

RESEARCH/INVESTIGACIÓN

PLANT-PARASITIC NEMATODES ASSOCIATED WITH EIGHT BANANA CULTIVARS IN SOUTHERN FLORIDA

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ABSTRACT

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Niche and specialty banana cultivation provides growers in southern Florida with economic opportunities that do not directly compete with foreign imports. These specialty bananas include dessert, cooking, and plantain types. Specific cultivars often lack foundational agronomic information that could guide the adoption of higher quality types. One major concern for banana growers in southern Florida is damage caused by plant-pathogenic nematodes. Nematodes limit the long-term economic return of some cultivars by reducing plant health until plants become unproductive. While some cultural and chemical methods exist for reducing pathogenic nematode populations, these can be expensive, ineffective, or cause environmental concerns. Understanding the prevalence of nematodes in southern Florida and their association with the roots of specific banana cultivars can help establish grower recommendations when starting new plantings. Seven nematode genera (*Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Trichodorus*, *Tylenchorhynchus*, and *Xiphinema*) were identified in histosol and limestone soil types in long-term banana plantings in southern Florida. Of these, only *Helicotylenchus* (0-4,704/100 g roots), *Meloidogyne* (0-365/100 g roots), and *Pratylenchus* (0-604/100 g roots) were consistently abundant over two sampling dates and for both soil types. Nematodes were isolated from the roots of all eight banana cultivars with ‘Giant Plantain’, ‘Pisang Raja’, and ‘Williams’ showing a trend towards fewer total isolated nematodes. This is the first report of nematodes associated with a diverse banana accessions in both histosol and limestone sites in southern Florida.

Key words: Banana, *Helicotylenchus*, histosol, limestone, *Meloidogyne*, nematodes, *Pratylenchus*, soil type

RESUMEN

Demesyieux, L., M. L. Mendes, W. T. Crow y A. H. Chambers. 2020. Nematodos fitoparásitos asociados a banano con cultivares de en el Sur de la Florida. *Nematropica* 50:19-28.

El cultivo de bananos especializados o para un nicho brinda oportunidades económicas a los productores del Sur de la Florida, al no competir directamente con importaciones extranjeras. Estos bananos especiales incluyen para postres, de cocción y tipos de plátanos. A menudo, los cultivares específicos carecen de información agronómica fundamental para guiar en la adopción de cultivares de mayor calidad. Una de las mayores preocupaciones de los productores de banano del Sur de la Florida es el daño causado por nematodos fitoparásitos. Los nematodos limitan el retorno económico a largo plazo de algunos cultivares al reducir la salud de la planta hasta que se vuelven improductivas. Si bien existen algunos

métodos culturales y químicos para reducir las poblaciones de nematodos fitoparásitos, estos pueden ser costosos, ineficaces o causar problemas ambientales. Al comprender la prevalencia de nematodos en el Sur de la Florida y su asociación con las raíces de cultivares de banano específicos puede ayudar a establecer recomendaciones a los productores al iniciar nuevas plantaciones. Siete géneros de nematodos (*Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Trichodorus*, *Tylenchorhynchus*, y *Xiphinema*) fueron identificados en plantaciones de banano a largo plazo en suelos de tipo histosol y calizos en el Sur de la Florida. De estos, solo *Helicotylenchus* (0-4704/100 g de raíces), *Meloidogyne* (0-365/100 g de raíces), y *Pratylenchus* (0-604/100 g de raíces) fueron consistentemente abundantes en dos fechas de muestreo y para ambos tipos de suelo. Los nematodos se aislaron de las raíces de los ocho cultivares de banano con ‘Giant Plantain’, ‘Pisang Raja’, y ‘Williams’ mostrando una tendencia hacia un menor número total de nematodos aislados. Este es el primer reporte de nematodos asociados a un diverso grupo de accesiones de banano en suelos de tipo histosol y calizos en el Sur de la Florida.

Key words: Banano, *Helicotylenchus*, histosol, calizos, *Meloidogyne*, nematodos, *Pratylenchus*, tipo de suelo

INTRODUCTION

Globally, banana (*Musa* sp.) is ranked as the fourth most important source of dietary calories after rice, wheat, and maize (Sagi *et al.*, 1998; FAOSTAT, 2017). Bananas are grown and consumed in more than 130 countries in the tropics and the subtropics and include dessert, cooking, and brewing types (Quénéhervé, 2009). Around 85% of global banana production is consumed locally with most banana growers maintaining small plantings (INIBAP, 1999; Quénéhervé, 2009; Tripathi *et al.*, 2015). In the United States, dessert-quality bananas are the most prevalent with a per capita consumption of 12 kg of fruit per annum (USDA ERS, 2018). While most bananas in the United States are imported, many domestic growers often seek specialty types for niche markets (Evans and Ballen, 2012). Specialty bananas include types not available through common retail supply chains with some types being specifically grown for their superior flavor quality (Schupska, 2008).

