



# Spatial-temporal distribution of plant-parasitic nematodes in banana (*Musa AAA*) plantations in Ecuador

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## ABSTRACT

**Objective:** To provide quantitative information about population densities of the major nematode pests in Ecuadorian banana plantations.

**Methodology and results:** Banana root samples taken from 2000 to 2007 in the banana (*Musa AAA*) plantations of Ecuador were analyzed in NEMALAB and used for this study. Nematodes were extracted from 25 g of fresh roots that were macerated in a kitchen blender; nematodes were recovered in a 0.025 mm pore size (No 500 mesh) sieve. Four plant parasitic nematodes were detected, and based on their frequencies and population densities their relative importance was established as follows: *Radopholus similis* > *Helicotylenchus* spp. > *Pratylenchus* spp. > *Meloidogyne* spp. *R. similis* was most abundant accounting for 43 to 67% of the overall root population throughout the sampling years. From a total of 9,999 root samples, 9,482 (95%) contained *R. similis*, 7,916 (79%) *Helicotylenchus* spp., 3,068 (31%) *Pratylenchus* spp. and 706 (7%) *Meloidogyne* spp. When all nematodes present were pooled (total nematodes) only 20 (0.2%) samples were free of phytonematodes.

**Conclusions and application of findings:** High populations of total nematodes were found in all the years, months and provinces. These results confirm these four nematode genera as the major cause of banana root damage and yield reduction yield. Research for nematode management tactics needs to be developed considering the total phytonematodes population.

**Key words:** banana, nematodes, *Helicotylenchus* spp., *Meloidogyne* spp., population densities, *Pratylenchus* spp., *Radopholus similis*

## INTRODUCTION

In Ecuador, banana (*Musa AAA*) is cultivated on small (< 5 ha), medium (5-50 ha) and big (> 50 ha) farms ranging from 0.5 to 300 ha or more for local consumption and export markets. Bananas are the most important crop in the country. In 2007, more than 4.6 million tonnes (Bananotas, 2008a) were exported, from about 220000 ha (Bananotas, 2008b), which gave a total income of about US \$1100 million.

Besides the constraints of banana market requirements and demands, there are other factors limiting production. The important abiotic factors

constraining yield of bananas include, edaphic soil condition mainly due to clay texture, poor structure and Na content. Among the biotic factors, phytonematodes are second after black Sigatoka (*Mycosphaerella fijiensis*). These pests reduce bunch weight and longevity, and increase the crop cycle duration.

In most of the banana plantations, phytonematodes usually occur in polyspecific communities, consisting mainly of a mixture of *Radopholus similis*, *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. To avoid

or reduce nematode damage, the only management strategy currently available is the regular application of non-fumigant nematicides, which are economically feasible.

However, nematicide application is done only in big farms. Small and medium farms end with low yield due in part to nematode damage since nematode control measurements are not used. Economic and environmental considerations dictate rational use of nematicides at minimum dosages. To achieve this, more research is

needed in the evaluation of nematicide application systems, biocontrol agents and cultural practices, to prevent nematode infection and excessive root damage.

The objective of this study was to provide quantitative information about population densities and frequencies of the major nematode pests in Ecuadorian banana plantations. This information will be used to identify more appropriate research areas on nematode management and as a basis to justify more investment.

## MATERIALS AND METHODS

**Study area description:** Samples included in the analysis were from long-term ratoon commercial banana plantations in all provinces where the crop is cultivated in the country. Farms vary in soil type, texture and structure, content of macro and micro elements and climatic conditions. The age of the plantations range from 10 to 30 years with a plant density of 1300 to 1500 ha<sup>-1</sup> and the cultivars used were mainly Valery and Grande Naine. Bunching plants were supported by tying them to adjacent plants with double polypropylene twine or by propping with wood poles.

**Crop management:** Various banana cultural practices (fertilization, weed control, nematodes and black Sigatoka management) were practiced and may have influenced the nematode population densities reported in this paper. Desuckering was carried out every six to eight weeks throughout each year, leaving the production unit with a bearing mother plant, a large daughter sucker, and a small granddaughter when possible. Usually from January to April, most of the total water requirement was supplied by rainfall, while from May to December sprinkler irrigation was necessary each year. Average rainfall (2000-2007) varied from 426 to 2,526 mm. A complex system of primary, secondary and tertiary drains was installed to carry off excess water, lower the water table and prevent water logging.

