FREQUENCIES AND POPULATION DENSITIES OF THE MAJOR PHYTONEMATODES ASSOCIATED WITH BANANA IN THE STATE OF ALAGOAS, BRAZIL

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ABSTRACT

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Banana (*Musa* spp.) is one of the most cultivated and consumed fruits in the tropics. The low productivity and low quality of the Brazilian bananas are largely due to disease problems, among which are nematodes. In Alagoas, there is a lack of information on the nematodes species affecting the banana crop. The objectives of this study were to update the information on the occurrence of plant-parasitic nematodes in some of the banana producing areas in Alagoas state, to provide quantitative information on the frequencies and population densities of these parasites, and to identify the nematode species associated with the banana crop. The study was carried out from April 2011 to January 2012. Forty-two soil and roots samples were collected from banana orchards planted with cultivars Prata, Pacovan, and Terra in 13 municipalities of Alagoas state. Nematodes were extracted from roots and soil samples using the maceration-centrifugal flotation and the centrifugal-flotation methods, respectively. The frequencies of the nematodes species extracted from the roots and soil samples were as follows: *Helicotylenchus* spp., 95% and 98%; *Meloidogyne* spp., 79% and 81%; *Pratylenchus* spp., 36% and 26%; and *Radopholus similis*, 38% and 21%; respectively. On average, *Helicotylenchus* spp. was most abundant with 2,359/ 50 g root, followed by *R. similis*: 1,359, *Meloidogyne* spp.: 810, and *Pratylenchus* spp.: 682. These high population densities show the need for research aimed at the management of plant-parasitic nematodes of banana in Alagoas state.

Key words: Helicotylenchus spp., *Meloidogyne* spp., *Musa* spp., nematodes, *Pratylenchus* spp., *Radopholus similis*

RESUMO

Lima, R. S., M. F. S. Muniz, J. M. C. Castro, E. R. L. Oliveira, P. G. Oliveira, K. M. S. Siqueira, A. C. Z. Machado, and J. G. Costa. 2013. Frequências e densidades populacionais dos principais fitonematoides associados à bananeira no Estado de Alagoas, Brazil. Nematropica 43:186-193.

A banana (*Musa* spp.) é uma das frutas mais cultivadas e consumidas nos países tropicais. As baixas produtividade e qualidade da banana no Brasil são, em grande parte, devidas a problemas fitossanitários, dentre os quais, os nematoides. Em Alagoas, há carência de informações sobre as espécies de nematoides que afetam a cultura. Assim, esse trabalho teve por objetivo atualizar as informações sobre a ocorrência de nematoides em alguns municípios produtores de banana no Estado de Alagoas, fornecer dados quantitativos sobre as frequências e densidades populacionais e identificar as espécies de nematoides associadas à cultura. O trabalho foi realizado durante o período de abril de 2011 a janeiro de 2012. Quarenta e duas amostras de solo e de raízes foram coletadas em pomares de bananeira das cultivares Prata, Pacovan e Terra, provenientes de 13 municípios. Os nematoides foram extraídos das amostras de raízes e solo por meio dos métodos de maceração aliada à centrifugação e de flotação centrífuga, respectivamente. As frequências das espécies de nematoides extraídas das amostras de raízes e de solo foram as seguintes: *Helicotylenchus* spp. (95% e 98%), *Meloidogyne* spp. (79% e 81%), *Pratylenchus* spp. (36% e 26%) e *Radopholus similis* (38% e 21%). Nas amostras de raízes,

Helicotylenchus spp. apresentaram as maiores densidades médias por 50 g de raízes: 2.359, seguidos por *R. similis*: 1.359, *Meloidogyne* spp.: 810 e *Pratylenchus* spp.: 682. Essas elevadas densidades populacionais demonstram a necessidade de pesquisas visando o manejo de fitonematoides que infectam a bananeira nesse estado.

Palabras clave: Helicotylenchus spp., *Meloidogyne* spp., *Musa* spp., nematoides, *Pratylenchus* spp., *Radopholus similis*.