Banana cultivation relies on clonally propagated, seedless cultivars that are maintained as perennial monocultures. These factors increase the risk of global disease epidemics. Historically, this was demonstrated with the demise of banana cv. Gros Michel banana when destroyed by Panama Disease (*Fusarium oxysporum* f. sp. cubense race 1) that required the entire industry to adapt to the modern ‘Cavendish’ type bananas (Jones, 2000; Ploetz, 2015; Laliberté, 2016). Similarly, modern banana cultivation practices

facilitate the increase of pathogenic nematodes and limits the effectiveness of chemical or cultural control methods. Under constant cultivation, banana yield losses of 30-60% can be common under high nematode pressure (Davide, 1996). Unfortunately, the most common types of banana are similarly susceptible to a few species of nematode and represent a major concern in all banana-growing regions (Stover, 1972; Gowen and Quénéhervé, 1990; Gowen *et al.*, 2005). The three most important banana nematodes globally are *Radopholus similis* (burrowing nematode), *Pratylenchus* spp. (lesion nematode), and *Helicotylenchus multicinctus* (spiral nematode) with others like *Meloidogyne* spp., and *Rotylenchulus reniformis* also causing significant negative impacts (Gowen, 1990; Ploetz, 1999). Nematode control relies on preventing the spread of nematodes or application of nematicides that can negatively impact the environment and applicators (Haegeman, 2010). Banana cultivar-specific differences in nematode abundance have been reported previously, and this suggests that banana diversity may play a role in reducing the overall economic risks from nematode damage (Brooks, 2004).

Growers in southern Florida often rely on specialty and niche crops to increase profitability while facing higher production costs than many fruit-exporting countries. Banana production in southern Florida comprises around 500 acres and is worth ~\$2 million per year (Evans and Ballen, 2012). Some growers in southern Florida cultivate diverse banana types in order to meet growing demand from various domestic ethnic populations

(Crane *et al.*, 2005). There are thousands of banana cultivars and types available for commercial production, and selecting specific cultivars for specialty banana commercial production can be challenging due to limited cultivar-specific information. Cultivars can vary for agronomic performance including yield, fruit quality, and important traits like disease resistance (Heslop-Harrison and Schwarzacher, 2007). The diverse types of banana being grown in southern Florida could be used as a method to identify those that naturally support lower rates of nematode infection.

Generating cultivar-specific information on yield, quality, and disease resistance for specific growing regions helps to reduce grower risk and support locally grown produce. Screening long-term banana cultivation sites for nematode populations could also help identify prevalent nematode species and potentially susceptible banana cultivars. This information can help reduce grower risks when selecting cultivars for new plantings. The purpose of this study was, therefore, to identify the most abundant, naturally occurring soil-borne nematodes from banana fields, and to identify the most abundant nematodes associated with banana roots at two selected sites in southern Florida under natural conditions. The outcomes of this research identify the most abundant nematodes

in banana-producing areas in southern Florida, and provide preliminary data facilitating future research investigating the genetic basis for nematode resistance in diverse banana accessions.

MATERIALS AND METHODS

Site selection

Two sites were selected for the nematode screening based on contrasting soil types and the availability of diverse *Musa* species under commercial cultivation. Both sites had been under commercial banana or plantain cultivation for at least 10 years, and both sites have favorable climatic conditions for *Musa* agricultural production. The average annual temperature was 28.9°C, and the annual precipitation was between 1,200 and 1,400 mm (Lusher, 2008). Neither site had applied nematicides for at least the previous 5 yr (Table 1).

Musa accessions

A total of eight accessions were selected for the nematode survey (Table 2). These accessions were selected to capture some genetic diversity and represent both dessert and cooking banana types.

Table 1. Soil type description, farm management, and age of the plantations for each of the two surveyed locations.