**Sampling and root processing:** Data of the samples recorded by Nematlab S.A. from 2000-2007 were used for this study. A total of 9999 root samples were processed from January 2000 to December 2007 and entered into a computer database along with 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 a following sucker. Samples were taken either from the follower suckers of 1-1.5 m height or in the area

between the recently flowered plants (within 3-7 days of flower emergence) and their follower suckers of 1 - 1.5 m of height. A hole approximately 30 cm wide x 30 cm length x 30 cm depth was dug with a shovel at the base of the plant. Roots from each hole were collected, placed in labeled plastic bags, and delivered to the laboratory in insulated boxes.

Root samples were registered and processed immediately or stored in a refrigerator (General Electric) at 6-8 °C until processing. Roots were rinsed free of soil, separated into functional (includes only living roots, healthy, free of symptoms of nematode damage) and non-functional roots (purple or brown colored tissue, dead, snapping, or extensively necrotic root tissue). Surface moisture was allowed to dry before weighing. During the root separation process, there were some roots that had healthy and damaged parts. In these cases, the symptomatic tissue was separated.

Nematodes were extracted from 25 g of fresh roots, which consisted of functional and non-functional roots in a representative proportion to what was determined in the root sample, proportionately. Roots were macerated in a Osterizer kitchen blender (Taylor & Loegering, 1953 method, modified by Araya, 2002) for 10 sec at low and 10 sec at high speed, and the resulting mixture was washed from the blender through a series of nested sieves of 0.25/0.106/0.025 mm openings (No 60/140/500 mesh). The residue on the 0.25 and 0.106 mm sieves was discarded and that on the 0.025 mm sieve was washed into a 250 ml beaker. Nematodes were identified and counted to determine the population densities of all plant-parasitic nematodes present. All counts were made with the aid of a Micromaster microscope at 4x magnification and values converted to numbers per 100 g of fresh roots.

Data were subjected to ANOVA and frequency distribution analysis for each nematode genus by year,

month and province in PC-SAS (SAS Institute, Inc. Cary, NC). The absolute frequency was calculated as a percentage (number of samples containing a species / number of samples collected \* 100 (Barker, 1985)). For each nematode genus, samples were distributed

according to the following specific frequency classes: nematodes not detected; 1-2,500; 2501-5,000; 5,001-10,000; 10,001-20,000; 20,001-50,000; and > 50,000 individuals per 100g of fresh roots.

## RESULTS

The major plant-parasitic nematodes present in the sampling areas were *R. similis* and *H. multincinctus* (Fig. 1). *Pratylenchus* spp. and *Meloidogyne* spp. were rarely detected and when present the populations were negligible. *Radopholus similis* was the most abundant nematode varying from 43-67% of the plant parasitic nematodes recovered, with *H. multincinctus*, *Pratylenchus* spp. and *Meloidogyne* spp. comprising from 30-47%; 2-11% and 0-0.2%, respectively, of the

overall root population during each of the different years.

The absolute frequency of *R. similis*, *Helicotylenchus* spp. and total nematodes differed ( $P < 0.0001$ ) among years (Table 1). In practice, that variation is small, ranging between 88-98% for *R. similis*, 76-85% for *Helicotylenchus* spp. and 94-100%, for the total nematodes, indicating that the significance came from the high number of observations in each year.

**Table 1:** Percentage of banana (*Musa* AAA) root samples each year from which various plant parasitic nematodes were recovered.

Nematode	Years								Mean
	2000	2001	2002	2003	2004	2005	2006	2007	
Number of samples	575	1556	369	1508	2150	1672	430	1734	
<i>Radopholus similis</i>	95	88	90	92	98	98	98	96	95
<i>Helicotylenchus</i> spp.	79	78	85	82	78	76	77	82	79
Total nematodes	94	99	99	100	90	100	100	100	100

Total nematodes = *Radopholus similis* + *Helicotylenchus* spp.