INTRODUCTION

Bananas (*Musa* spp.) are major fruit crops in the tropics and subtropics (Robinson and Galán Saúco, 2010). India is the largest world producer, and Brazil ranks sixth with nearly 7.3 million tons (FAO, 2011), 39% of which come from the Northeast region. The state of Alagoas alone contributed 49.4 thousand tons to the Brazilian banana production (IBGE, 2011). Nematodes are among the most important constraints to banana production worldwide, with 146 species in 43 genera recorded on the crop (Gowen and Quénéhervé, 1990). The most detrimental nematode species are those that destroy the primary roots, thereby disrupting the anchorage system and causing the toppling of the plants (Gowen *et al*., 2005).

In Brazil, the major nematode pests of banana are *Radopholus similis* (Cobb) Thorne, *Meloidogyne javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, *Helicotylenchus multicinctus* (Cobb) Golden, and *Pratylenchus coffeae* (Zimmerman) Filipjev & Schuurmans Stekhoven (Cofcewicz *et al*., 2004; Cordeiro *et al*., 2005). In the state of Alagoas, information on the occurrence of phytonematodes on banana is scarce. A study conducted by Andrade *et al.* (2009) showed *H. multicinctus*, *Pratylenchus* sp., and *R. similis* were present, but at low frequencies (2.1%, 1.6%, and 6.5%, respectively). However, symptoms of toppling and associated yield decline have been observed at high incidence, mainly on cv. Terra (Comprida), which has a wider acceptance in local and regional markets. In some cases, this situation has led farmers to simply abandon their plantations (M. F. S. Muniz, personal communication).

Survey data are useful for the identification of nematode species associated with different crops, and the distribution of different nematode species in a given locality can enable studies on the biology and ecology of these nematodes. In addition, survey data can serve as source of information for the implementation of control measures before the damage threshold is reached (Davide, 2005).

The objectives of this study were three-fold: *i*) to update the information on the occurrence of plant-parasitic nematodes in some of the banana producing areas in Alagoas state, *ii*) to provide quantitative information on the frequencies and population densities of these parasites, and *iii*) to identify the nematode species associated with the banana crop in Alagoas state.

MATERIALS AND METHODS

Forty-two samples, each containing banana roots and soil from the rhizosphere, were collected between April, 2011 and January, 2012 in 13 municipalities of the state of Alagoas, Brazil. The 13 municipalities included União dos Palmares, Maragogi, Japaratinga, Santana do Mundaú, Porto Calvo, Matriz do Camaragibe, Joaquim Gomes, Ibateguara, São José da Laje, Murici, Cajueiro, Viçosa, and Coruripe. The age of the plantations ranged from 2 months to 20 years, and the cultivars grown were mainly Prata, Pacovan, and Terra, accounting for 30%, 11%, and 59% of the overall banana cropping area, respectively. The size of the farms ranged from 0.5 to 20 hectares.

Composite samples of roots and soil were collected in areas with known history of toppling and/or yield loss, using a zig-zag pattern within each field. Each composite sample, approximately 100 g roots and 500 g soil, was made of 10 subsamples per hectare. The samples were placed in labeled plastic bags and transported in cool boxes to the Phytopathology Laboratory of the Federal University of Alagoas, where they were processed within 72 hours. Nematodes were extracted from 100 cm^3 soil by the Jenkins (1964) method. The roots were washed, dried on paper tissue, cut into 1 to 3-cm pieces, and mixed thoroughly before a 50-g subsample was processed acccording to Coolen and D'Herde (1972).

After extraction, nematodes were killed and fixed in hot 4% formalin, and identified to genus, based on morphological characteristics under a light microscope, according to Mai and Mullin (1996). The population densities of the nematode suspensions were determined from triplicate 1 ml aliquots, with the aid of a Peter's counting slide (Astel, Botucatu, SP, Brazil) under a light microscope at 100x magnification. The absolute frequency was calculed as (number of samples containing a nematode species/number of samples collected) × 100 (Barker, 1985). Population densities of the nematodes from roots and soil samples were subjected to chi-square analysis using the statistical software GENES version 5.1 (Cruz, 2013). *Helicotylenchus* spp. were identified according to Fortuner (1991), and *Pratylenchus* spp., according to Castillo and Vovlas (2007). *Meloidogyne* populations were maintained in a greenhouse on

tomato (*Solanum lycopersicum* L. Santa Cruz cv. Kada Gigante) grown in pots containing autoclaved soil mixed with chopped infected roots collected from the field. Females of each population were examined for perineal patterns, according to Hartman and Sasser (1985). For biochemical characterization, single mature egg masses were inoculated around the roots of tomato plants growing in pots in the greenhouse.

