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# Frequencies and population densities of plant-parasitic nematodes on banana (*Musa* AAA) plantations in Ecuador from 2008 to 2014

Frecuencias y densidades poblacionales de los nematodos parásitos en banano (*Musa* AAA) en plantaciones de Ecuador desde 2008 hasta 2014

Orlando Aguirre<sup>1</sup>, César Chávez<sup>2</sup>, Alejandro Giraud<sup>3</sup>, and Mario Araya<sup>3</sup>

## **ABSTRACT**

An analysis of the plant-parasitic nematodes found on the banana (Musa AAA) plantations in the provinces of Cañar, El Oro, Guayas, Los Rios and Santo Domingo of Ecuador from 2008 to 2014 was carried out. The nematode extraction was done from 25 g of fresh roots that were macerated in a blender and from which nematodes were recovered in a 0.025 mm (No 500) mesh sieve. The data were subjected to frequency analysis in PC-SAS and the absolute frequency was calculated for each individual genus. Four plant parasitic nematodes were detected and, based on their frequencies and population densities, the nematode genera in decreasing order was: Radopholus similis > Helicotylenchus spp. > Meloidogyne spp. > Pratylenchus spp. Radopholus similis was the most abundant nematode, accounting for 49 to 66% of the overall root population, followed by Helicotylenchus spp. with 29 to 45% of the population throughout the different analyzed years. From a total of 13,773 root samples, 96% contained R. similis, 91% Helicotylenchus spp., 35% Meloidogyne spp., and 25% Pratylenchus spp. and, when all of the nematodes that were present were pooled (total nematodes), 99.9% of the samples had nematodes. A large number of samples with a nematode population above the economic threshold suggested by Agrocalidad, INIAP and Anemagro (2,500-3,000 nematodes/100 g of roots) was observed in all of the years, the months and the five sampled provinces. The statistical differences (P<0.0001) detected for the nematode frequencies among the years, months and provinces, more than likely, were associated with the high number of samples included in each year, month and province because the variations in the frequencies for each nematode genus were small.

 $\textbf{Key words:} \ Helicotylenchus \ spp., Meloidogyne \ spp., Pratylenchus \ spp., Radopholus \ similis, population \ distribution, pests \ of \ plants$ 

### RESUMEN

Se realizó un análisis de los nematodos parásitos encontrados en las plantaciones de banano (Musa AAA) en las provincias de Cañar, El Oro, Guayas, Los Ríos y Santo Domingo de Ecuador desde 2008 hasta 2014. La extracción de nematodos se hizo de 25 g de raíces frescas que fueron maceradas en una licuadora y los nematodos recuperados en la criba No 500 mesh (0,025 mm). Los datos se sometieron a un análisis de frecuencias en PC-SAS y se calculó la frecuencia absoluta para cada género. Cuatro géneros de nematodos parásitos de plantas fueron detectados, y basado en sus frecuencias y densidades poblacionales, los géneros de nematodos en orden decreciente sería: Radopholus similis > Helicotylenchus spp. > Meloidogyne spp. > Pratylenchus spp. Radopholus similis fue el nematodo más abundante contabilizando de 49 a 66% de la población total de las raíces, seguido por *Helicotylenchus* spp. con 29 a 45% de la población a través de los diferentes años analizados. De un total de 13,773 muestras de raíces, 96% tenían R. similis, 91% Helicotylenchus spp., 35% Meloidogyne spp., 25% Pratylenchus spp., y cuando se agrupó todos los nematodos presentes (nematodos totales) 99,9% de las muestras tenían nematodos. Un gran número de muestras con poblaciones de nematodos superior al umbral económico sugerido por Agrocalidad-INIAP y Anemagro (2,500-3,000 nematodos/100 g de raíces) fueron observadas en todos los años, meses y en las cinco provincias muestreadas. La diferencia estadística (P<0.0001) detectada en la frecuencia entre años, meses y provincias muy probablemente esté asociada con el alto número de muestras incluidas en cada año, mes y provincia, ya que las variaciones en las frecuencias en cada género fueron pequeñas.

**Palabras clave:** *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis*, distribución de lapoblación, plagas de plantas.