Site	Soil type	Farm management	Age of the plantation
Pahokee/Palm Beach County	Mostly histosol characterized by a thick black muck soil layer over limestone bedrock. Soils are poorly drained, amply rainfed for 6 to 12 months each year except for very dry years. They originate from organic matter. https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/florida/FL611/0/Palm_Beach.pdf	Occasional weed control mostly by mowing and herbicide application. Zero input of fertilizer drawing upon the moderate fertility of the soil.	~10 years
Homestead/Miami-Dade County	Calcareous entisol of recent limestone origin with poor water retention and nutrient content. https://edis.ifas.ufl.edu/pdf/SS/SS65500.pdf https://journals.ashs.org/horttech/view/journals/horttech/20/1/article-p10.xml	Low fertilizer input with additional water supply through micro-aspersion. Herbicides and mowing were used as means of weed control.	>20 years

Table 2. A list of the eight banana and plantain accessions used in this study. Cultivar is the name of the accession, Genome Group is the subgenome composition, and Description is the primary use of the banana accession.

Cultivar	Genome group	Description
African Rhino	AAB	Cooking banana
Dwarf Cavendish	AAA	Dessert
FHIA 1	AAAB	Dessert
FHIA 18	AAAB	Dessert
Giant Plantain	AAB	Cooking
Hua Moa	AAB	Cooking/dessert
Pisang Rajah	AAB	Dessert
Williams	AAA	Dessert

The eight accessions represent all cultivars that were common between both long-term banana growing sites.

Root sampling

Samples were collected in May and again in August 2018. Two to four plants were sampled for each accession at each site preferentially from recently flowering plants. Roots and soil were collected from an approximately 30 cm x 30 cm area on two sides of each plant. The root and soil samples from each mat for a given cultivar were bulked to form composite samples and placed into a plastic bag properly identified with the date, location, and cultivar name. All samples were transported in insulated boxes to protect samples from direct sunlight and temperature fluctuations. Samples were stored at 4°C until processing for nematode extraction.

Nematode extraction

Soil and root samples were processed separately. Nematodes were extracted from plant roots and soil following the modified Baermann tray/funnel technique/pie-pan method (Whitehead and Hemming, 1965). Prior to the extraction of nematodes, all soil samples were sieved using a No. 30 (595 µm) mesh to remove debris and stones that might be in the sample. Two subsamples of 50 g of soil were taken from each bulk sample for nematode extraction. Each subsample was placed into a 500 ml beaker and thoroughly mixed with 200 ml of water. The mixture was then poured onto a coffee filter supported by a screen placed inside of a plastic bowl. The water level inside the plastic recipient was kept even with the soil in the coffee

filter throughout a room temperature incubation for 72 hr (Coyne *et al.*, 2007; Mekete *et al.*, 2012). After incubation, the filtered water from subsamples was pooled. Nematodes were collected and concentrated using a 38-µm sieve. Nematodes were then identified and quantified as the number of nematodes/100 g of soil.

For root samples, the same method was used with some modifications (Whitehead and Hemming, 1965). Roots from each accession and location were independently processed. Roots were cleaned with tap water and left to be air-dried for 2 hr before processing. Roots were then cut into small portions of 1 or 2 cm and mixed thoroughly. Twenty-five grams of cut roots were then macerated in a kitchen blender with ~200 ml of water for ~20 sec. The blended root samples were then poured onto a coffee filter placed inside of a mesh sieve in a plastic bowl as described for the soil samples (Coyne *et al.*, 2007; Mekete *et al.*, 2012). Nematode quantification is reported as nematodes per 100 g of roots.

Nematode identification and quantification

An inverted microscope was used to identify the nematodes at the genus level and to enumerate the number of each individual per genus contained in each sample. A petri dish with scored lines was used to prevent recounting of the same individuals.

RESULTS

Abundance of nematodes from soil samples

Nematode counts from soil samples were taken from the histosol and limestone sites to identify the percentages of plant-parasitic

