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PHYTO-PARASITIC NEMATODES ASSOCIATED WITH CULTIVATION OF SUGAR CANE (*Saccharum officinarum* L.) IN GUANTANAMO PROVINCE, CUBA

Nemátodos fitoparásitos asociados al cultivo de la caña de azúcar (*Saccharum officinarum* L.) en la provincia Guantánamo, Cuba

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ABSTRACT. Phyto-parasitic nematodes are associated with affectations in the development and production of agricultural crops, recognized as one of the limiting factors on yields. The need to update the record of these organisms associated with sugar cane in Guantanamo province was the main objective of this research. For this, soil and root samples taken in two cane production units were processed. The determination of genera and species was conducted at the of Laboratory Nematology of the Provincial Directorate of Plant Protection, also the population was characterized by determining indices of genus and species richness of Margaleff and dominance index of Berger Parker. 24 species of phyto-parasitic nematodes grouped in 16 genera were identified, and *Pratylenchus* as the most represented genus with 5 species. These results constitute a contribution to knowledge of the nematodes composition associated with the cultivation of sugarcane in the province.

Key words: population density, *Meloidogyne*, *Pratylenchus*

RESUMEN. Los nematodos fitoparásitos se asocian a afectaciones en el desarrollo y producción de los cultivos agrícolas, reconociéndose como uno de los factores limitantes en los rendimientos. La necesidad de actualizar el registro de estos organismos asociados a la caña de azúcar en la provincia Guantánamo, constituyó el objetivo principal de esta investigación. Para ello se procesaron muestras de suelo y raíz, tomadas en dos unidades productoras de caña. La determinación de géneros y especies se realizó en el Laboratorio de Nematología de la Dirección Provincial de Sanidad Vegetal, además se caracterizó la población mediante la determinación de los índices de riqueza de género y especies de Margaleff y el índice de dominancia de Berger Parker. Se identificaron 24 especies de fitonematodos agrupados en 16 géneros, y *Pratylenchus* como el género de mayor representación con cinco especies. Estos resultados constituyen una contribución al conocimiento de la nematofauna asociada al cultivo de la caña de azúcar en la provincia.

Palabras clave: densidad de la población, *Meloidogyne*, *Pratylenchus*

INTRODUCTION

Phyto-parasitic nematodes are pests of agroecosystems and they are considered to exert an important influence on their structure and stability. There are 4,105 species of phyto-nematodes known, which cause annual losses between 11 and 14 % of production in economically important crops such as legumes, grains, bananas, cassava, coconut, sugar beets, sugar cane, potatoes, vegetables and various fruit trees; equivalent to 80 billion USD per year (1).

The damages caused frequently by nematodes are attributed to more evident causes such as nutritional deficiencies or water stress among others, since it is impossible to observe them at first sight in the field since the symptoms

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they produce are nonspecific, therefore, the Farmers and technicians often underestimate their effects (2). Some studies indicate that the ability to cause damage from nematodes is favored by edaphoclimatic and agronomic factors such as monoculture (3), high rainfall (4) and sandy soils (5).

The problem of parasitism by nematodes in sugar cane is a recognized fact, in those countries where this crop is of economic importance (6).

In recent years, efforts have been made to study and control this pest in order to reduce its damage and obtain better harvests (6-8).

Most of these investigations indicate that nematodes affect the malformation of sugarcane plants and the reduction of their yield (9).

In the United States, the estimated average losses due to nematodes in sugarcane areas was 4 % (10).

In Costa Rica, preliminary studies carried out in different sugarcane areas revealed the existence of high populations of phyto-parasitic nematodes associated with stunted plants with low production (11); therefore, the analysis of population density is important to determine the control strategies of these pathogens (12). It has also been observed that the increase in yield in sugar cane is concomitant with the abrupt reduction in density of the nematode community shortly after planting and harvesting (13).

On the other hand, agricultural yields of sugarcane in Guantánamo province have declined in recent years, from 45,3 ton/ha in 2007 to 25,1 ton/ha in 2010 (14). This reduction could be associated, among other causes (15), with the presence of phyto-parasitic nematodes.

Despite the importance of these factors, studies of nematodes in sugar cane in Cuba are very scarce and go back almost three decades. For these reasons, the objective of this work was to identify the nematofauna associated with this crop in two cane production units in Guantanamo province; as well as, estimate the density and population distribution of the most common genera and species in the field.

