PLANT-PARASITIC NEMATODES ASSOCIATED WITH PLANTAIN (*MUSA PARADISIACA*) IN TALAMANCA, LIMÓN, COSTA RICA

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ABSTRACT

Peraza-Padilla, W., R. Artavia-Carmona, E. Arboleda-Julio, R. Rodríguez-Porras, and S. Orozco-Cayasso. 2020. Plant-parasitic nematodes associated with plantain (*Musa paradisiaca*) in Talamanca, Limón, Costa Rica. Nematropica 50:151- 159.

Plantain is one of the most important food and commercial crops in the Huetar Atlantica region of Costa Rica. Plant-parasitic nematodes (PPN) are one of the major constraints of plantain production and are therefore a threat to food security. The present study was part of an investigation that was carried out in Talamanca, Limón, Costa Rica with the objective to quantify and identify the main PPN genera associated with plantain cultivation. Nematodes were extracted from a total of 76 samples; 63 from soil and 13 from roots of plantain collected from 10 smallholder farms. In terms of absolute frequencies of distribution, the most abundant PPN genus in soil was *Pratylenchus*, which was detected in over 76.9% samples, followed by *Helicotylenchus* (69.2%), *Meloidogyne* and *Radopholus* (61.5%). In the roots, *Helicotylenchus* (100%), *Meloidogyne* (90.5%), *Pratylenchus* (74.6%), and *Radopholus* (66.7%) were the most frequently detected genera. In general, frequency and population densities of PPN were high in comparison to other similar surveys. Overall, the data suggest that PPNs could pose a serious threat to plantain production in Costa Rica and that control measures should be further developed and implemented.

Key words: *Helicotylenchus*, *Meloidogyne*, plant-parasitic nematodes, *Pratylenchus*, *Radopholus*

RESUMEN

Peraza-Padilla, W., R. Artavia-Carmona, E. Arboleda-Julio, R. Rodríguez-Porras, and E. Orozco-Cayasso. 2020. Nematodos vegetales asociados con plátano (*Musa paradisiaca*) en Talamanca, Limón, Costa Rica. Nematropica 50:151-159.

El plátano es uno de los cultivos alimentarios y comerciales más importantes de la región Huetar Atlántica de Costa Rica y del mundo. Los nematodos parásitos de plantas (NPP) son una de las principales limitaciones de la producción de plátano y, por lo tanto, son una amenaza para la seguridad alimentaria. El presente estudio fue parte de una investigación que se llevó a cabo en Talamanca, Limón y cuyo objetivo fue cuantificar e identificar los principales NPP asociados al cultivo de plátano. Los nematodos se extrajeron de un total de 76 muestras, 63 de suelo y 13 de raíz de plantas de plátano recolectadas en diez fincas de pequeños agricultores. En términos de frecuencias absolutas de distribución, el género más abundante en el suelo fue *Pratylenchus*, que se detectó en más del 76,9% de las fincas, seguido de

Helicotylenchus (69,2%), *Meloidogyne* y *Radopholus similis* (61,5%). En el caso de la raíz, el género más frecuentemente encontrado fue *Helicotylenchus* (100%), *Meloidogyne* (90.5%), *Pratylenchus* (74.6%), finalmente, *R. similis* (66.7%). En general, la frecuencia y la densidad de población de NPP fue alta. Esto probablemente se debió al manejo de las condiciones agronómicas y climáticas observadas durante los últimos años. En general, los datos sugieren que las PPN podrían representar una grave amenaza para las plantaciones de plátano y que deberían desarrollarse e implementarse medidas de control.

Palabras clave: Helicotylenchus, *Meloidogyne*, nematodos parásitos de plantas, *Pratylenchus*, *Radopholus*

INTRODUCTION

Plantain, *Musa paradisiaca*, is in the Musaceae family. The fruit is less sweet than that of banana, the other important species in the genus *Musa*. It is estimated that *Musa* is cultivated in more than 125 tropical and subtropical countries (Jones, 2000). Plantains are a major source of carbohydrates for millions of people in Africa, the Caribbean, Latin America, Asia, and the Pacific (FAO, 2003).

