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Identification and Economic Importance of Banana Phytoparasitic Nematodes in Antioquia Uraba, Colombia

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The research work was carried out with the objective of evaluating the populations of phytoparasitic nematodes that affect the banana crop. The research was carried out in the three zones of Antioquia Urabá (North, Central and South), in the rainy season (May-September, 2018). A total of 169 samples were collected from the three zones. Root sampling was done by the technique proposed by Araya and Calvo (2001), the separation of nematodes was done by the sieving and centrifugation method in sucrose solution (Alvarado and López 1985) and its identification was made at the gender level (Maggenti et al. 1987). The following genera were found: Radopholus, Helicotylenchus and Meloidiogyne. In the same way, root damage was found that oscillated 25.2 and 33.4%, without influence on crop production. It was concluded that the populations of dominant nematodes in the Urabá area belong to the genera Radopholus, Helicotylenchus and Meloidiogyne, with high populations, but without significant effect on the yields of the banana crop.

Keywords: *Nematodes in Urabá, Radopholus sp, Helicotylenchus, Meloidogyne.*

Introduction

Banana is a plant native to Southeast Asia, as a crop estimated to be more than 10,000 years old, whose first traces were found in Papua New Guinea in the vii century before Christ. At present, it is still in the wild in the Philippines, Papua New Guinea and Indonesia and cultivated in tropical and subtropical areas. Natural crosses have produced significant genetic diversity and have allowed the production of no-seedless varieties (UNCTAD 2011).

Banana is the most widely eaten tropical fruit worldwide; it has been established as a technified crop in Colombia as well as in other countries of the world. However, this crop has certain associated pests and diseases that can infer in quality and production, among them are: Sigatoka, Moko, Fusarium, some insects and nematodes. After the necrotic lesions caused by fungi such as *Mycosphaderella fijiensis* and *M. Musicola*, the phytoparasitic nematodes (*Radopholus similis* (Cobb) Thorne), the growth and development of this crop can be threatened, generating losses in production between 20 and 100% (Guzmán 2011, Seenivasan et al. 2013).

This fruit is the fourth foodstuff of basic necessity in the world; it is consumed raw, or as fried banana, roasted, dry, in juice or in leaflets, alcoholic drinks or flours. It is rich in carbohydrates and contains little fat. It also contains essential vitamins, such as vitamin C, B6, B1, B2 and contains large amounts of potassium

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and magnesium, which includes it within the group of fruits with great nutritional properties that provide a good amount of carbohydrates, potassium, magnesium, folic acid and fibers. The cultivation of banana requires fertile soil, well drained and frank texture to loam clay; the ideal climate for this fruit is tropical humid, must have a temperature around 18 °C (Rodríguez and Rojas 2015).

The nematodes are segmented bodies of filiform animals and generally do not present coloration. They have marks in their body of striated form, round bodies of transverse form, varied buccal apparatus, without extremities or other complements. In some cases adult females may take a globose or rounded form (Perry and Moens 2006). They are individuals, whose size varies from 300 to 1,000 μm in length and from 15 to 33 μm in width, which hinders their visibility (Luc et al. 2005, Perry and Moens 2006). The nematodes have been characterized as being evolved beings, and adapt to different thermal floors, types of soil and water (sweet and salt), and are of great importance to ecosystems (Bongers and Ferry 1999, Baldwin et al. 2004).

In research related to nematodes, regardless of whether they are evaluated as beneficial or harmful, it is essential to perform the identification with high degree of precision, which is very useful to determine the timely management when required (Mai et al. 1996). Identification at the family level, is the category that most researchers and ecologists reach. However, when they have tools, they can reach the species level, which would greatly facilitate the selection of management practices, since with this information, they can know the behavior, evolution and trophic functions (Power 2001).

In banana, there are many nematodes that can affect the development and production of the crop; the literature reports about 146 species distributed in 43 genera of parasitic nematodes or associated to the *Mussa* species, where migratory endoparasitics (*Radopholus similis, Pratylenchus coffeae*), ecto-endoparasitic (*Helicotylenchus multicinctus* and *H. dihysteria*), sedentary endoparasitics (*Meloidogyne incognita, M. javanica*) and semi-endoparasitic (*Rotylenchulus reniformis*) are reflected (Martínez et al. 2006).