### Introduction

Bananas (*Musa* AAA cv. Grande Naine, Valery, and Williams) are cultivated in Ecuador for export markets. It is the most important crop, accounting for almost 25% of the

agricultural gross national product. In 2014, 286 million boxes of 43 pound were exported (AEBE, 2015), produced on 266,124 ha, which gave a total income of US \$2.300 million FOB.

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Besides the constraints of the banana market requirements and demands, there are other factors that limit production. Among the important abiotic factors constraining banana yield are reduced radiation, low temperatures for part of the year, a shallow soil water table level and edaphic conditions, mainly due to clay texture, poor structure and high sodium (Na) content. These constraints differ between the farms and provinces and not all happen on a specific farm. Plantations are found in flat areas with no more than 4% slope, with the cultivated area close to sea level, no more than 100 m a.s.l.

Within the biotic factors, phytonematodes are second, after black Sigatoka, caused by *Mycosphaerella fijiensis*. On local plantations, usually only polyspecific communities occur, consisting mainly of a mixture of *R. similis*, and *Helicotylenchus* spp., with very low populations of *Meloidogyne* spp., and *Pratylenchus* spp. Nematodes increase the time for leaf emission, reduce the bunch weight and plant longevity, and increase the crop cycle duration (Quénéhervé *et al.*, 1991a; Araya, 2004).

To avoid or reduce nematode damage, the only management strategy currently available is the regular application of non-fumigant nematicides, which growers know is economically feasible. However, a nematicide application is done only on farms with high yields, in an intensive manner. Then, there are many large, medium and small farms that have low yields due in part to severe nematode root damage because nematode control measurements are not used.

Economic and environmental constrains dictate the rational use of non-fumigant nematicides at the recommended dosages. To achieve this, more research is needed for the evaluation of biocontrol agents, cultural practices, nematicide rotations, number of cycles per year, and application systems to prevent nematode population build up and root damage.

The objective of this study was to provide quantitative information on population densities and frequencies of the major nematode pests on Ecuadorian banana plantations from 2008 to 2014. This information will be useful for identifying more appropriate research areas for nematode management and as a basis to justify more investment.

### Materials and methods

The nematode data included in the analysis were from root samples of long-term commercial banana plantations of the

provinces of Cañar, El Oro, Guayas, Los Ríos and Santo Domingo, where the crop is cultivated in the country. The farms vary in soil type, texture, structure, content of macro and micro nutrients and climatic conditions. The age of the plantations ranged from 5 to 40 years with a plant density of 1,300 to 1,700 plants/ha and the sown cultivars were mainly of the Cavendish subgroup: Grande Naine, Valery, and Williams. The bunching plants were supported by tying them to adjacent plants with double polypropylene twine, propping them with wood poles or by aerial gugying.

Various banana cultural practices (fertilization, control of weed and nematodes and aerial spraying of fungicides to control black Sigatoka) were carried out during the years, which may have influenced the nematode population behavior reported in this paper. Desuckering was carried out every six to eight weeks throughout the years, leaving the production unit with a bearing mother plant, a large daughter sucker, and a small grand-daughter.

Usually, the water requirement was supplied by rainfall during the rainy season, from January to April, while, from May to December, sprinkle irrigation was necessary each year. The average rainfall (2008-2014) varied from 672 to 4,024 mm. A complex system of primary, secondary and tertiary drains was installed to carry off excess water, lower the water table and prevent waterlogging.

The data of the samples recorded by Nemalab S.A. from 2008 to 2014 were used for this study. A total of 13,773 root samples were processed from January 2008 to December 2014 and entered into a computer database along with the farm identity, province, month and year of sampling. Each root sample consisted of the roots of ten randomly selected stools, which consisted of a mother plant and follower sucker. The samples were taken either from the follower sucker, 1.25 to 1.75 m height, or from the area between the recently flowered plant (within 8 d of flower emergence) and its follower sucker, 1.25 to 1.75 m of height. A hole about 30 cm long, 30 cm wide and 30 cm deep (soil volume of 27 L) was dug with a shovel at the plant base. Roots from each hole were collected, placed in labeled plastic bags, and delivered to the laboratory in coolers.