Plantain and banana have been ranked as the fourth most important food crop in the world after rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum* spp.) (Frison and Sharrock, 1999). Plantain and banana are used for food, beverages, fermentable sugars, medicines, flavorings, and cooking (Nelson *et al*., 2006; Phillip *et al*., 2009). According to Oladejo and Sanusi (2008), plantain contributes to nutrition and food security in rural and urban areas. The main producing countries of plantain are Democratic Republic of Congo, Ghana, Cameroon, Uganda, Colombia, Philippines, Nigeria, and Perú (FAO, 2018). In sub-Saharan Africa, for example, it is estimated that over 30 million people subsist on banana and plantain as the principal source of dietary carbohydrate (Karamura *et al*., 1999). *Musa* plantations are a major economic base in Central American, Caribbean, and some South American countries. According to (FAO, 2018), the production area and yield of plantain globally was estimated at 5,643,475 ha and 39,482,164 tons, respectively.

In Costa Rica, *Musa* spp. is one the most important crops (Sánchez and Zúñiga, 2001), representing almost 18.8% of the agricultural gross national products just below pineapple, which represents 19.1%. Exported *Musa* products in 2017 were valued at $1,042,171 (USD) produced on 42,921 ha (Mora and Quirós, 2018).

Historically, plantain cultivation has had an important role in the social development and economy of Costa Rica. Plantain production in Costa Rica is concentrated in the Talamanca region and often is creatively integrated into various cropping systems on small-scale farms. The region has the potential for development of industrial processing of plantain into snack products and plantain flour. This commercial potential has increased the interest of small producers to grow plantain; however, there are problems in commercialization and prices. In addition, there are several limiting production factors, including plant-parasitic nematodes (PPN) and Black Sigatoka disease (*Mycosphaerella fijiensis*), which reduces bunch weight and increases the time needed for fruit to develop to maturity (Araya, 2002).

Plant-parasitic nematodes are the primary pest of *Musa* crops worldwide (Gowen and Quénéhervé, 1990; Kashaija *et al*., 1994; Pattison, 2011). In Costa Rican plantations, mixtures of the following PPN usually occur: *R. similis* (Cobb, 1893, Thorne, 1949, Sher, 1968), *Helicotylenchus multicinctus* (Cobb, 1893, Golden, 1956), *H. dihystera* (Cobb, 1893; Sher, 1961), *Meloidogyne incognita* (Kofoid and White, 1919, Chitwood, 1949), *M. javanica* (Chitwood, 1949, Treub, 1985), and *Pratylenchus* spp. (Linford and Oliveira, 1940). Plantain production in Costa Rica is characterized as being highly dependent on agrochemicals, which increase costs and productive vulnerability. The regular application of nematicides is the most widely used strategy to control PPN in Costa Rica; however, the environmental implications and restrictions in international markets dictate a rational use of nematicides at minimum dosages.

Considering the economic importance of plantain as well as the destructive nature of PPN to plantain, the objective of this study was to provide quantitative information about population densities and frequencies of the major PPN associated with plantain in smallholder farmer fields in Talamanca, Limón. This information will be used to identify the most important PPN associated with plantain in Costa Rica.

MATERIALS AND METHODS

Study area and description

The survey on PPN associated with plantain was conducted from January 2016 to December 2017 among smallholder plantain farmers of Bratsi, Telire, and Sixaola Districts, Talamanca Canton, Costa Rica. The farms were located at an altitude of 10 to 70 m above sea level (Fig. 1). Ten farms from the production area were surveyed. According to Holdridge (1982), this region is located in a humid tropical forest with annual rainfall ranging from 1 m to 2 m. In this region, mostly native varieties are grown in rural and indigenous communities in small fields. In some locations near the Sixaola District, farmers produce plaintain cv. Curraré enano.