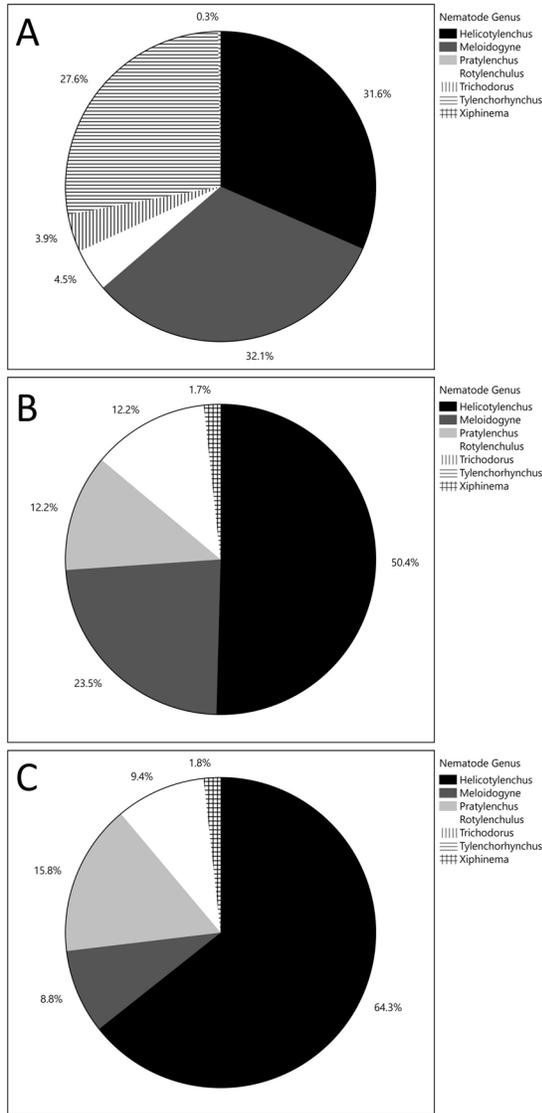


Figure 1. Percentage of the total number of plant-parasitic nematode percentages by genus in soil samples from histosol (A) and limestone (B) sites. An addition, an independent limestone site collection is also shown (C). Data represent nematode percentages from eight independent soil samples for each A, B, and C.

nematodes present by genus. The percentage of isolated genera are shown in Figure 1, and representative microscope images are shown in Figure 2. *Helicotylenchus*, *Meloidogyne*, *Rotylenchulus*, and *Xiphinema* were isolated at both sites. *Pratylenchus*, *Trichodorus*, and *Tylenchorhynchus* were only isolated in soil samples from the histosol site (Fig.1A). The May soil sampling for the limestone site was

qualitatively similar to the August sampling, but with increased *Helicotylenchus* and decreased *Meloidogyne* percentages (Fig. 1C). *Helicotylenchus* and *Meloidogyne* nematodes were the most abundant species and represented 2/3 to 3/4 of total nematodes isolated from soil samples. *Pratylenchus* nematodes were much less abundant in histosol soils (0-0.2%) compared to limestone soils (1.9 to 17.3%).

Nematodes associated with Musa roots

Roots from banana and plantain were sampled in May and August from eight cultivars growing at both sites (Table 1). *Helicotylenchus* was the predominant nematode species and accounted for 63.9 to 96.3% of all plant-parasitic nematodes associated with banana roots. *Meloidogyne* spp. were found at each sampling time and site and ranged from 3.7 to 17.3% of all nematodes sampled. *Hoplolaimus* spp. nematodes were only identified once from histosol root samples in May (0.8% of all nematodes).

In roots from histosol soils, *Meloidogyne* individuals were the most frequent with a frequency rating of 87.5% of the root samples followed by *Helicotylenchus* that were found in 75% of samples. *Hoplolaimus* and *Pratylenchus* were also identified, but with lower frequency rating of 12.5% and 6.3%, respectively. In roots from limestone soils, *Helicotylenchus* was extracted in 81.3% of the samples, while *Meloidogyne* individuals were found in 56%. The absolute frequency of *Pratylenchus* in banana root samples was three times higher in the limestone than in the histosol location. *Helicotylenchus*, *Meloidogyne*, and *Pratylenchus* were, in general, the most consistently isolated and abundant genera. Nematodes associated with *Musa* cultivars in histosol and limestone soils.

Number of plant-parasitic nematodes associated with each of the eight banana cultivars grown at the histosol site are shown in Table 3. *Meloidogyne*, *Helicotylenchus*, *Hoplolaimus*, and *Pratylenchus* were isolated from root samples from histosol soils. *Meloidogyne* spp. were isolated from every cultivar during one or both sampling dates and ranged from 0 to 365 nematodes. *Helicotylenchus* spp. were the most abundant nematodes associated with banana roots and ranged from 0 to 4,704 nematodes. *Hoplolaimus* was only isolated from two cultivars during a single



Figure 2. Representative microscope images of the most common nematode species isolated from banana roots in southern Florida. *Helicotylenchus* (A), *Meloidogyne* (B), and *Pratylenchus* (C) are shown.

timepoint, and *Pratylenchus* was only isolated from a single cultivar at one timepoint.