In the lab, the root samples were registered and processed as soon as possible, and when it was necessary, stored in a refrigerator (General Electric) adjusted to 6-8°C until being processed. The roots were rinsed free of soil, separated in functional (living roots, either healthy or with symptoms of nematode damage, but without necrosis or root decay) and non-functional roots (dead, snapping or very extensively

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necrotic root tissue), left to dry off the surface moisture and weighed. During the root separation process, in some roots, it was necessary to cut some damaged parts, which were classified as non-functional roots. The remaining part was the functional root. The nematode extraction was made from 25 g of fresh functional root subsamples by the Taylor and Loegering (1953) method, as modified by Araya (2002). The nematodes were identified at the genus and species level when possible, based on the morphological characteristics under a light microscope, following the key of Siddiqi (2000). The population densities of all of the present plant-parasitic root nematodes were determined and the values were converted to numbers per 100 g of fresh roots.

The data were subjected to a frequency distribution analysis for each particular nematode by year, month, and province in PC-SAS (SAS Institute, Cary, NC). The absolute frequency was calculated as No. samples containing a species / No. samples collected \* 100 (Barker, 1985). Additionally, in each nematode genus, the samples were distributed according to specific ranges of population densities as follows: free of nematodes, from 1 to 2,500, from 2,501 to 5,000, from 5,001 to 10,000, from 10,001 to 20,000, from 20,001 to 30,000, and samples over 30,000 individuals per 100 g of fresh root. The percentage of samples containing a nematode genus was compared between the years, months and provinces by Proc Gmod of SAS using the log transformation as the link function and the negative binomial probability distribution to model the errors.

### Results

Irrespective of the year, the major plant-parasitic nematodes present in the sampling areas were R. similis, which varied from 49 to 66%, and Helicotylenchus spp., varying from 29 to 45% (Fig. 1). Meloidogyne spp. and Pratylenchus spp. contributed with 3 to 8% and 2 to 3% to the total nematode population, respectively (Fig. 1). Even though a difference (P<0.0001) was detected between the years for the frequency of the different nematode genera, the values for each genus were very similar in the different studied years (Tab. 1). The highest frequency was always found in R. similis, above 92%, followed by Helicotylenchus spp. which ranged from 74 to 94%, then Meloidogyne spp. which varied from 28 to 44% and Pratylenchus spp. from 11 to 34%. Considering all of the nematodes, all of the samples in every year had at least one nematode genus reaching a frequency of 100%.

By month, again *R. similis* (> 86%) and *Helicotylenchus* spp. (>84%) were the main nematodes in the samples, followed

by *Meloidogyne* spp. (28-48%) and *Pratylenchus* spp. (13-31%) at smaller proportions and, in practically all of the samples (> 99%), at least one of those nematodes was present (Tab. 2). Although statistical differences (P<0.0001) were reported for those frequencies between the months, their variations were small.

A similar trend was observed for the frequencies (*P*<0.0041) among the provinces, where the variation within each genus was small (Tab. 3). In descending order, the highest frequency was detected for total nematodes with 100% followed by *R. similis* above 90%. For *Helicotylenchus* spp., it varied from 64% in Cañar to 95% in Los Ríos, for *Meloidogyne* spp. it varied from 29 to 44%, while for *Pratylenchus* spp. with the exception of Santo Domingo that reached 8% in the other provinces was very similar varying from 25 to 28%.

The distribution of the root samples for each nematode population density clearly indicated that *R. similis* showed the highest population (Fig. 2). From the 13,773 recorded root samples, only 4.2% were free of nematodes and 45.3% were above 5,000 per 100 g of roots (Fig. 2). For *Helicotylenchus* spp., only 9.3% of the samples were found to be negative and 31.1% had levels above 5,000 nematodes. More than 64.7% of the smaples were free of *Meloidogyne* spp. and 1% showed densities higher than 5,000 nematodes. *Pratylenchus* spp. was present in 25.4% of the samples, with only 0.2% above 5,000 nematodes. When all of the nematodes were pooled (total nematodes), it was observed that only nine samples (0.07%) were free of nematodes and 79.3% contained more than 5,000 nematodes per 100 g of roots.

