with guava. It is worth mentioning that this genus is considered omnivorous, that is, it has varied eating habits, and its eating habits still need to be better understood.

The species: *Tubixaba tuxaua* was reported for the first time by Monteiro and Lordello (1980) in the municipality of Marechal Cândido Rondon, western Paraná, associated with soybean plants cv. Bragg with symptoms of dwarfism. Since its discovery, T. tuxaua has been associated with other crops, affecting the development of plants, such as sunn hemp, jack bean, velvet bean and velvet bean (FURLANETTO et al., 2008), as well as production. of corn, soybeans and wheat (FURLANETTO et al., 2010).

The presence of the main guava plant nematode was not detected in any of the samples., *Meloidogyne enterolobii*, which can be explained by the non-observation of cracks, darkening and stripping of the roots, yellowing and browning of the leaves, which are characteristic symptoms of its presence. The first report of *M. enterolobii* in Brazil, it was assimilated into guava roots in the municipalities of Petrolina (PE), Curaçá and Maniçoba (BA), causing great economic losses (CARNEIRO et al., 2001). Since then, it has been reported infecting guava and other plants in all regions of the country (CASTRO and SANTANA, 2010; SILVA and OLIVEIRA, 2010). This has already been reported by Siqueira et al. (2009) in Goiás in cultivation of guava cv. Paluma in consortium with papaya cv. Formosa in the region of the municipalities of Formosa and Luziânia. At the moment, *M. enterolobii* is considered one of the most important species of its genus because it is highly virulent and polyphagous, capable of overcoming the resistance conferred by the Mi-1 gene t *M. incognita*, *M. javanica* and *M. arenaria* (CARNEIRO et al., 2006). Thus, eradication measures and, mainly, exclusion are recommended since the first report of *M. enterolobii* in Brazil.

Despite the absence of *M. enterolobii* in the samples mentioned above, another species of the genus was detected: *Meloidogyne* associated with guava, *M. javanica* in 3.3% of the samples. The species was confirmed by analyzing the perineal pattern of females (Figure 1). Its spread to the cultivation area may have occurred either by infested seedlings, or by contaminated machinery and implements from other neighboring areas. Thus, the adoption of management in the area must be taken into account, in order to keep the population density below the level of economic damage. Also, special attention must be paid not to spread to the rest of the cultivation area and to other neighboring areas.

In recent years, the installation of guava processing industries in the municipality of Morrinhos has increased the demand for the fruit, which has gradually led to the expansion of the cultivation area in the region. Thus, the awareness of fruit growers in relation to the acquisition of seedlings free from phytonematodes, in particular *M. enterolobii*, is essential to ensure the longevity and productivity of orchards, in addition to preventing the spread of this and other phytonematodes to non-infested areas. Therefore, it is suggested to carry out other phytonematological surveys in guava cultivation, in this and other regions of Goiás, at different times of the year, in order to obtain more information related to phytonematodes.

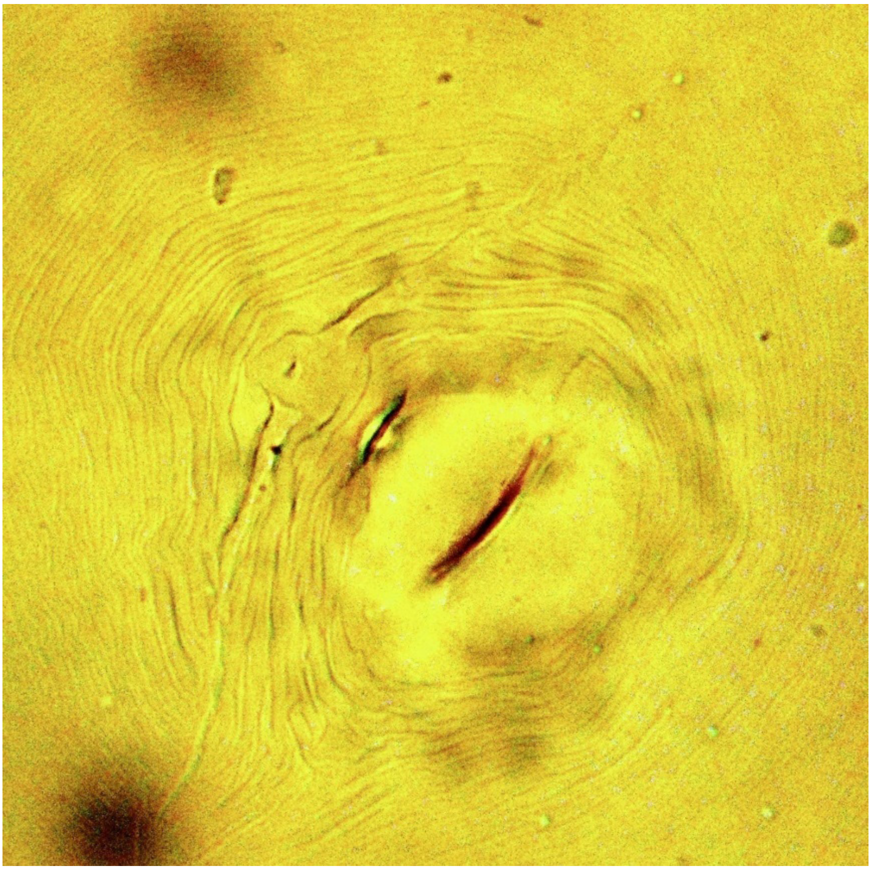

Figure 1. Perineal pattern of female of *Meloidogyne javanica* associated in 3.3% of the guava samples in the southern region of Goiás.

CONCLUSION

The main phytonematodes associated with guava in the southern region of Goiás were those of the genus: *Helicotylenchus* and *Xiphinema*.

The gender: *Helicotylenchus* is associated in more than 70 % of the soil and guava root samples carried out in the south-goiana conditions.

For the first time, the occurrence of *Tubixaba* spp. in guava cultivation area in Goiás.

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