Nematodes were also isolated from banana roots growing in limestone soil (Table 3). *Helicotylenchus*, *Meloidogyne*, and *Pratylenchus* were isolated from banana roots growing in limestone soil. *Meloidogyne* spp. were isolated from each cultivar and ranged from 0 to 204 nematodes per sample. *Helicotylenchus* was the most abundant nematode in limestone root samples and ranged from 0 to 1,344 per sample. *Pratylenchus* was only isolated from two cultivars (African Rhino and Williams' growing in limestone soil and ranged from 0 to 604 nematodes per sample.

DISCUSSION

This study identified the most frequent and abundant nematode species isolated from soil and banana roots from two long-term banana plantings in southern Florida. Three of them (*Helicotylenchus*, *Meloidogyne*, and *Pratylenchus*) are known to cause the serious economic damage to banana plantations (Quénéhervé, 2009). *Helicotylenchus* and *Meloidogyne* were, in general, the most abundant phytonematodes recovered from soil and root samples. Similar cases have been found in South Africa and in Hawaii where complexes of *Meloidogyne* and *Helicotylenchus* represented more than 2/3 of the nematode

population recovered in these locations (De Jager *et al.*, 1999; Wang and Hooks, 2004). Nematodes were isolated from all banana cultivars, but trends suggest that 'Giant Plantain', 'Pisang Raja', and 'Williams' may support lower levels of nematode population. These preliminary results will need to be confirmed in future studies using increased replication with sampling throughout the growing season. Unfortunately, this was not possible for the current study due to the limited plant materials and planting designs available in the long-term banana cultivation areas.

In general, the results of this study agree with previous studies that isolated *Helicotylenchus* and *Meloidogyne* from roots of a commercial planting of 'Burro' banana in southern Florida (McSorley and Parrado, 1981). However, *Radopholus similis* was not isolated from any soil or root samples even though this has been reported as a problem in southern Florida (McSorley, 1982). These results agree with conclusions by other authors that *Helicotylenchus* is the most important nematode in the subtropics where conditions are suboptimal for banana production and *Radopholus similis* survival (Gowen and Quénéhervé, 1990; Ploetz, 1997). We also isolated *Hoplolaimus* from two cultivar root samples in histosol soils as has been reported in other locations previously (Adiko, 1988; Martelle, 1988; Quénéhervé 1990; Nguyen *et al.*, 2015).

The presence of *Trichodorus*, *Tylenchorhynchus*, and *Xiphinema* in soil samples

Table 3. Plant-parasitic nematode counts from roots of banana cultivars. Data from the three consistently isolated genera (*Helicotylenchus*, *Meloidogyne*, and *Pratylenchus*) are shown for histosol and limestone sites during both May and August extractions.

Banana cultivar	<i>Helicotylenchus</i>				<i>Meloidogyne</i>				<i>Pratylenchus</i>			
	H ^w	L ^x	H	L	H	L	H	L	H	L	H	L
	M ^y	M	A ^z	A	M	M	A	A	M	M	A	A
African Rhino	75	300	0	108	150	0	4	68	10	10	0	604
Dwarf Cavendish	0	10	100	1,344	365	0	40	36	0	0	0	0
FHIA 1	1,495	0	132	176	55	0	4	4	0	0	0	0
FHIA 18	3,135	5	504	0	215	0	0	204	0	0	0	0
Giant Plantain	10	125	4	108	180	35	48	128	0	0	0	0
Hua Moa	15	0	470	328	80	0	88	32	0	0	0	0
			4									
Pisang Raja	335	10	0	20	5	0	12	4	0	0	0	0
Williams	0	40	136	172	0	0	20	184	0	0	0	8

^w=Histosol

^x=Limestone

^y=May

^z=August

might be explained by the presence of surrounding weed species in the surveyed areas. These nematode species were not a focus in the present study because they were not associated with banana root samples, though each has been associated with banana production areas previously (Chau *et al.*, 1997; Pathan *et al.*, 2004; Khan and Hasan, 2010). The differences between the sites might explain some of the more subtle differences in this study. For example, the increased abundance of total nematodes at the histosol site might be due to the rich organic soil or due to farm management. The histosol site relied primarily on mowing to control weeds and prevent soil erosion, whereas the limestone site leveraged leaf mulch to suppress weeds around banana stems. Soil and climatic influences on the dynamics of banana parasitic nematodes have been investigated in the past by several researchers and results reveal that species of *Helicotylenchus* thrive better than *Radopholus* sp. in wet organic soils (Quénéhervé, 1988). The diversity of non-banana plants at the histosol site

might, therefore, support a more favorable environment for nematodes. The increased abundance of *Pratylenchus* nematodes in the limestone site could also be related to this soil type, because the same cultivars were under long-term cultivation at both sites, and the histosol site had fewer of these nematodes in general.