The two main nematode genera encountered were detected in all months of the year (Table 2). Even though there was little variation in the absolute frequencies, 91-97% for *R. similis* and 74-85% for *Helicotylenchus* spp. statistical differences ( $P < 0.0001$ ) were detected most likely due too the high number of observations recorded in each month. Similarly, both nematodes were detected in the four provinces,

differing significantly ( $P < 0.0001$ ) amongst themselves. The absolute frequency of *R. similis* was high and varied from 95-99%, while for *Helicotylenchus* spp. the absolute frequency it varied from 43-82% (Table 3). The absolute frequency of the total number of plant parasitic nematode was higher than 99%, which indicated that practically, no farms were free of plant parasitic nematodes.

**Table 2:** Mean percentage per month (2000-2007) of banana (*Musa* AAA) root samples from which various plant parasitic nematodes were recovered.

Nematode	Months											
	J	F	M	A	My	J	Jl	A	S	O	N	D
Number of samples	1072	617	760	727	709	1112	799	668	630	1118	1166	621
<i>Radopholus similis</i>	97	94	97	98	93	95	91	91	96	93	96	97
<i>Helicotylenchus</i> spp.	82	79	78	78	78	78	81	86	81	80	74	79
Total nematodes	100	99	100	100	99	100	100	99	100	100	100	100

Total nematodes = *R. similis* + *Helicotylenchus* spp

The specific frequencies (population density classes for each taxa) for the root samples clearly indicated that *R. similis* is the most abundant species in banana (Fig. 2). From the 9999 recorded root samples, *R. similis* was absent in 517 (5%) and 1278 samples (13%) contained greater than 10000 *R. similis* per 100 g of fresh roots (Figure 2). For *H. multincinctus*, 2083 (21%) of the samples were negative and only 678 (8%) had numbers greater than 10000 nematodes (Fig. 2).

When the four nematode genera were pooled to give the total nematode population, nematodes were not detected in only 20 (0.2%) samples while 3777 (39%) contained greater than 10000 nematodes per 100 g of fresh roots. Because *R. similis* usually comprised from 43-67% of the overall nematode population, it strongly

influenced the total nematode density ratios distribution by months, years and province. A stable trend in the number of samples (32-52%) with greater than 10000 plant parasitic nematodes per 100g of fresh roots in the different years analyzed (Fig. 3). In each month of the year, between 2–5% of the samples had greater than 10000 plant parasitic nematodes per 100 g of fresh roots was evident and nematodes were undetected in very few root samples (Fig. 4). From the provinces, the highest nematode populations were detected in El Oro, where 30% of the samples had more than 10000 nematodes per 100 g of roots (Fig. 5). In the other provinces, the number of samples with a total nematode population level greater than 10000 nematodes per 100 g of fresh roots was less than 5%.

**Table 3:** Mean percentage per province (2000-2007) of banana (*Musa AAA*) root samples from which various plant-parasitic nematodes were recovered.

Nematode	Provinces			
	Cañar	El Oro	Guayas	Los Ríos
Number of samples	412	7873	1328	385
<i>Radopholus similis</i>	99	95	95	95
<i>Helicotylenchus</i> spp.	43	82	79	72
Total nematodes	100	100	100	100

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

## DISCUSSION

The four nematode genera detected in this study are well known pathogens of banana roots (Pinochet, 1977b; McSorley & Parrado, 1986; Sarah, 1989; Gowen & Quénehervé, 1990; Gowen, 1995; Sarah *et al.*, 1996; Marín *et al.*, 1998; De Waele & Davide, 1998; De Waele, 2000; Gowen, 2000a,b; Gowen *et al.*, 2005). Nematode genera identified are consistent with those reported by Gómez (1997) and Chávez & Araya (2001) in Ecuador and are similar with those found in Belize (Pinochet, 1977a; Bridge *et al.*, 1996; Ramclan & Araya, 2006), in Colombia (Gómez, 1980), in Bolivia (Quispe, 2004), in Philippines (Boncato & Davide, 1992), and in Costa Rica (Araya *et al.*, 2002).