The genus *Pratylenchus* is a species of great importance in banana cultivars, especially for the Cavendish variety; its life cycle takes approximately 30 days and for its development requires a temperature of 25 to 30 °C (Gowen 1994). Likewise, the genera *Helicotylenchus* and *Radopholus* are associated to the banana crop, generating small necrotic lesions around the root bark, which under severe conditions can become a problem for the crop (McSorley and Gallaher 1994, Holguín 2018).

The *Meloidogyne* genus or root-knot nematodes are widely distributed worldwide. This genus, in juvenile state, penetrates the root near or along the radical meristem, invades the endodermis, entering the wake and inducing the formation of multinucleated giant cells derived from the cortical parenchyma or differentiating vascular cells in the central part of the wake (De Waele and Elsen 2002).

Another important genus is the *Radopholus* or banana borer nematode, which can induce the overturning of the plant. This nematode breaks the cell wall with its stylet and feeds on the cytoplasm of the cells, making cavities that subsequently coalesce to form reddish-brown lesions and finally become necrotic by the action of other organisms (Marín et al. 1998).

Nematode management should be done with cultural and preventive practices, such as use of tolerant or antagonistic species, rotation of crops, use of bionematicides based on fungi and bacteria (Rhizobacteria, *Bacillus*), and in the worst case, use of chemical nematicides, which greatly degrades soil biota (Ferraz et al. 2010, Gowen et al. 2005). The use of fungi, forming mycorrhizal symbioses can also be implemented, which generates a root system that could contribute to the mitigation of the nematode problem in the soils (Bautista et al. 2015).

Aim of the Study

The main objective of this study was the identification of nematodes of economic importance in the management of banana plantations. The study was carried out in three areas of the Urabá of Antioquia, in the second semester of 2018.

Materials and Methods

Location

The research was carried out in the three zones, in which the Urabá area is divided: North Zone, Central Zone and South Zone. We chose lots that had irrigation, a condition that can favor an ideal moisture regime for the development of phytosanitary diseases; among them, the attack of phytopathogenic nematodes or phytoparasitics. The Urabá is a coastal area on the Caribbean Sea with heights that do not exceed the 20 m, mostly with a slightly flat topography, average temperature of 26.6 ºC, average annual rainfall of 2,617 mm, 86.2% relative humidity and a solar brightness of 6.2 hours / days. Its climate is classified as semi-humid warm. The soils are of different texture (clayey-silty, clayey, frank, clayey, silty loam and silty clay loam), well-structured and with a pH ranging between 5 and 7.5 (Cárdenas et al. 2017).

Selection of Lots

65 lots were selected in the North Zone, 42 in the Central Zone and 62 in the South Zone. All the lots had an irrigation system and were in productive stage. The area of each plot ranged between 3 and 10 ha cultivars with 12 years of age of establishment within the Canvendis var. Valery and William.

Root Sampling

The methodology proposed by Araya and Calvo (2001) was used; roots of five plants in flowering stage (maximum eight days after having emitted the inflorescence or maximum two open bracts) were collected. The collection of the roots was carried out in a trunk of 17 x 17 cm and 25 cm deep, at the base of the pseudostem, oriented in the interval between the mother plant and the son of succession. All the roots were deposited in a plastic bag previously labeled with the identification of the lot and farm of sampling. The sampling was done in three plants per point and all the roots that were found in the soil volume (7225 cm^3 / plant) were collected. These samples were taken to CENIBANANO Phytopathology Laboratory for processing.

Processing of Root Samples

The collected roots were washed with abundant potable water. Then, the functional roots were separated (live roots, turgid, consistent and brown) and non-functional (dead roots, flaccid, completely necrotic and blackish). Both roots (functional and non-functional) were weighed using a balance with a precision of ± 0.1 g. For the evaluation of the roots the scale proposed by Araya and Calvo (2001) was used. It is illustrated in Table 1.

Table 1. *Classification of Ranges for Banana Roots to Weigh*

	Deficient	Regular	Optimal
Total Root	< 70	70-90	> 90 g/plant
Functional Root	< 60	60-75	> 75 g/plant

Source: Araya and Calvo 2001.