MATERIALS AND METHODS

The study was conducted in Guantánamo province, in two Basic Units of Cooperative Production (UBPC) “La Esperanza” and “Mártires de Barbados”, belonging to the municipalities Manuel Tames (Argeo Martínez locality) and Guantánamo (Paraguay locality) respectively, in the period comprised from June to November 2014.

For the sampling of soil and roots, three fields of three cane blocks of the shoot strain were selected. The primary information of the area under study is shown in Table 1. The four main cultivars of the province were taken into account in terms of the planted area percentage.

Table 1. Origin of soil and root samples for the determination of the population of nematodes

UBPC	Block	Field	Area (ha)	Cultivar	Soil Grouping
La Esperanza	15	11	14,89	C1051-73	Calcic Sialityc
La Esperanza		12	3,22	C1051-73	Calcic Sialityc
La Esperanza		13	2,42	C1051-73	Calcic Sialityc
La Esperanza	12	1	16,00	C1051-73	Calcic Sialityc
La Esperanza		2	14,10	C1051-73	Calcic Sialityc
La Esperanza		3	7,57	C1051-73	Calcic Sialityc
La Esperanza	36	5	6,31	C86-12	Calcic Sialityc
La Esperanza		6	2,68	C86-12	Calcic Sialityc
La Esperanza		7	2,59	C86-12	Calcic Sialityc
Mártires de Barbados	11	1	5,30	C87-51	Alluvial
Mártires de Barbados		2	10,76	C87-51	Alluvial
Mártires de Barbados		3	5,11	C87-51	Alluvial
Mártires de Barbados	20	2	2,69	C87-51	Alluvial
Mártires de Barbados		3	8,66	C87-51	Alluvial
Mártires de Barbados		4	3,80	C87-51	Alluvial
Mártires de Barbados	19	1	1,02	C90-647	Alluvial
Mártires de Barbados		2	6,00	C90-647	Alluvial
Mártires de Barbados		3	3,50	C90-647	Alluvial

The experiment was carried out in five sampling stations, one in each corner and in the field center. Each station was formed by two rows of three meters long and in each of them four sub-samples corresponding to four seedlings was taken. The portions of soil and roots were taken in the area of the rhizosphere of each selected seedling, below the first 5 cm and up to 30 cm deep. In this way, the sample of each field was made up of 20 sub-samples of soil and roots, extracted with the help of pick and shovel, mixed homogeneously. From them, a portion of 500 g of soil and 100 g of roots were taken, which were stored together in a plastic bag of 2 kg to help preserve the roots, avoiding desiccation. The bags were properly labeled with the corresponding data for each plantation and moved for processing to the Provincial Plant Health Laboratory. A fraction of additional soil was also taken in each field for the assembly of the indicator plant technique.

The nematodes were extracted by the modified Fenwick method for cytogens and for the extraction of vermiforms by the decantation-sieving method (16). The recoveries were deposited in *Baermann funnels* (17). The identification of the phyto-nematode species was carried out by means of morphological and morphometric methods, based on the keys and taxonomic references used in this type of research (18-22). Nematode populations were counted and expressed as number of individuals in 25 g of soil and number of nematodes in 25 g of fresh root.

Simultaneously, the assembly of indicator plants was carried out in bags containing only soil, in which three pumpkin seeds were planted. 35 days after the plants germinated and grown, they were taken to the Laboratory to determine the presence or not of gall-forming nematodes and to determine genera and species, as well as the gradology, by the Zeck methodology (23).

The data were analyzed by means of descriptive statistics, the appearance frequency and the population density expressed as a percentage were determined, of each genera of nematodes in each locality studied and in a general way.

To characterize the pattern of the communities of species and genera in the two locations sampled, the calculations of two indices were carried out (24):

Wealth Indexes of Gender and Margaleff species.

DMg = (S-1)/ln N

Berger Parker's Dominance Index, BP = N max/N

N = Number of individuals

S = Number of genera

RESULTS AND DISCUSSION

Genera and species of more frequent phyto-parasitic nematodes. Population density

The analysis of the samples defined 16 genera of phyto-parasitic nematodes associated with the cultivation of sugar cane in the sampled areas, of which 15 were found in the soil and 12 in the roots. The Criconemoides, Paratrophurus, Psilenchus and Trophurus genera were present in the soil samples but not in the roots, as well as Rotylenchus were found in the root samples and not in the soil samples (Table 2).