Because *R. similis* comprised more than 49% of the overall nematode population and all four cause damage to the banana root system, it was decided to show the total nematode density ratios distribution by year, month and province. A stable trend in the number of samples (67-87.3%), with levels higher than 5,000 nematodes was observed among the different analyzed years (Fig. 3). In every month of the year, between 70 and 87.7% of the samples had a high nematode content and less than 0.14% of the samples were nematode free (Fig. 4). A similar pattern was detected in the provinces, where 64.8 to 91.9% of the samples showed populations over 5,000 individuals per 100 g of roots (Fig. 5).

## **Discussion**

The four detected nematode genera are well known pathogens in banana roots (Sarah, 1989; Gowen and Quénéhervé, 1990; Fogain, 1994; Gowen, 1995; Davide, 1996; Sarah et

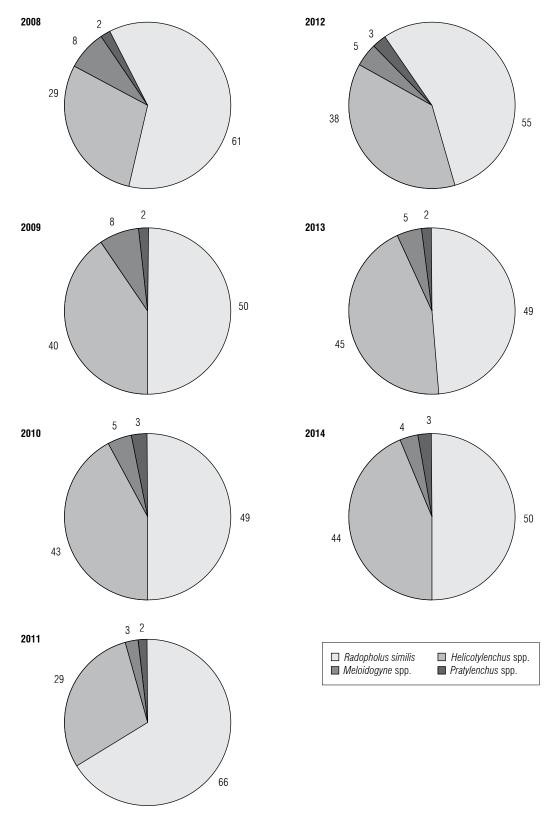


FIGURE 1. Nematode population distribution in Banana plantations between 2008 to 2014 (Ecuador).

*al.*, 1996; Bridge *et al.*, 1997; Marin *et al.*, 1998; De Waele and Davide, 1998; Gowen, 2000a, 2000b; De Waele, 2000; Gowen *et al.*, 2005; Dubois and Coyne, 2011; Volcy, 2011).

These nematodes continue to be a serious threat to banana production in Latin America and the Caribbean (Dita *et al.*, 2013).

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**TABLE 1.** Percentage of banana (*Musa* AAA) root samples per year from which various plant-parasitic nematodes where recorded on Ecuadorian plantations from 2008 to 2014.

Nematode				Years	2012	2013	2014	Mean	<i>P</i> >F
	2008	2009	2010	2011					
Number of root samples	1,322	1,396	1,840	2,744	2,799	1,685	1,987	1,3773	
Radopholus similis	97	99	99	96	95	92	92	96	P<0.0001
Helicotylenchus spp.	74	85	90	94	94	94	94	89	P<0.0001
Meloidogyne spp.	34	33	32	28	39	44	37	35	<i>P</i> <0.0001
Pratylenchus spp.	11	11	21	25	35	28	34	24	<i>P</i> <0.0001
Total nematodes	100	100	100	100	100	100	100	100	P = 0.0034

Total nematodes = R. similis + Helicotylenchus spp. + Meloidogyne spp. + Pratylenchus spp.

**TABLE 2.** Mean percentage per month (2008-2014) of banana (*Musa* AAA) root samples from which various plant-parasitic nematodes were recovered from Ecuadorian plantations.