Isozyme characterization for α-esterase was conducted at Embrapa Tropical Semi-Arid in Pernambuco state, Brazil. Young egg-laying females of *Meloidogyne* spp. were removed from tomato galls, under a stereomicroscope, and macerated in 6.0 µl of extraction buffer containing 20% sucrose and 2% Triton X-100 (Kunieda de Alonso and Alfenas, 1998). Females of *M. javanica* were used as reference phenotype to facilitate comparison of *Meloidogyne* spp. based on the relative migration (Rm) of electrophoresis bands. Electrophoresis was performed in separating and stacking homogeneous gels, 7.5% and 4.0% polyacrylamide, respectively, with Tris–glycine buffer in a vertical slab electrophoresis unit. Gels were stained with substrate α-naphthyl acetate for α-esterase (Alfenas *et al*., 1991; Esbenshade and Triantaphyllou, 1985), washed in distilled water, placed in a 10% acetic acid and 40% methyl alcohol solution for 30 min, and dried between two cellophane sheets.

RESULTS

The major phytonematodes present in the sampled areas in this study were *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *Radopholus similis*. Species of *Helicotylenchus* included *H. multicinctus* and *H. dihystera* (Cobb) Sher. Females of *H. multicinctus* had a C-shaped body when killed. Their stylet was well developed (21.48 to 24.78 µm long), the vulva in the mid to posterior part of the body, and the tail rounded. Males of *H. multicinctus* also were present. In contrast, females of *H. dihystera* were characterized by a spiral shaped body when killed, a 22.64 to 26.25 µm long stylet, and a pointed tail terminus. Males were absent in *H. dihystera*.

Based on perineal patterns and isozyme phenotypes, three root-knot nematodes species were found in this study: *M. incognita*, *M. javanica*, and *M. arenaria*. The perineal pattern of *M. incognita* was characterized by the presence of a high dorsal arch, with no distinctive lateral lines. Females of *M. arenaria* showed a low, rounded dorsal arch, with lateral lines forming a shoulder. *Meloidogyne javanica* had a perineal pattern with distinctive lateral lines. Electrophoresis separation of esterases revealed three distinctive phenotypes: phenotype I2 with two bands (Rm 1.00 and 1.09) specific to *M. incognita*, phenotype J3 with three bands (Rm 1.00, 1.16, and 1.29) characteristic of *M. javanica*, and phenotype A2 with two bands (Rm 1.17 and 1.28) specific to *M. arenaria* (Fig. 1). Of the three species, *M. incognita* was the most frequent, since it was detected in five samples, either alone (as in Japaratinga) or in association with *M. arenaria* (Maragogi and Matriz do Camaragibe) and/or *M. javanica* (Maragogi and Porto Calvo).

Pratylenchus species mainly consisted of *P.coffeae*, with only one population of *P. brachyurus* recorded in the municipality of Santana do Mundaú. Females of *P. coffeae* had body length between 560.0 and 596.0 µm, labial region with two annuli, stylet 12.6 to 13.0 µm in length, vulva position at 71.29 to 81.69% body length, and tail tip truncate or hemispherical. Males of *P. coffeae* were abundant. In *P. brachyurus*, female body length was between 512.9 and 553.8 µm. The labial region was angular, with two annuli, the basal one narrower than the first annulus of the body. The stylet was 18.54 to 20.39 µm long, with rounded basal knobs, the vulva at 73.24 to 88.06% body length, with a nonfunctional spermatheca, and the tail smooth and broadly rounded. Males of *P. brachyurus* were absent.

The usual suspect nematode species known to be the most important parasites of banana worldwide were isolated from the root samples examined in this study. The nematode species and the average percentage of positive root samples were as follows: *Helicotylenchus* spp., 95%; *Meloidogyne* spp., 79%; *Pratylenchus* spp., 36%; and *R. similis*, 38% (Table 1). *Helicotylenchus* spp. was detected in 100% of root samples from 12 of the 13 municipalities surveyed, the only exception being Santana do Mundaú, where only 71% of the samples were positive (Table 1). The number of *Helicotylenchus* individuals per 50 g roots ranged from 0 to 21,200. On average, population densities of *Helicotylenchus* spp. in banana roots were higher than those of *Meloidogyne* spp., *Pratylenchus* spp., and *R. similis* ($P \leq 0.01$) (Table 1).