Evaluation of Root Damage by Nematodes

Local scale methodology for evaluation of root damage by nematodes described by Moens et al. (2001) was used; 10 functional roots of the collected sample were taken randomly. Afterwards, they were cut into pieces of 10 cm in length and finally they were cut longitudinally. Each half was evaluated, measuring the length of the necrotic tissue with a ruler. To calculate the total necrosis of the functional root, the value of the necrotic tissue measured in each half was added, divided by 2, and this result was divided by 10 and then multiplied by 100.

$$\%\ DE\ DAÑO = \frac{r1 + r2 + r3 \ldots + r10}{10} x100; donde\ r = \frac{b1 + b2}{2\ x\ 10}$$

r: Root damage ratio
b: Length of edge damage (cm)

Extraction and Counting of Nematodes

The methodology of liquefied-sieving adjusted by Alvarado and Lopez (1985) was used. The functional roots were cut into pieces of 0.5 to 1 cm in length, homogenized and 25 g were taken, to perform the extraction. These 25 g of root were deposited in a beaker together with 100 ml of potable water. This process was carried out in a conventional two-speed blender. The first solution was made at low speed (1000 to 1500 rpm) and the second at high speed (2000 to 2500 rpm), both for 10 seconds.

Subsequently, all the solutions were added to a set of superimposed sieves of 250, 125 and 25 μm opening (No. 60, 120 and 500 respectively). In the 250 micron sieve, the pieces of roots were washed with potable water for two minutes, then in the 125 micron sieve they were washed for one minute and finally the material retained in the 25 micron sieve was deposited in a beaker and completed to 200 ml of potable water for later reading. The suspension was homogenized for 30 s with a glass stirrer. A 4 ml aliquot was then taken from the center of the beaker and deposited in a reading chamber with 2 ml effective for counting. The number of nematodes was expressed by 100 g of functional root.

Identification and Quantification of Nematodes

Processed samples were examined under the 4x objective Microscope and were identified using the taxonomic keys described by Guzmán (2016), published in the manual for the identification of phytoparasitic nematodes (Maggenti et al. 1987).

Bunch Sampling

At each sampling point within a radius of 30 m, a total of 3 bunches were marked with ages of 3 and 4 weeks after apparition of banana bacotte, with a white ribbon tied at the end of the stem, which contained the information of the sample coordinate and three replicates per point demarcated as R1, R2 and R3. These bunches were harvested at 11 weeks and then the weights in kilograms were taken with the aim of correlating populations of phytoparasitic nematodes with production.

Data Analysis

The information was tabulated in Excel tables and analyzed with descriptive statistics. There was also proof of abundance, diversity and similarity among the lots of the three evaluated areas, for which the Shannon-Wiener Index was used, which is calculated by formula, in which the number of genera *i* found between the total number of species in the relevant zone. The diversity index was estimated with the following formulas:

$$pi = \frac{ni}{N} \qquad H = -\sum pi * Ln(pi)$$

where:

pi = relative abundance of species i.
ni = number of individuals of the gender i.
N = number of total individuals.
H = Shannon-Wiener diversity index.
Ln = natural logarithm.

For interpretation, the relative abundance index will oscillate between 0.0-1.0, being more abundant as it approaches 1.0, while the diversity index (H) will be interpreted as a positive value with the following scale, <2: low diversity, 2-3: medium diversity, and >3: high diversity. Ggenerally, ecological systems, in general terms, are below 5.0 (Pla 2006, Pla and Matteucci 2001).

Results and Discussion

Presence of Nematodes

Three genera of phytoparasitic nematodes were found (Table 2). The North Zone was where the greatest number of nematodes was found, with an average of 70,055 individuals/100 g of root, while the South Zone had the lowest values of total nematodes, with an average of 16,122 individuals/100 g of roots. Of the phytoparasitic nematodes found, the genus *Helicotylenchus* outperformed the other genera in the North and South Zones, but in the Central Zone it was surpassed by the genus *Radopholus*.

Table 2. *Weight of Samples of Banana Roots and Average Number of Nematodes per 100 g of Roots Collected form South, North and Central of Antioquia Uraba, Colombia (2018)*

Zone	NS	Rs	Hspp	Mspp	NT	TR
South	62	4,632.3	8,903.2	2,587.1	16,122.6	36.9
North	65	27,987.7	42,055.4	12.3	70,055.4	58.0
Central	42	25,847.6	23,161.9	100.0	49,109.5	48.0

NS: Number of Samples, Rs: *Radopholus similis*, Hspp: *Helicotylenchus* spp, Mspp: *Meloidogyne* spp, NT: Total Nematodes, TR: Total Root (g/plant).