Namatada	Months												
Nematode	Jun	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	P>F
Number of root samples	1,093	667	965	754	887	825	881	1,117	1,235	3,139	1,017	1,193	
Radopholus similis	95	97	97	98	86	96	96	95	95	98	96	97	P<0.0001
Helicotylenchus spp.	92	92	84	92	86	92	92	88	92	94	89	89	P<0.0001
Meloidogye spp.	33	28	28	33	39	35	39	36	37	31	39	48	P<0.0001
Pratylenchus spp.	13	21	25	22	28	23	25	22	24	31	26	30	P<0.0001
Total nematodes	100	100	100	100	99	100	100	100	100	100	100	100	P<0.0001

Total nematodes = R. similis + Helicotylenchus spp. + Meloidogyne spp. + Pratylenchus spp.

**TABLE 3.** Mean percentage per province (2008-2014) of banana (*Musa* AAA) root samples from which various plant-parasitic nematodes were recovered on Ecuadorian plantations.

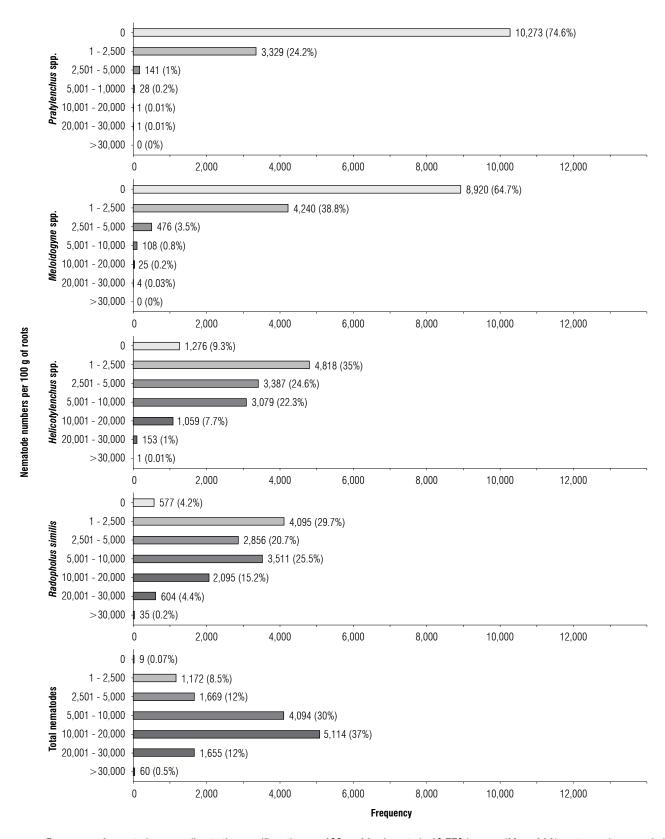
Nematode -						
	Cañar	El Oro	Guayas	Los Ríos	Santo Domingo	<i>P</i> >F
Number of root samples	162	8,328	1,925	3,287	71	
Radopholus similis	96	97	90	96	100	P<0.0001
Helicotylenchus spp.	64	91	86	95	94	P<0.0001
Meloidogyne spp.	44	36	41	29	31	P<0.0001
Pratylenchus spp.	28	25	27	26	8	P = 0.0041
Total nematodes	100	100	100	100	100	P<0.0001

Total nematodes = R. similis + Helicotylenchus spp. + Meloidogyne spp. + Pratylenchus spp.

The nematode genera encountered in this study are consistent with those found earlier in Ecuador by Quimí (1981), Asanza et al. (1994), Gómez (1997), Jiménez et al. (1998), and Chávez and Araya (2001, 2010) and also with those reported in Colombia (Volcy 2011), Venezuela (Haddad et al., 1975), Bolivia (Quispe, 2004), Brasil (Lima et al. 2013), México (Guzmán et al., 1995), Costa Rica (Araya et al., 2002), Belize (Ramclam and Araya, 2006), Martinique (Chabrier et al., 2002), Australia (Jackson et al., 2003), Phillipines (Davide, 1994), India (Gantait et al., 2011), Ivory Coast (Quénéhervé et al., 1991a, 1991b), South Africa (Daneel et al., 2015), Democratic Republic of Congo (Kamira et