Table 2. Genera of phytoparasitic nematodes found in soil and roots in sugarcane plantations

Gender	Soils	Roots
Aphelenchoides	X	X
Aphelenchus	X	X
Criconemoides	X	
Ditylenchus	X	X
Dorylaimus	X	X
Helicotylenchus	X	X
Paratrophurus	X	
Paratylenchus	X	X
Pratylenchus	X	X
Psilenchus	X	
Rhabditis	X	X
Rotylenchulus	X	X
Rotylenchus		X
Trophurus	X	
Tylenchus	X	X
Xiphinema	X	X
16	15	12

In general, the most frequent nematode genera were *Pratylenchus* (100 %), Tylenchus (72 %), Paratylenchus (67 %), Aphelenchoides and Dorylaimus (55 %), while the highest populations were observed in the *Pratylenchus* genera (21,5 %), Paratylenchus (14,4 %), Dorylaimus (11.4%) and Rotylenchulus (11,2 %), out of a total of 2106 individuals (Figure 1). Other genera of nematodes found in order of percentage of occurrence in the total samples were Rotylenchulus (44 %), Aphelenchus and *Helicotylenchus* (27 %).

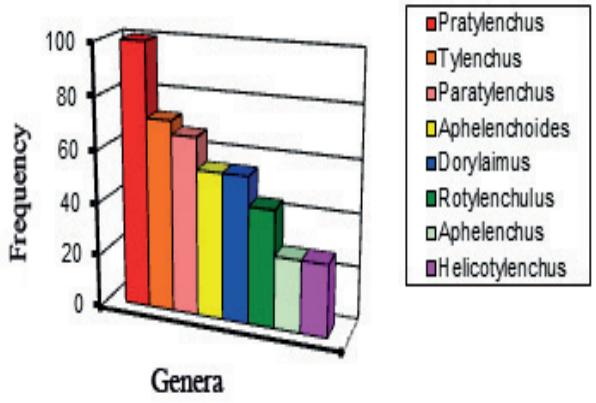

Figure 1. Genera of phytoparasitic nematodes more frequent in soil and roots of sugarcane

Several authors point out that *Helicotylenchus*, *Pratylenchus* and *Trichodorus* are the most dispersed genera in sugarcane plantations worldwide (12). In Brazil, *Pratylenchus* and *Meloidogyne* predominate, while species of *Pratylenchus*, *Meloidogyne* and *Helicotylenchus* are widely distributed in various areas of Australia (25).

In India, eight genera of phytopathogenic nematodes of importance in sugarcane were identified (26), among them: *Heterodera*, Rotylenchus, Hoplolaimus, Longidorus, Tylenchus, Aphelenchoides, *Pratylenchus* and *Meloidogyne*. In Venezuela *Pratylenchus zeae* Graham is reported as one of the most abundant species, found in Aragua, Carabobo and Yaracuy states (27).

As it can be seen, *Pratylenchus* is the common genera in all these studies of nematofauna associated with this crop.

Nematodes can cause numerous symptoms, including generalized yellowing, chlorosis and stunting in plants, symptoms that are closely related to their population densities (28). These pathogens, after penetrating the root are nourished by the cells causing lesions, initially small, that gradually increase in size. In addition, the damage caused by phyto-nematodes in the roots is reflected in aerial tissues such as insufficient growth of stems, leaf chlorosis and death of plants, due to reduced absorption of water and nutrients by the secondary roots (29).

The nematodes of the genera *Pratylenchus* known as lesioners of the roots are migratory endoparasites that penetrate the root system and feed on the cells, passing from one root to another. Consequently, the roots are underdeveloped, poor in secondary roots, deficient and unable to perform their functions. The openings in the roots are a source of entry to fungi and pathogenic bacteria, which aggravates to a greater degree the root system of the plant. *Pratylenchus* sp. has been part of complexes with *Fusarium* sp., *Pythium* sp. and other genera of fungal pathogens (30).

In this study, the genera *Pratylenchus* was found as the most frequent and with the largest population, appearing in 100 % of the analyzed samples. This fact may be affecting the low agricultural yields obtained in sugar cane in the two areas studied. Impacts on sugarcane yield have been reported in studies related to the nematofauna associated with this crop in different regions of the world (6,10,12,13).

In the study, a total of 24 species of nematodes were identified in the sampled fields, 24 of them in soils and 18 in roots (Table 3).

The presence of more than 275 species of 48 genera is reported on a global scale in cane (31).