Soil and root samples

A total of 76 samples were collected. Each sample consisted of 20 soil cores (30 cm width × 20 cm deep). Holes were dug in the rhizosphere of each of the sampled plants. Samples were spaced at roughly equal intervals in a zig-zag pattern. Soil and root samples were placed in sealed plastic bags and protected from both direct sunlight and high temperatures. The samples were stored at 4ºC before being analyzed to minimize changes in nematode population densities. A handheld global positional system device (GPS) (Garmin, GPS Map 60CSx, Garmin, Olathe, KS) was used to identify the locations.

Nematode extraction

Nematodes were extracted from 100 g of soil and 10 g of roots. Soil samples were processed by the flotation-centrifugation method (Jenkins, 1964). Root samples were washed in running tap water and chopped into small pieces (~ 1 cm),

Figure 1. Map of Costa Rica showing the site studied, Talamanca, Limón province, 2016-2017.

macerated in a kitchen blender for 20 sec at low speed, and the resultant suspension passed through two sieves (400 µm and 100 µm) to collect nematodes. Nematodes were killed and fixed in hot 4% formaldehyde (70ºC) and subsequently infiltrated with glycerin, using Seinhorst's modified slow method (Seinhorst, 1959, 1962) and mounted on slides for observation and preservation. All specimens of this study were deposited in the Laboratory of Nematology Collection at Universidad Nacional, Heredia, Costa Rica.

Nematode identification and enumeration

Nematodes were identified and counted to determine the population densities of all PPN in both the soil and root samples. PPN were identified to genus and/or species level (if possible) using an Olympus BX50 microscope (Tokyo, Japan). The identification of PPN was carried out with the help of taxonomic keys developed by Thorne (1961) and Mai *et al*. (1996).

Data analysis

Frequency distribution analysis for each nematode genus and species by farmer field was determined, and the absolute frequency of each nematode genus was calculated (Barker, 1985). *Radopholus similis* and *Pratylenchus* sp. males and females, *Helicotylenchus* sp. females, males, and juveniles, and *Meloidogyne* sp. juveniles were included in the data analysis.

RESULTS

Six genera and one species of PPN were found to be associated with the surveyed plantations, including: *Meloidogyne* sp., *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchus* sp., *Aphelenchus* sp., *Aphelenchoides* sp. and *R. similis*.

Stereoscopic examination of the root system revealed the presence of necrotic lesions. These lesions in some roots were yellow, typical of the beginning of PPN injury. However, other reddish-brown to black lesions were also observed, showing advanced and necrotic states of the lesion produced by the migratory endoparasitic nematodes *Pratylenchus* spp. and *R. similis*. The magnitude of necrosis observed in the current study may have been the result of either high population densities of *R. similis* (9,520 nematodes/10 g root) or *Pratylenchus* sp. (1,762 nematodes/10 g root).

Frequency of PPN in plantain soil

From the 10 farmer fields surveyed in Talamanca canton, the major PPN associated with plantain in the soil were in the following order of frequencies: *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp. and *R. similis* (Table 1). Population densities of these nematodes varied among farms. Out of the 10 farms, the most abundant nematode based on total number of nematodes per each genus was *Helicotylenchus* spp. with an average population density of 78 nematodes/100 g soil, followed by *Meloidogyne* spp., and *Pratylenchus* spp. which had average population densities of 33 and 25 nematodes/100 g soil, respectively. In the case of *R. similis* in Costa Rica, this nematode is considered to be one of the most important PPN associated with plantain plantations. However, it was the least abundant with a population density of 12 nematodes/100 g soil. The average population density of all nematode species for the 4 genera from the 10 farms was 147 nematodes/100 g soil (Table 1). *Pratylenchus* sp. and *Helicotylenchus* sp. were widespread and occurred in soil in 77% and 69% of the farms surveyed, respectively. *Radopholus similis* and *Meloidogyne* sp. occurred in soil in 61.5% of the farms surveyed (Table 1).