These results are in the ranges reported in the literature. However, the population of *Radopholus similis* of the South Zone is below the average of 10,000 individuals/100 g of root reported by Araya and Calvo (2001), enough to cause economic damage to banana plantations. The Central and North Zones exceed the economic damage threshold reported by the above-mentioned authors, whose averages were 25,847.6 and 27,987.7 individuals/100 g of roots respectively.

The populations of the nematodes evaluated, differ; this can be explained because of the biological cycles of each genus. The tendency of *Helicotylenchus* spp to be found in larger populations may be due to its biological habit, as it is a semi-endoparasitic nematode (Yeates et al. 1993) or ectoparasite which feeds on the nearby tissue of the rhizodermis (Dropkin 1989). As it is shown in Table 2, populations of *Helicotylenchus* spp were the highest in the three zones evaluated. However, it has not been shown that this nematode migrates through the cortical tissue of banana roots (Siddiqi 1973).

The Population of *Meloidogyne* spp was relatively low in the three zones of Uraba-Antioquia. However, it is lower than the economic damage threshold for the AAA banana crop which is in accordance with the findings of Araya et al. (1995), who state that populations of this genus are detected in local conditions with few frequencies and population densities.

Abundance and Diversity

Despite the high populations of phytoparasitic nematodes, the Shannon-Weiner Index indicates that there is low diversity (H = 0.67) in the North Zone. *Helicotylenchus* spp was the dominant species with 60%, followed by *Radopholus similis* with 39.95%, and the species *Meloidogyne* spp with 1%. Similar dynamics were found in the Central Zone - low diversity (H = 0.69). However, in this zone the species *Radopholus similis* was dominant with 52.7%, followed by *Helicotylenchus* spp with 47.2%. In the South Zone, there is greater diversity (H = 0.97) and as in the North Zone, the species *Helicotylenchus* spp was dominant (55.2%), followed by *Radopholus similis* (28.7%). It is noted that in this area, there is a high population of the *Meloidogyne* species, although its dominance was low (16%).

Necrosis Caused by Nematodes

The average percentages of necrosis in functional roots which were evaluated in each batch and in each zone, showed that the average of necrosis for the North Zone was 25.2%, while for the Central and South Zones, was 26.6% and 33.4%, respectively, with a standard deviation that ranged between 9.2 and 10.3 (Table 3). For the analysis of the severity of damage in each batch of each zone, three evaluation ranges were established: less than 20%, 20 to 50%, and more than 50%, indicating low, medium and high, respectively, as illustrated in Table 2. In this table, it can be seen that only in the North Zone there is a low percentage of lots that exceeds the damage threshold and that could be a problem for the management of the banana crop. The great majority of the lots in the three zones has medium infestation (Table 3) and it is not economically important.

Table 3. *Percentages of Lots Grown with Bananas Infested by Phytoparasitic Nematodes in the North, Central and South Zones of the Uraba-Antioquia (2018)*

Ranges	**North Zone**		**Central Zone**		**South Zone**	
	Lots	%	Lots	%	Lots	%
< 20%	10	37.03	5	26.32	2	9.1
20-50%	16	59.26	14	73.68	20	90.9
> 50 %	1	3.7	0	0	0	0
Rank (%)	5.9-52.5		8.5-43.0		16.9-47.1	
Average (%)	25.2		26.6		33.4	
Standard Deviation	10.3		10.1		9.2	

Correlations between Nematodes and Yield (Bunch Weight)

No significant correlations were found between the amount of banana plant parasitic nematodes and their yield (bunch weight). The highest correlation was found in the Central Zone with the species *Helicotylechus* spp, but no-significant statistics (Table 4). Likewise, in none of the cases, this figure indicates dependence among the intervening factors and that means that the weight of the bunch is not affected by the three types of phytoparasitic nematodes that were found in the studied banana.

The distributions reflected for the yield component with respect to the phytoparasitic populations that were evaluated in the Central Zone, had a high disaggregation of the values with respect to the mean, generating, thus, a low degree of confidence for this variable, demonstrating that the phytoparasitic nematodes do not affect the yield of the banana. Likewise, the same behavior was observed in the populations of *Radopholus similis* and *Meloidogyne* spp, but with lower values of correlation in comparison to those released for *Helycotilenchus* spp. This analysis predicts that other factors may influence the decline in yields, other than the nematode populations.

Very low correlation is presented in the healthy root components for the Central Zone, with the highest numbers, being these of the populations of *Radopholus similis* and *Helicotylenchus* spp (Table 4), although these are not sufficient figures to explain the variability of yields bananas crop from the study zone and therefore, are not important economically.