However, in Cuba, 72 species were found in the soil and 62 in the roots (32). Other researchers report 83 species (33), while in Granma province, 36 nematode species were grouped into 15 genera in areas dedicated to cane production (34).

On the other hand, notable differences were found in the population of the localities studied, even at the level of sampled fields; in this sense, the UBPC “Mártires de Barbados” stands out with a total population of 1400 individuals. In this locality, the largest population (201 individuals) was found in field one of block 11. Paratylenchus was the most frequent genera with 100 % appearance in the sampled fields, which gives it the character of dominance over the rest of the genera, followed by Tylenchus (77,8 %), Aphelenchoides (55,6 %) and Dorylaimus (44,4 %). In turn, the largest number of individuals was found in the genera Paratylenchus (21 %), Dorylaimus and *Pratylenchus* (10 %).

In the UBPC “La Esperanza”, the genera *Pratylenchus* (77, 8 %), Aphelenchoides, Dorylaimus and Tylenchus predominated with 66,7 % frequency. The largest population in this locality was found in the genera Dorylaimus (13,8 %), followed by *Helicotylenchus* and Aphelenchoides with 12,2 % and Rotylenchulus (7,2 %) of a total of 706 nematodes present in the samples.

There are species that were diagnosed in a specific locality, this is the case of *Aphelenchus* sp., *H. multicinctus* (Cobb) Golden, *H. dihystera* (Cobb) Sher, *Paratrophurus* sp. and *Trophurus* sp., which were only found in the soil samples of the UBPC "La Esperanza", as well as *Pratylenchus* scribneri (Steiner) that was only observed in the root samples.

Table 3. List of nematode species identified in soil and roots in sugarcane in the locations studied

No.	Nematode species	UBPC Mártires de Barbados Block and Field										UBPC La Esperanza Block and Field							
		11			20			19				15		12			36		
		1	2	3	2	3	4	1	2	3	11	12	13	1	2	3	5	6	7
1	*Aphelenchus* sp.										x			x	x		x		x
2	*Aphelenchoides* sp.								x	x	x	x	x		x	x			
3	*Aphelenchoides subtenuis* (Cobb) Steiner & Buhrer							x					x					x	
4	*Criconemoides* sp.												x						
5	*Dorylaimus* sp.	x	x			x				x	x		x	x		x	x		x
6	*Ditylenchus* sp.				x			x			x						x		
7	*Helicotylenchus* sp.			x				x					x		x		x		
8	*H. multicinctus* (Cobb) Golden													x					
9	*H. dihystera* (Cobb) Sher,																	x	
10	*Pratylenchus* sp.			x			x					x	x						x
11	*P. zeae* Graham	x	x	x	x						x								
12	*P. pratensis* (Sher y Allen)									x					x	x			
13	*P.scribneri* (Steiner)																		x
14	*P. brachyurus* (Goodey) Filipjev					x		x					x					x	
15	*Psilenchus* sp.					x													x
16	*Paratylenchus* sp.	x	x	x	x	x	x	x	x	x	x					x	x		
17	*Paratrophurus* sp.											x							
18	*Rotylenchus* sp.	x	x														x		
19	*Rotylenchulus reniformis* (Linford y Oliveira)				x		x		x			x		x	x		x		x
20	*Tylenchus filiformis* (Butschli)	x	x			x	x							x			x		
21	*Tylenchus* sp.			x		x				x		x			x	x	x		
22	*Xiphinema* sp.				x		x											x	
23	*Trophurus* sp.															x			
24	*Rhabditis* sp.	x									x								

Diversity and wealth of genres in the sampled localities

The indices of biological diversity allow us to determine the variety of organisms that are part of an ecosystem. The calculated values of the Margaleff index for the sampled localities reveal the greatest wealth of genera (2.21) in the soil samples of the UBPC “La Esperanza”, followed by soil samples from the UBPC "Mártires de Barbados" (1.56).), which indicates that the community of nematode genera identified and quantified in the soil samples are the most diverse (Table 4).

For this index, values lower than two are areas of low diversity and values higher than five are indicative of high biodiversity (35).

The highest values of the Berger Parker Dominance index are presented in the soil and root samples of the UBPC "Mártires de Barbados". This is a measure that expresses the proportional importance of the most abundant genera, given by the genera *Pratylenchus*, which represents 61 % of the nematodes identified in the soil samples and 77 % in the roots. This result indicates that in this area *Pratylenchus* dominates over the rest of the identified genera.