Frequency of PPN in plantain roots

From the 10 farmer fields surveyed, the major PPN associated with plantain roots were: *R. similis*, *Pratylenchus* spp., *Helicotylenchus* spp., and *Meloidogyne* spp. (Table 1). The most abundant nematode was *R. similis* with a highest average population density of 9,520 nematodes/100 g root, followed by *Pratylenchus* spp. and *Helicotylenchus* spp., which had average population densities of 176 and 561 nematodes/100 g root, respectively. *Meloidogyne* spp. was the least abundant with a population density of 222 nematodes/100 g root; however, this nematode was present in 10 of 13 samples (Table 1). The average population density of all nematode species for the 4 genera from the 10 farms was 1,207 nematodes/100 g root (Table 1). *Helicotylenchus* spp. and *Meloidogyne* spp. were widespread and occurred in roots in 100% and 91% of the farms

Table 1. Analysis of plant-parasitic nematode (PPN) from soil and root samples of plantain grown in Talamanca, Limón, Costa Rica in 2016-2017.

	No. samples collected		No. positive samples		Avg no. of nematodes		Frequency of distribution (%)	
Nematode	Soil	Root	Soil	Root	100 g soil	100 g root	Soil	Root
Radopholus similis	63	13	42	8	12	9,520	62	67
Pratylenchus spp.	63	13	47	8	25	1,762	77	75
Helicotylenchus spp.	63	13	63	9	78	561	69	100
Meloidogyne spp.	63	13	57	10	33	222	62	91

surveyed, respectively, while *Pratylenchus* spp. and *R. similis* occurred in root in 75% and 67% of the farms surveyed, respectively (Table 1).

DISCUSSION

The PPN detected in the rhizosphere of plantain in the Talamanca region, *Meloidogyne* sp., *Helicotylenchus* sp., *Pratylenchus,* and *R. similis*, have been reported in Costa Rica (López, 1980; Araya *et al*., 2002; Araya, 2002, 2003, 2005; Chavez and Araya, 2010), Uganda (Kashaija *et al*., 1994), Ecuador (Chavez and Araya, 2010), India (Srinivasan *et al*., 2011), Greece (Tzortzakakis, 2008), Australia (Pattison, 2002, 2011), Hawaii (Wang and Hooks, 2009), Belize (Bridge, 1996), and Rwanda (Gaidashova *et al*., 2004).

Previous studies by Quénéhervé (1989) and by Volker and Gamboa (1991) identified *R. similis*, *P. coffeae*, and *P. brachyrus* as the most damaging PPN to plantain. Additionally, species like *H. multicintus* and *H. dishystera* were also damaging to plantain (Lamptey and Karikari, 1977; McSorley and Parrado, 1986; Kamira *et al*., 2013). Roman (1986) also reported *Helicotylenchus* sp. and *Rotylenchus reniformis* on plantain. Root-associate and fungal-feeding nematodes, including *Tylenchus* sp., *Aphelenchus* sp., and *Aphelenchoides* sp., considered to be of minor importance in plantain production, were not considered further in this study. Although found in association with plantain cultivation, they were found at low frequencies and densities; therefore, they probably do not have economic importance in the system. The results of all the genera reported in this study, except *R. reniformis*, are consistent with those by Gowen *et al*. (2005) and Roman (1986), which both reported that nematode parasitism in *Musa* is characterized by several nematode species causing simultaneous infections.