Species of root-knot nematodes occurred in 100% of the root samples collected from six of the 13 municipalities, including Porto Calvo, Matriz do Camaragibe, Joaquim Gomes, Santana do Mundaú, Viçosa, and Coruripe (Table 1). No *Meloidogyne* spp. was detected in the municipality of Cajueiro. Of the four nematode genera observed in this study, *Pratylenchus* spp. had the lowest average number (682) of specimens per 50 g root ($P \leq 0.01$) (Table 1). The lesion nematode was present in 100% of root samples from the municipalities of Japaratinga, Matriz do Camaragibe, Viçosa, and Coruripe. It was also present in Maragogi, Santana do Mundaú, although only in 40% and 14% of the samples, respectively. *Radopholus similis* was the least frequent of the nematode species encountered during the survey, since it was found in 100% root samples only in the municipalities of Porto Calvo, Matriz do Camaragibe, and Joaquim Gomes (Table 1). Its maximum population density, 12,267 nematodes per 50 g roots, was observed in the municipality of Porto Calvo ($P \leq 0.01$).

In soil samples, the nematodes species and the average percentage of positive samples were the following:

Table 1. Frequency, mean density, and range of variation of *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *Radopholus similis* in 42 banana fields from 13 municipalities in the state of Alagoas, Brazil.

Municipality - Fields	*Helicotylenchus*		*Meloidogyne*		*Pratylenchus*		*Radopholus similis*	
	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]
Maragogi - 5	100	567 (47-2480)	80	2172 (0-8667)	40	211 (0-967)	60	568 (0-1867)
Japaratinga - 6	100	158 (60-367)	60	272 (0-1247)	100	4247 (20-13467)	60	2281 (0-13533)
Porto Calvo - 1	100	67 (67)	100	1867 (1867)	0	0	100	12267 (12267)
M. Camaragibe - 2	100	258 (133-383)	100	2284 (150-4417)	100	200 (183-217)	100	2642 (417-4867)
J. Gomes - 3	100	24 (7-47)	100	263 (3-717)	0	0	100	889 (123-1367)
U. Palmares - 6	100	4158 (540-10244)	83	76 (0-267)	0	0	0	0
Ibateguara - 2	100	4784 (3880-5687)	50	667 (0-1333)	0	0	0	0
São J. da Lage - 2	100	4348 (733-7963)	50	377 (0-753)	0	0	0	0
S. do Mundaú - 7	71	626 (0-1917)	100	1235 (197-5333)	14	69 (0-483)	57	2905 (0-12433)
Murici - 2	100	1565 (897-2233)	50	50 (0-100)	0	0	0	0
Cajueiro - 2	100	9150 (9000-9300)	0	0	0	0	0	0
Viçosa - 3	100	1480 (610-2700)	100	623 (23-1167)	100	260 (30-600)	0	0
Coruripe - 1	100	21200 (21200)	100	1153 (1153)	100	450 (450)	0	0
TOTAL - 42	95	2359 (0-21200)	79	810 (0-8667)	36	682 (0-13467)	38	1359 (0-13533)

[y]Frequency was determined as 100 × (number of fields containing the species/number of fields sampled in the municipality).
[z]Figures are numbers of nematodes/50 g roots, and ranges of variation are shown in parenthesis.
Plant-parasitic nematode population densities differed between and within the municipalities ($\chi 2$ =120255, degrees of freedom - df = 36, $P \leq 0.01$).

Table 2. Frequency, mean density, and range of variation of *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *Radopholus similis* in 42 banana fields from 13 municipalities in the state of Alagoas, Brazil.