Nematodes were present in all the years, months and provinces because of the continual monoculture of bananas, favorable edaphic and climatic conditions. Low variation in nematode population densities and frequencies may be due in part to the stable soil moisture, since in the dry season; water was supplied by sprinkler irrigation. The variation in soil temperature may also play a major impact on banana root development which may affect nematode populations. Based on the nematode frequencies and their

population densities, the relative importance of nematode genera in commercial banana clones appears to be as follows: *R. similis* > *H. multincinctus* > *Pratylenchus* spp. > *Meloidogyne* spp. Individual and in concomitancy pathogenicity studies are necessary to verify if this relative importance corresponds with the damage caused by each pest. The relative importance of *R. similis* as the main banana root nematode in local conditions is supported by observations of Jiménez *et al.* (1998) and Chávez & Araya (2001). In other countries producing bananas for domestic consumption and export, *R. similis* was also considered as the most economically important plant parasitic nematode, e.g. such as in African countries (Fargette & Queneherve, 1988; Speijer & Fogain, 1999; Kashaija *et al.*, 1999), in Australia (Stanton, 1994), in Philippines (Davide, 1994), in the Pacific Islands (Pone, 1994), in Colombia (Gómez, 1980) and in Costa Rica (Araya *et al.*, 2002). Different parasitic habits of the nematode genera present in this study exhibited different parasitic behaviour on the roots of, banana, migratory endoparasitic (*Radopholus* - *Pratylenchus*), ectoparasitic, feeding on subsurface tissue

(*Helicotylenchus*) and sedentary endoparasitic (*Meloidogyne*). The presence of mixed populations of plant parasitic nematodes is likely to exacerbate root damage, because lesions can develop at feeding sites throughout the root tissue.

The high frequencies and population densities found for *R. similis* and *Helicotylenchus* spp. are fostered by the lengthy banana monoculture, reduced options for cultural control and neglect of quarantine measures. The abundance of *R. similis* in Ecuadorian banana plantations could also be a consequence of the affinity between this nematode and the commercial banana (*Musa AAA*), its type host (Orton & Siddiqi, 1973). The high population densities of *R. similis* in banana roots in this field study agrees with the high reproductive fitness found for the species under controlled conditions (Fallas *et al.*, 1995; Binks & Gowen, 1997; Stoffelen *et al.*, 1999a,) and *in vitro* on carrot disk cultures (Fallas & Sarah, 1995; Stoffelen *et al.*, 1999b).

In all the years, there were fewer numbers of *H. multicinctus* compared to *R. similis*, which corresponded with others (Gómez, 1980; Araya *et al.*, 2002) and may be due to differences in host suitability of banana, differences in life cycles and competition for feeding sites and degradation of the roots of the banana plants following feeding of *R. similis*. The life cycle of *H. multicinctus* was reported to take 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 & Bar-Eyal, 1995), while *R. similis* completes the life cycle 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 of *R. similis* per generation could be expected in the same period of time

The low frequency and population density of *Pratylenchus* spp. could be due to the high abundance of *R. similis*, being that both nematodes have the same

habitat. Thus, *R. similis* may be suppress suppresses *Pratylenchus* spp. In addition, *Pratylenchus* spp. has a longer life cycle (Siddiqi, 1972). The very low frequency and population density of *Meloidogyne* spp. could also be due to the feeding behavior of *R. similis*. Santor & Davide (1992) found that the presence of *R. similis* in root-knot galls caused deterioration and disintegration of the giant cells, which affected the development and reproduction of *M. incognita*. Considering *R. similis* alone, only 13% of the samples surpassed the economic threshold, and those were mainly concentrated in El Oro province. This probably indicates that the environmental conditions in Cañar, Guayas and Los Ríos are not conducive for the development of plant parasitic nematodes or those are areas which have a short history of banana production. In Ecuador, non fumigant nematicides are recommended when *R. similis* exceeds 10000 nematodes per 100 g of fresh roots. This is an action threshold suggested by Gómez (1980) and Tarte & Pinochet (1981). *Radopholus similis* has been well documented to cause banana root damage and yield reduction. Similarly *Helicotylenchus* spp. has been reported to cause root damage on bananas (McSorley & Parrado, 1986; Davide, 1996; Mani & Al Hinai, 1996; Chau *et al.*, 1997) to and more specifically reduce banana yields between 19% (Speijer & Fogain, 1999) and 34% (Reddy, 1994). It is also well established that *Pratylenchus* spp. is also a well established pathogen of banana causing damage to its roots and reducing yields (Pinochet, 1978; Tarté, 1980; Rodríguez, 1990; Bridge *et al.*, 1996; Moens & Araya, 2002) and similarly *Meloidogyne* spp. (Santor & Davide, 1992; Davide & Marasigan, 1992; Fogain, 1994; Patel *et al.*, 1996; Moens & Araya, 2002). Therefore, development of nematode management tactics requires consideration of the damage caused by the total phytonematode population.