Based on the nematode population of *Helicotylenchus* sp. with 78 nematodes/100 g soil and *R. similis* with 9,520 nematodes/100 g root, these were the most abundant PPN in plantain fields in the Talamanca canton region. *Helicotylenchus* and *R. similis* are diverse pests of *Musa* (Gowen and Quénéhervé, 1990) causing considerable damage (Kashaija *et al*., 1994; Fogain and Gowen 1997; Elsen *et al*., 2000; Barekye *et al*., 2005). These findings are similar to those obtained by Marín *et al*. (1998) and Castillo-Russi *et al*. (2010) where these two genera were more abundant and widespread than *Pratylenchus*. The low frequency and abundance of *Pratylenchus* sp. could be attributed to its low competitiveness and suppression by high densities of *R. similis* since both nematodes have similar feeding strategies. However, the frequency and abundance of each of these nematodes can change depending on the plantain variety and the agroecological conditions during the year (Araya, 2003).

Nematodes like *R. similis* and *H. multicinctus* are of enormous concern among the many plant-parasitic nematodes associated with *Musa* (Wang and Hooks, 2009), and they usually occur together in plantain roots (Gowen, 1993). *Radopholus similis* can significantly weaken root systems, reduce yields, topple plants before harvest, reduce fertilizer uptake and utilization, and make plants more prone to wind knockdowns. Nelson *et al*. (2006) indicated that *R. similis* can cause lesions to root and rhizomes, plantain decline, yield losses, and toppling; the nematode has a relatively wide non-*Musa* host range. Brooks (2004) reported that *Helicotylenchus* sp. is a worldwide problem in *Musa*-growing regions causing yield losses of 40% and 30-60% in Africa and India, respectively. It is possible that the abundance of weeds associated with plantain plantations is a reservoir of PPN around plantain in the studied area. This condition could increase the presence of these PPN in the plantations.

López (1980) determined the presence of *R. similis* in Costa Rica, along with some species of *Helicotylenchus*, *Meloidogyne*, and *Pratylenchus*. However, lower population densities of *H. multicinctus* were observed associated with the presence of high population densities of *R. similis* that may have impeded its development. In this investigation, *Helicotylenchus* sp. was found in 100% of the root samples. We believe this is an important finding and merits future research related to the damage caused by this nematode to plantain. This study focused on determining the nematodes associated with plantain cultivation only. According to Sikora *et al*. (1989) and Gowen and Quénéhervé (1990), there are nematodes with different feeding habits that generally exist as mixed populations in *Musa* fields. We support this view because, in a tropical country such as Costa Rica, it is easy to find a mixture of species in a single crop such as plantain.

Previous research (López, 1980; Araya *et al*., 2002; Araya, 2002, 2003, 2005; Chavez and Araya, 2010) supports the relative importance of *R. similis* as a *Musa* pest in Costa Rica. For example, the high population densities and frequencies of *R. similis* in many plantain fields in Sixaola, Talamanca can be attributed to the long-term monoculture practices that lead to ideal conditions for nematode survival and much variation in nematode populations. However, in Alto Talamanca (indigenous zone), the management practices are different because agrochemicals are not used, and plantain is grown in association with timber and fruit trees. Nevertheless, we also found high population densities of PPN. We believe the introduction of contaminated plantain plants could have also contributed to the high diversity of nematodes found in the fields. It is also important to investigate if weeds growing in *Musa* plantations are hosts of the identified nematodes, and whether they are of concern for plantain cultivation. In this sense, López (1980) found that weeds such as *Croton trinitatis*, *Stachytarpheta* sp., and *Cyathula achyranthoides* are hosts of *M. javanica* and that *Laportea aestuans* and *Piper auritum* are of *M. incognita*. These weeds can be found frequently in plantain plantations and may be alternate hosts of PPN.

In conclusion, *R. similis*, *Pratylenchus* spp., *Helicotylenchus* spp., and *Meloidogyne* are the major PPN associated with plantain in Talamanca canton. Considering the destructive nature of these nematodes in various *Musa*-producing countries, research and management practices have to be in place to minimize crop damage as well as improve income and well-being from the small plantain producers of Talamanca. Future studies should evaluate the extent of plantain damage caused by PPN in Talamanca canton, followed by development of integrated pest management strategies against these nematodes. An alternative would be establishing nematode-free plantain planting material in fields not previously cultivated with plantain.

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