Municipality - Fields	*Helicotylenchus*		*Meloidogyne*		*Pratylenchus*		*Radopholus similis*	
	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]	Frequency[y]	Mean density[z]
Maragogi - 5	100	156 (53-267)	80	203 (0-527)	40	20 (0-60)	40	8(0-27)
Japaratinga - 6	100	596 (107-1427)	67	104 (0-327)	67	149 (0-527)	33	6 (0-27)
Porto Calvo - 1	100	420 (420)	100	27 (27)	100	13 (13)	0	0
M. Camaragibe - 2	100	135 (50-220)	100	1433 (833-2033)	50	4 (0-7)	50	25 (0-50)
J. Gomes - 3	100	54 (17-93)	67	360 (0-1017)	0	0	33	312 (0-917)
U. Palmares - 6	83	38 (0-67)	67	28 (0-93)	0	0	0	0
Ibateguara - 2	100	137 (117-157)	100	202 (93-310)	0	0	0	0
São J. da Lage - 2	100	163 (73-253)	100	63 (53-73)	0	0	0	0
S. do Mundaú - 7	100	148 (17-373)	100	251 (33-487)	0	0	43	11 (0-30)
Murici - 2	100	154 (100-207)	0	0	0	0	0	0
Cajueiro - 2	100	290 (220-360)	100	17 (13-20)	0	0	0	0
Viçosa - 3	100	116 (100-127)	100	18 (3-33)	67	10 (0-20)	0	0
Coruripe - 1	100	633 (633)	100	243 (243)	100	167 (167)	0	0
TOTAL - 42	98	213 (0-1427)	81	200 (0-2033)	26	29 (0-527)	21	27 (0-917)

[y]Frequency was determined as 100 × (number of fields containing the species/number of fields sampled in the municipality).
[z]Figures are numbers of nematodes/100 cm^3 soil, and ranges of variation are shown in parenthesis.
Plant-parasitic nematode population densities differed between and within the municipalities ($\chi 2 = 5726$, df = 36, $P \leq 0.01$).

Helicotylenchus spp., 98%; *Meloidogyne* spp., 81%; *Pratylenchus* spp., 26%; and *R. similis*, 21% (Table 2). *Helicotylenchus* spp. and *Meloidogyne* spp. had the highest average population densities: 213 and 200 individuals per 100 cm^3 soil, respectively ($P \leq 0.01$) (Table 2). *Helicotylenchus* spp. were detected in 100% soil samples from all municipalities, except União dos Palmares where only 83% of the samples were positive. The highest population densities of *Helicotylenchus* spp. in soil were found in the municipalities of Coruripe and Japaratinga, with 633 and 596 specimens per 100 cm^3 soil, respectively ($P \leq 0.01$). *Meloidogyne* spp. were present in 100% soil samples from eight municipalities, and the highest population densities were recorded in the municipality of Matriz do Camaragibe: 1433 specimens per 100 cm^3 soil, followed by Joaquim Gomes: 360 specimens per 100 cm^3 soil, and Santana do Mundaú: 251 specimens per 100 cm^3 soil ($P \leq 0.01$) (Table 2). *Pratylenchus* spp. were detected in 100% soil samples only in the municipalities of Porto Calvo and Coruripe, but it was present in four other municipalities (Table 2). The burrowing nematode, *R. similis*, was present in soil samples from five municipalites, although at lower frequencies (Table 2). The highest population density of *R. similis*, 312 specimens per 100 cm^3 soil ($P \leq 0.01$), was observed in the municipality of Joaquim Gomes.