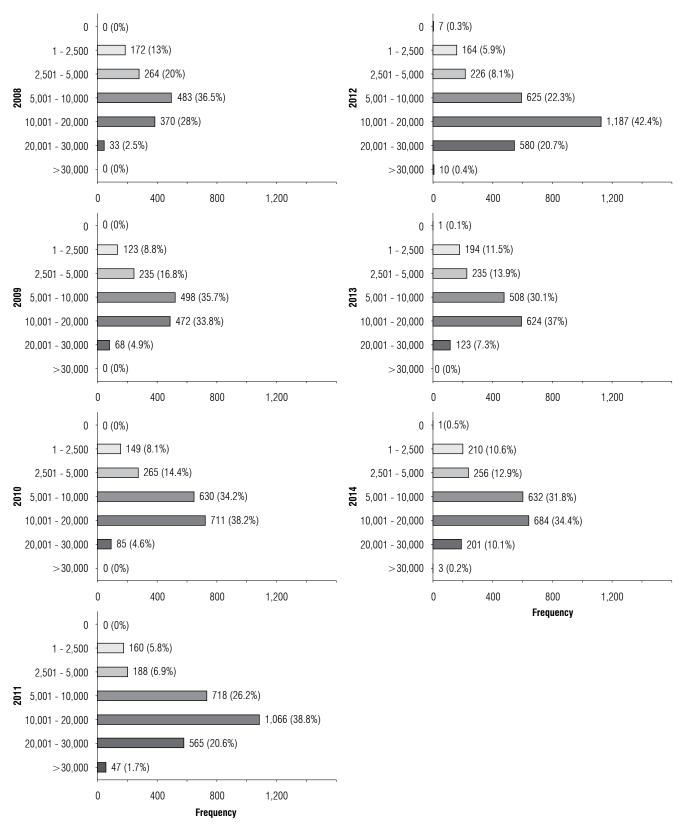
*al.*, 2013), and other African countries (Dubois and Coyne, 2011; Blomme *et al.*, 2013).

Nematodes were present in all of the years, provinces and months because of the continual monoculture of bananas and favorable edaphic and climatic conditions. The statistical significance that was detected in all frequencies by year, month and province more likely came from the high number of observations in each case. The low variation in the nematode frequencies may have been due in part to the stable soil moisture since, in the dry season, water was supplied by sprinkle irrigation, which also reduces the soil temperature variation.

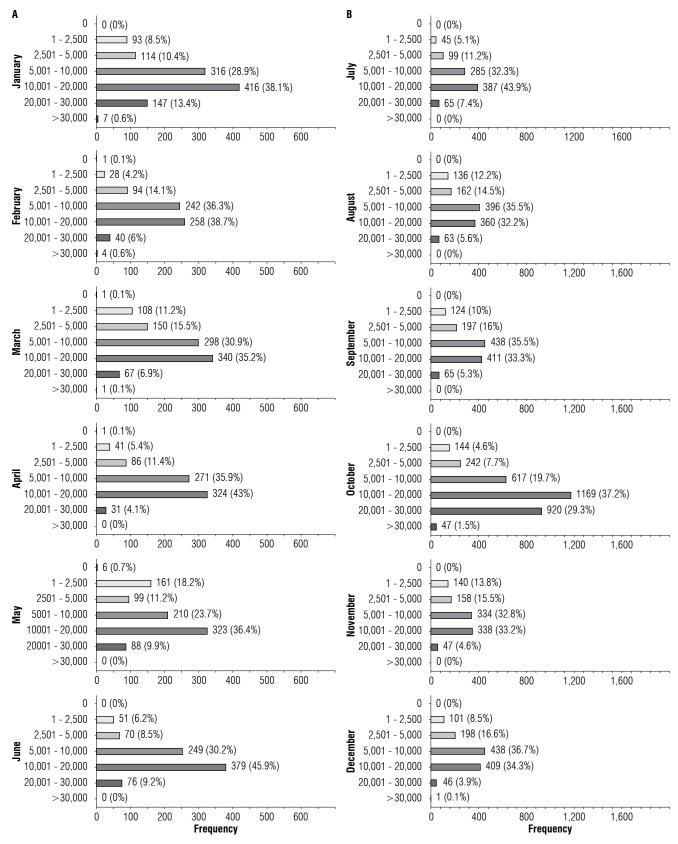


**FIGURE 2.** Frequency of nematodes according to the specific ratios per 100 g of fresh roots in 13,773 banana (*Musa* AAA) root samples recorded from 2008 to 2014. Total nematodes = *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.