The South Zone, like the other zones studied (North and Central), does not present a correlation for the production variable compared with the populations of nematodes; none of the values obtained in the Pearson correlations explain the variability of banana crop yield. Similar explanations are given for the three zones, in terms of necrosis of the functional roots and the effect of this component on the components of yield (bunch weight), a condition that leads to the prediction that banana crop yields for these three zones studied do not depend on the populations of phytoparasitic nematodes, but on other factors, perhaps those associated with soil problems. For example, physical soil problems (low aeration), salt accumulation, nutritional imbalance, and can be a good subject for other research work.

Table 4. *Pearson Correlation (R) between Populations of Phytoparasitic Nematodes and the Variables Bunch Weight, Root Necrosis (%) and Total Root (g/p) of the Three Zones (North, Central and South) of the Antioquia Urabá*

	Hspp	**Mspp**	**NT**	**%RF**	**RS**	**Wbunch**	**%Necro**
North Zone							
Mspp	0.0253						
NT	0.6898	0.1061					
%RF	-0.1042	0.0907	-0.0200				
RS	-0.1403	0.1149	0.6201	0.0853			
Wbunch	-0.1092	-0.1411	-0.1342	-0.0732	-0.0648		
%Necro	-0.0396	-0.0019	0.1974	0.0445	0.3064	-0.1901	
WRT	0.1434	0.0252	0.1876	-0.0301	0.1012	0.0272	0.1368
Central Zone							
Mspp	0.3172						
NT	0.6959	0.1055					
%RF	-0.4146	-0.1243	-0.3074				
RS	0.3472	-0.0555	0.9150	-0.1686			
Wbunch	-0.3336	-0.1007	-0.3926	0.2260	-0.3247		
%Necro	0.2948	0.0226	0.2525	-0.0838	0.1653	-0.0240	
WRT	0.1081	0.2027	0.0894	-0.1426	0.0530	0.1926	0.1595
South Zone							
Mspp	-0.0745						
NT	0.6789	0.4096					
%RF	-0.5245	0.1099	-0.3900				
RS	0.1396	-0.0806	0.6265	-0.1935			
Wbunch	-0.1988	-0.0098	-0.1621	0.1274	-0.0533		
%Necro	-0.1431	-0.0414	-0.1685	0.0126	-0.0981	0.0932	
WRT	-0.0655	-0.1084	-0.0158	0.0611	0.1091	0.1384	-0.1845

Mspp: *Meloidogyne* spp, Hspp: *Helicotylenchus spp*, RS: *Radopholus similis*, NT: Nematodes Total, %RNF: % Root Functional, WRT: Weight Total Root, WBunch: Weight Bunch.

Production of Total Roots of Banana

As it is shown in Table 5, the three zones, in general, presented values below 70 g/plant, which would be classified according to Araya and Calvo (2001), lots with a deficient root system; only for the North Zone a 11.1% of the lots evaluated are root systems in the regular range, 3.7% are root systems in the optimal range and finally 85% are in the deficient range. These differences can be explained by the intrinsic conditions of the sampling site (soil type and nutrition, among others).

Table 5. *Total Root Production of Banana Plants from Three Zones (North, Central and South) of Urabá Antioquia*

Ranges	**North Zone**	**Central Zone**	**South Zone**
Range (g/plant)	36.0-95.3	25.7-65.7	22.3-50.6
Average (g/plant)	58.9	47.2	36.7
Standard Deviation	14.4	10.5	9.0

For the Central and South Zones, all the lots (100%) evaluated are in the deficient range, with values lower than 70 g/plant, having averages of total root weight per plant 47.2 g and 36.7 g, respectively. These low values predict that there are soil problems, possibly because of the long time that these lots have been exploited (more than 30 years) For example, they may be problems of soil aeration.

Conclusions

From the research that has been done the following conclusions can be drawn. Firstly, the lots under study belonging to the three zones of Urabá Antioqueño have low root production, perhaps due to abiotic rather than biotic problems. Secondly, the development of the biological cycle of each genus of phytoparasitic nematodes in banana cultivation can influence the final populations registered in each evaluation. Thirdly, Averages of root necrosis are not enough to affect yield components (bunch weight) in the three zones studied (North, Central and South) and finally, the Urabá banana zone has the three genera of phytoparasitic nematodes of the greatest care in the management of banana crops. However, their populations are not important economically.

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