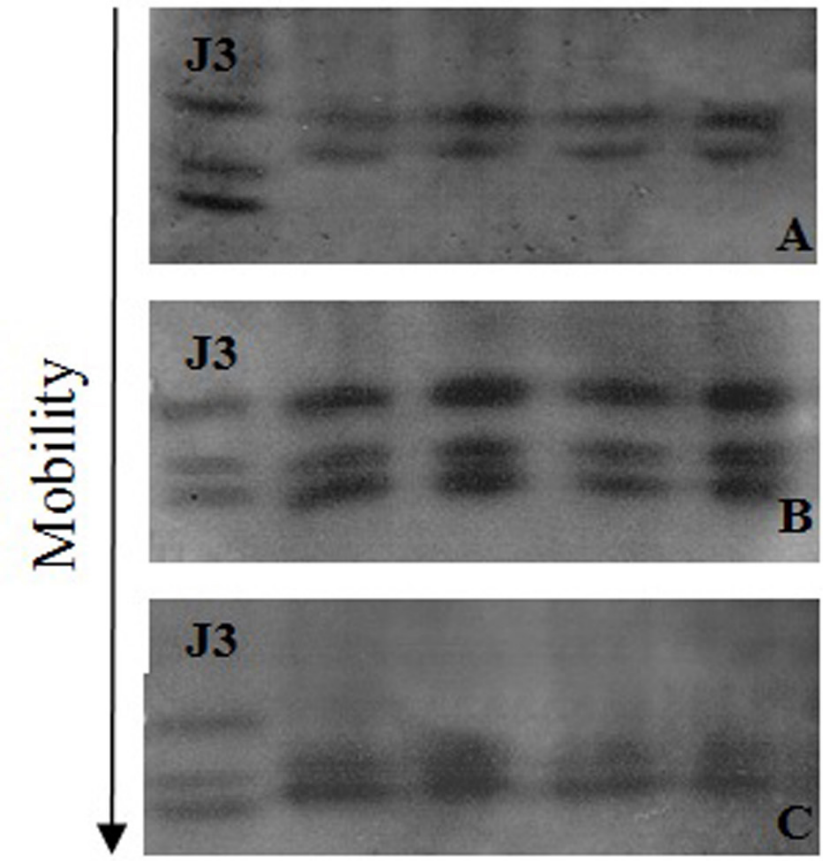

Fig.1. Esterase phenotypes of *Meloidogyne* spp. infecting banana in Alagoas state, Brazil. A – *M. incognita* (phenotype I2), B – *M. javanica* (phenotype J3), and C – *M. arenaria* (phenotype A2). Esterase phenotype of *M. javanica* (J3) was used as standard for comparison.

DISCUSSION

The results of this study are similar to those reported in many other banana fields in Brazil, particularly the high frequencies of *Helicotylenchus* spp. and *Meloidogyne* spp. (Cavalcante *et al*., 2005; Ritzinger *et al*., 2007; Souza *et al.*, 1999). The three *Meloidogyne* species identified in this study (*M. incognita*, *M. javanica*, and *M. arenaria*) also have been reported elsewhere in Brazil (Cofcewicz *et al*., 2004; Dinardo-Miranda and Teixeira, 1996), Costa Rica (Araya *et al*., 2002), and Ecuador (Chávez and Araya, 2010). But, this is the first record of *Meloidogyne* spp. on banana in the state of Alagoas. The nematodes species *H. multicinctus*, *Pratylenchus* sp., and *R. similis* have been previously reported in Alagoas state, although in very low frequencies (Andrade *et al*., 2009).

The genus *Helicotylenchus* was the most abundant nematode in all of our sampling areas, consistent with earlier reports indicating *H. dihystera* and *H. multicinctus* were the most abundant nematodes on banana in the Northwest of Paraná, Brazil (Dias-Arieira *et al*., 2010). Also, our finding that population densities of *Helicotylenchus* spp. in roots were higher than those of *Meloidogyne* spp., *Pratylenchus* spp., and *R. similis* is similar to Wang and Hooks (2009) report in Hawaii. As recommended by these two authors, *H. multicinctus* should receive greater attention. On the other hand,

Pratylenchus spp. had the lowest average number of all the phytonematodes recorded in this study. This observation agrees with the results of a recent survey of banana fields in the northern region of Minas Gerais, Brazil (Dias *et al*., 2011). In some of the root samples, *R. similis* occurred at high densities (up to 13,533 individuals per 50 g roots). Root infestations in excess of 2,000 nematodes per 100 g roots is a potential cause of crop losses in all commercially grown cultivars (Gowen *et al*., 2005). With *R. similis*, any infestation, however small, might become a serious threat to production over the long term.

The high population densities observed in this study indicate the need for research aimed at the management of phytonematodes that parasitize banana in the state of Alagoas. In most of the banana plantations in this state, phytonematodes usually occur in polyspecific communities, consisting mainly of a mixture of *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *R. similis*. *Helicotylenchus* spp. were widespread and most frequent, indicating that these species are the major parasitic nematodes of banana in the state of Alagoas. As it emerged during this study, the presence of such important plant-parasitic nematodes in the state of Alagoas could be attributed to the continuous monoculture of bananas. The lack of nematode control measures and inadequate knowledge by farmers could also have contributed to heavy infestations.

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