As stated by some authors (28), in order to achieve an effective control of the diseases caused by phyto-parasitic nematodes, it is necessary to reduce their population densities in order to minimize the severity of the disease and the yield losses that these pathogens cause; therefore, this result should be taken into account in the integrated pest management in this UBPC, with measures that are aimed primarily at this genera, which has the largest population in the samples analyzed.

For the Berger Parker index, its reciprocal is usually adopted, and the values obtained show that an increase in the value of this index accompanies an increase in diversity and a reduction in dominance and with it a greater uniformity (24). The values of this index indicate that the community of nematodes in the UBPC “La Esperanza” in the samples of soil and roots is more diverse and the dominance of genres is smaller with respect to the locality of Paraguay, and in turn it is the most uniform community, since 18 and 13 species of nematodes were identified in soil and roots respectively (Table 5).

Table 4. Indices that quantify the pattern of communities of nematode genera associated with sugarcane in the studied areas

Evaluated indices	UBPC Mártires de Barbados		UBPC La Esperanza	
	Soil	Root	Soil	Root
Number of individuals	1169	231	556	150
Number of genera	12	9	15	7
Margaleff's Wealth Index (DMg)	1,56	1,47	2,21	1,20
IBerger Parker's Index of Dominance (BP)	0,68	0,61	0,32	0,39
Inverse of Berger Parker	1,48	1,65	3,10	2,54

Table 5. Quantity of genera, species and number of individuals in soil and roots in the studied locations

	La Esperanza		Mártires de Barbados	
	Soil	Roots	Soil	Roots
Genera	15	7	12	9
Species	18	13	16	12
Individual	556	150	1169	231

In similar studies carried out in Granma province (34), values of Margaleff's index of 1.37 were found in soil samples in Registered Seed Banks, and 1,29 in root samples of production areas. This indicates that areas of low gender diversity are below the values of the Margaleff index calculated below

Results of the assembly of the indicator plants

Hundreds of genera of phyto-parasitic nematodes are reported worldwide; however, only a small number are recognized as causing the main impacts on agricultural crops. The *Meloidogyne* genera is considered to be the most economically important (within this, M. incognita is the most harmful species), and then others are located as Bursaphelenchus, Globodera, *Heterodera*, *Pratylenchus*, Radopholus, Rotylenchulus and Xiphinema (36). As a result of the growing concern of farmers for gall-forming nematodes and the limitations of chemical nematicides due to their negative effects on agroecosystems, it is necessary to develop alternatives to solve this problem (37-40).

The assembly of indicator plants for cecidogenic nematodes was carried out in 100 % of the samples; however, only positive for the species *Meloidogyne incognita* (Kofoid and White) Chitwood, fields one and three of block 19 of the UBPC "Mártires de Barbados".

The morphology of the gall-forming nematodes changes during their life cycle and is characterized by marked sexual dimorphism (21). On the other hand, the population levels and duration of the life cycle of the *Meloidogyne* genera depend on its adaptation to the physical and biological environment of the soil, its compatibility with the host plant and the consequent access to nutrient sources (41). This causes that the diagnosis of this genera is difficult, which may have influenced that its presence could not be detected in the analysis of soil samples. However, with the technique of indicator plants, even with low population levels of the nematode, when placed in front of an alternative host and in good conditions of humidity and temperature, the development of the same is favored, completing its life cycle until reaching form the gills in the roots of pumpkin plants easily detectable by the naked eye.

In similar studies (13), the species *Meloidogyne* sp. was reported; appearing in four of the nine sampled districts cultivated with sugarcane, in Louisiana, United States.

The results obtained in this study demonstrate the presence of phyto-parasitic nematodes in the sugar cane plantations of the production units studied, which may be influencing the crop's agricultural production results. Several authors have found depressive effects of yield between 17 and 20 %, caused by phyto-parasitic nematodes (42,43).

CONCLUSIONS

- They were identified 24 species of phyto-nematodes associated with the cultivation of sugar cane in Guantánamo province, grouped into 16 genera, the genera *Pratylenchus* being the most frequent and the most abundant.
- The greatest wealth of genera was found in the soil samples.
- The *Meloidogyne* incognita species was diagnosed only in Paraguay locality.

RECOMMENDATIONS

- To study the existing nematofauna in the localities of Manuel Tames and Cuneira, representative of other edaphoclimatic zones of the province.
- To execute studies with different control methods to be able to recommend a strategy to fight phyto-parasitic nematodes associated with sugar cane.

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