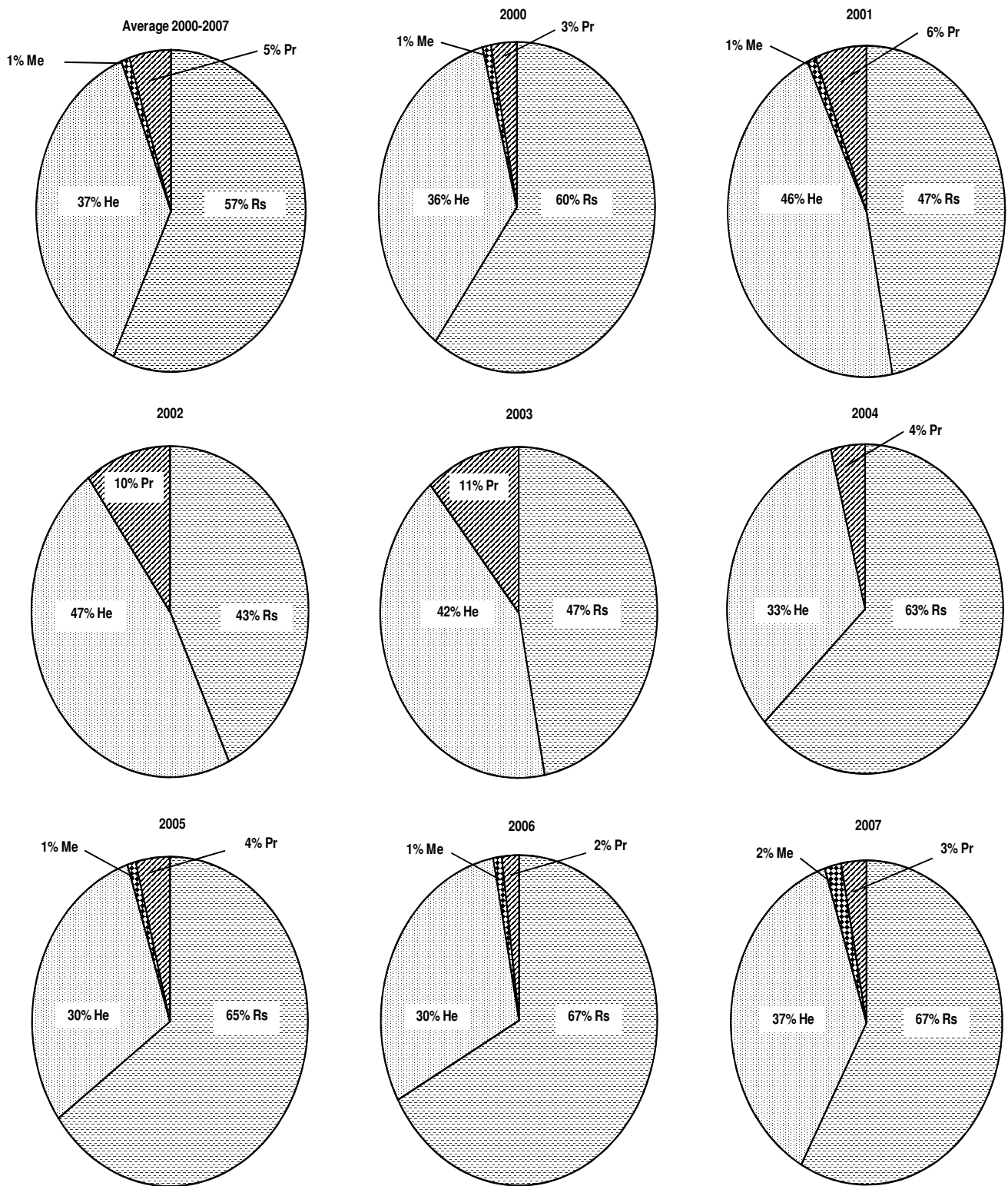