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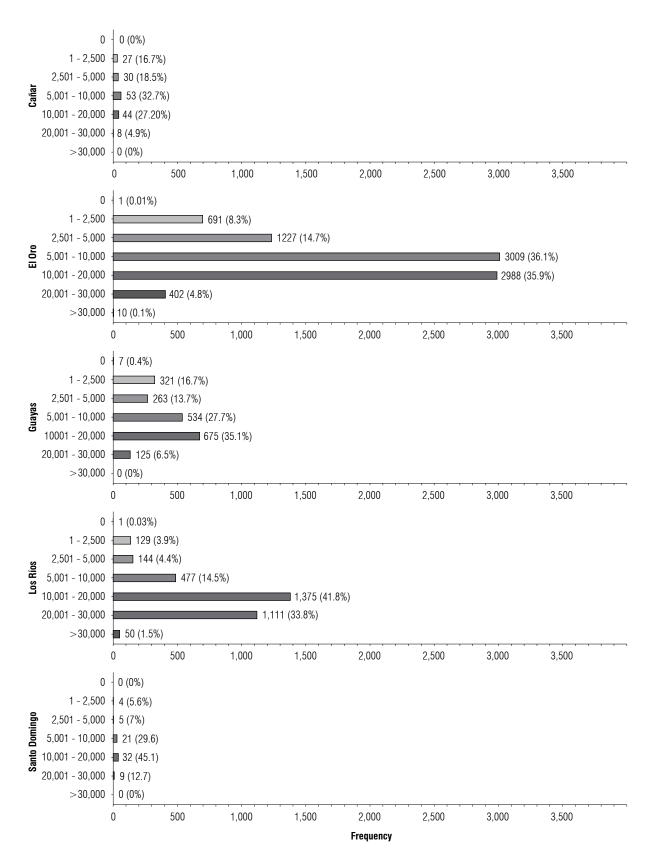


**FIGURE 3.** Sample distribution by total nematode population densities (Sum of *R. similis + Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) per 100 g of fresh banana (*Musa* AAA) roots in the different analyzed years.



**FIGURE 4.** Sample distribution by total nematode population densities (Sum of *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) per 100 g of fresh banana (*Musa* AAA) roots in the different analyzed months.

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**FIGURE 5.** Sample distribution by total nematode population densities (Sum of *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) per 100 g of fresh banana (*Musa* AAA) roots in the different analyzed provinces (Ecuador).

The high population densities and frequencies found for *R. similis* are encouraged by the long time banana monoculture and coincided with other local studies (Asanza *et al.*, 1994; Gómez, 1997; Jiménez *et al.*, 1998; Chávez and Araya, 2001, 2010) and with studies from Colombia (Jaramillo and Quirós, 1984), Costa Rica (Araya *et al.*, 2002), Belize (Ramclam and Araya, 2006) and with studies from Philippines (Davide, 1994). The lack of nematode control measures, superficial water table level, and inadequate knowledge of the farmers could also have contributed to the heavy infestations. High nematode population densities were found in all of the provinces, which calls for research on how to control them. However, it is advisable first to run experiments on the largest banana producing province.

Based on the observed nematode frequency and population densities, the relative importance of the nematode genera in the commercial banana clones appeared to decrease in the following order: *R. similis* > *Helicotylenchus* spp. > *Meloidogyne* spp. > *Pratylenchus* spp., in agreement with that found earlier by Chávez and Araya (2001, 2010). Individual and in concomitancy pathogenicity studies are necessary to verify if this relative importance corresponds with the damage caused by each pest and with the established economic threshold. The behavior of *R. similis* as the principal banana root nematode was confirmed by the observations of Blomme *et al.* (2013) in African countries, Stanton (1994) in Australia, Davide (1994) in Philippines, Pone (1994) in the Pacific Islands, Jiménez *et al.* (1998) in Ecuador, Gómez (1980) in Colombia, and Araya *et al.* (2002) in Costa Rica.