The results of this study, while useful, should be cautiously interpreted as it relates to generalizations about cultivar vigor and quality. Some banana accessions can support relatively high nematode populations without showing symptoms of stress. Therefore, nematode abundance alone cannot be used to predict banana yield. Also, only limited replication was available for this study because of the minimal availability of plants for each cultivar as is common in commercial plantings involving many diverse accessions. Regardless, these results provide novel information for future work that could combine the most promising cultivars in terms of yield and fruit quality with multi-year trials in order to validate

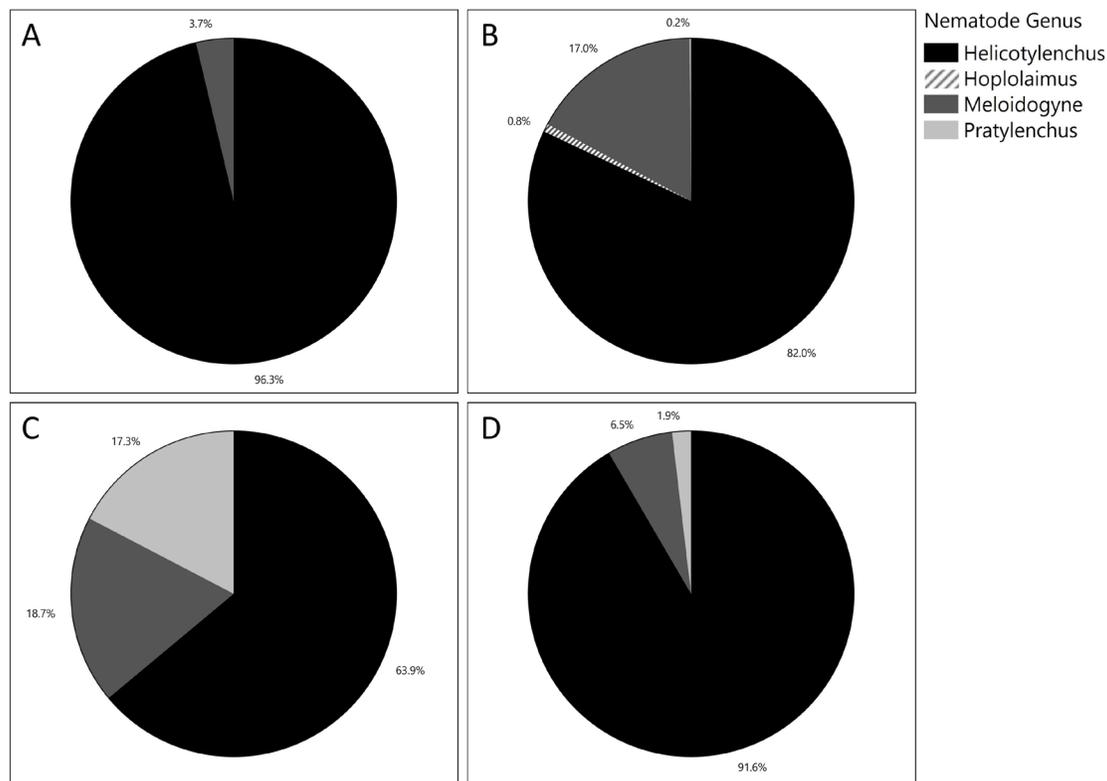


Figure 3. Percent of the total number of plant-parasitic nematodes extracted from banana roots from histosol August (A), histosol May (B), limestone August (C), and limestone May (D) samples. Data represent bulked root samples across all banana cultivars at each site for each sampling date.

these results and enable confident grower recommendations.

In conclusion, the purpose of this study was to identify the most common nematode species associated with soil and banana roots in southern Florida. We identified *Helicotylenchus*, *Meloidogyne*, and *Pratylenchus* as the most common nematodes associated with banana roots. Also, *Pratylenchus* nematodes seem to be less abundant in histosol than limestone soils. ‘Giant Plantain’, ‘Pisang Raja’, and ‘Williams’ all seemed to consistently yield fewer nematodes from root extractions. These accessions might be favorable for growers that are currently looking for accessions that could be more resistant to nematode colonization.

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