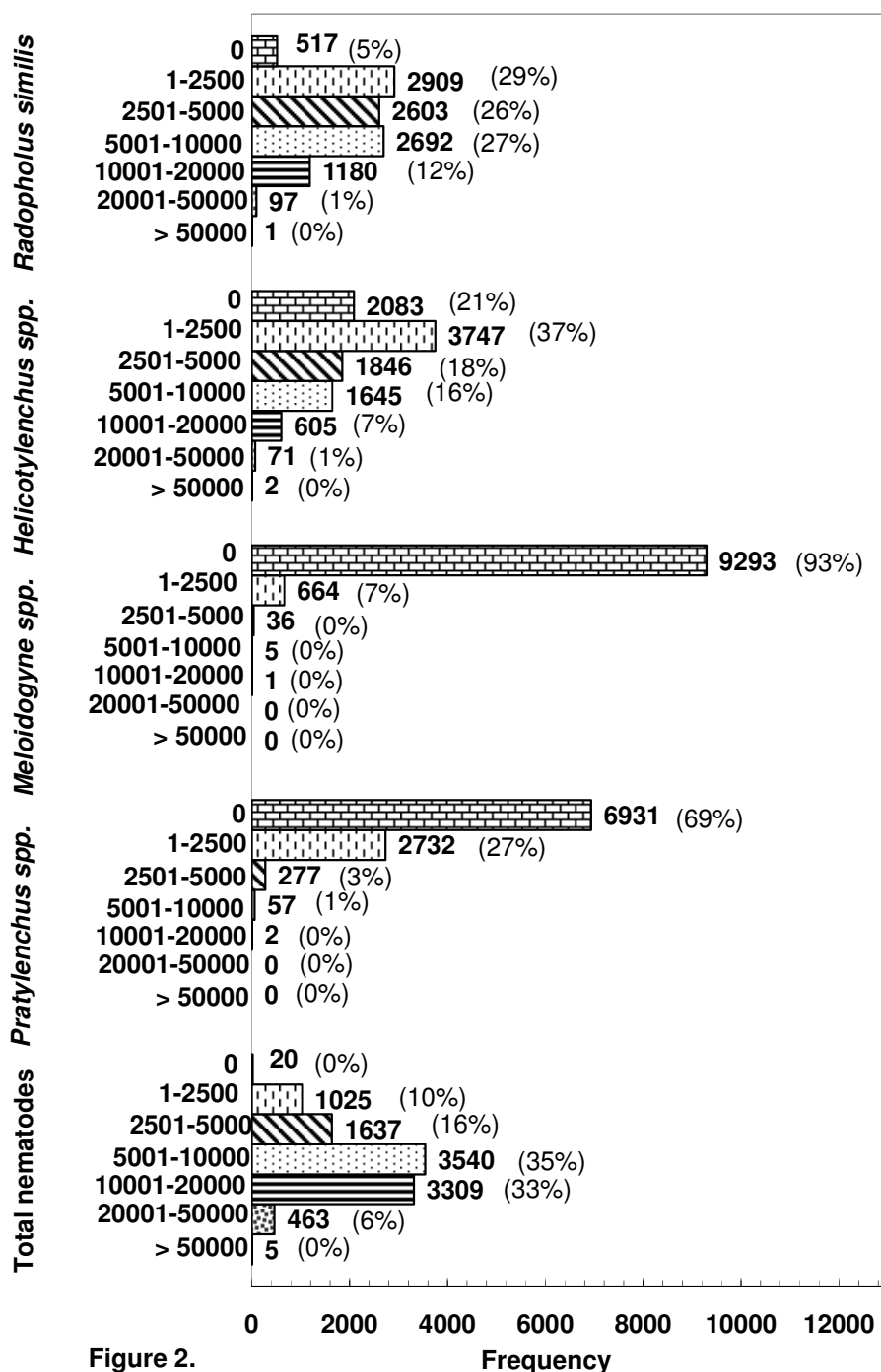