The high frequency and population density of *R. similis* could be a consequence of the affinity between this nematode and the commercial banana (*Musa* AAA), its type host (Baker *et al.*, 2014). The high levels of *R. similis* agree with the high reproductive fitness of *R. similis* on banana plants cultivates under controlled conditions (Stoffelen *et al.*, 1999a) and *in vitro* on carrot disc cultures (Stoffelen *et al.*, 1999b).

The different parasitic habits of the present nematode genera, migratory endoparasites: *Radopholus* and *Pratylenchus*, sedentary endoparasite: *Meloidogyne* and ectoendoparasite, feeding on subsurface tissue *Helicotylenchus* are likely to exacerbate root damage, because lesions can develop at feeding sites in the root cortex and through the root tissue. The *Helicotylenchus* spp. levels were lower than the *R. similis* populations, in agreement with other results (Araya *et al.*, 2002). More likely, this is because banana roots are not as good of a host for this nematode as they are for *R. similis*. Also, there is a difference in the life cycles. For

example, in *H. multicinctus*, the life cycle has taken 42 days at 28 °C on *Arabidopsis thaliana*, the adult females laid eggs at the rate of 4 per day for a period of 10-12 days (Orion and Bar-Eyal, 1995), while in *R. similis*, the life cycle has been completed in 20-25 days at 24-32°C on banana roots, and the adult females laid 4-5 eggs per day during 15 days (Loos, 1962). This means that more generations and more individuals per generation could be expected in the same period of time in the case of *R. similis*.

The low frequency and population density of *Meloidogyne* spp. could be related to the feeding behavior of *R. similis*. Santor and Davide (1992) found that the presence of *R. similis* on the galls caused deterioration and desintegration of the giant cells, which affected the development and reproduction of *M. incognita*. *Pratylenchus* spp. were rarely present and in low densities, which is reasonable because it has the same habitat as *R. similis* and a longer life cycle (Siddiqi, 1972).

For the local conditions, the Instituto Nacional Autónomo de Investigaciones Agropecuarias-INIAP (2015) has recommended the application of non-fumigant nematicides when R. similis is over 10,000 individuals per 100 g of total roots in samples taken between the mother plant and its follower sucker, or over 2500 per 100 g of total roots in samples taken from follower suckers. The local laboratory, Anemagro (2014) suggested that the use of non-fumigant nematicides when R. similis is over 10,000 individuals per 100 g of total roots, or over 2,000 per 100 g of functional roots in samples taken from recently flowered plants and, when samples are taken from follower suckers, over 3,000 per 100 g of total roots or over 1,000 per 100 g of functional roots. The Agencia Ecuatoriana de Aseguramiento de la Calidad del Agro (Agrocalidad, 2014) promotes nematicide applications when R. similis is over 10,000 per 100 g of total roots, obtained from samples taken in the interspace between the mother plant and its follower, or over 2,500 per 100 g of roots if samples are taken from the follower sucker.

These economic thresholds considers only *R. similis*; however, there is scientific knowledge that *H. multicinctus* and *H. dihystera* (McSorley and Parrado, 1986; Davide, 1996; Mani and Al Hinai, 1996; Chau *et al.*, 1997; Hartman *et al.*, 2010; Das *et al.* 2014) damage the banana root system and reduce yield by between 19% (Speijer and Fogain, 1999) and 34% (Reddy, 1994). Also, it is well known that *Meloidogyne* spp. (Santor and Davide, 1992; Davide and Marasigan, 1992; Fogain, 1994; Patel *et al.*, 1996; Moens and Araya-Vargas, 2002) and *Pratylenchus* spp. (Pinochet, 1978; Tarté, 1980; Rodríguez, 1990; Bridge *et al.*, 1997; Moens

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and Araya-Vargas, 2002) damage banana roots and reduce yield. Therefore, deciding on the nematode management depends on the total phytonematode population because all four genera damage the banana root system.

### Conclusion

The main nematodes that parasitize banana roots around the world are found in the banana production provinces of Ecuador and, in many cases, reach population densities above the economic threshold at any month of the year, which cause damage to the banana root system, restricting water and nutrient up take, increasing time for leaf emission, reducing bunch weight and plant longevity and increasing the crop cycle duration.

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