Figure 1: Percentage of the total plant-parasitic nematode population recovered from banana (*Musa AAA*) root samples for each nematode genus during each year of a survey in four provinces of Ecuador. Rs= *Radopholus*

*similis*, He= *Helicotylenchus* spp., Pr= *Pratylenchus* spp. and Me= *Meloidogyne* spp. Average N= 9,999; 2000 N= 575; 2001 N= 1,556; 2002 N= 369; 2003 N= 1,508; 2004 N= 2,153; 2005 N= 1,674; 2006 N= 430 and 2007 N= 1,734 root samples.



**Figure 2.** Distribution of the nematode counts from 100 g of fresh roots in 9,999 banana (*Musa* AAA) root samples processed from 2000-2007. Total nematodes= *Radopholus similis* + *Helicotylenchus* spp. + *Pratylenchus* spp. + *Meloidogyne* spp. Values in ( ) are the frequencies.

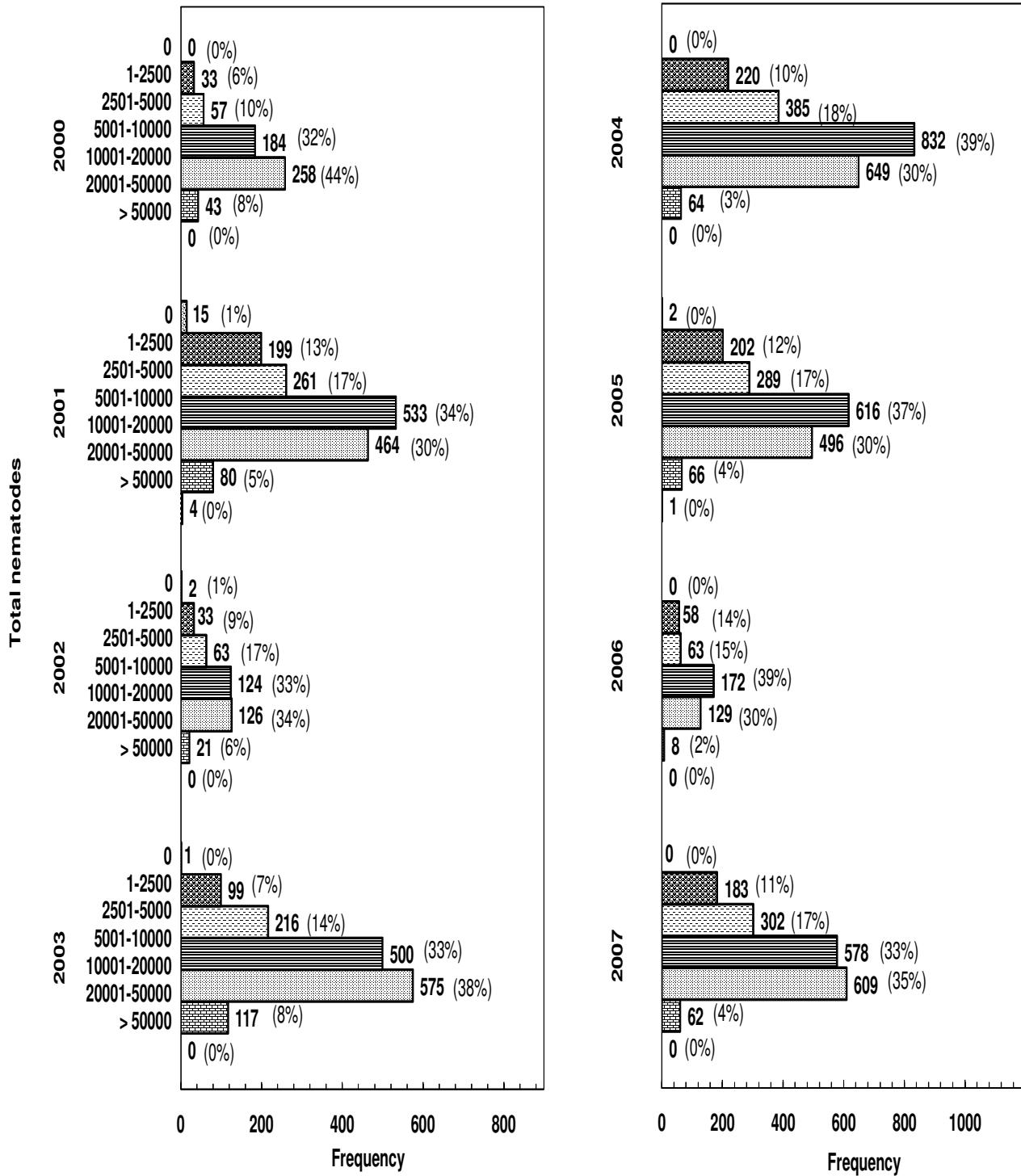


Figure 3: Distribution of the total (sum of *Radopholus similis* + *Helicotylenchus* spp. + *Pratylenchus* spp. + *Meloidogyne* spp.) nematode counts from 100 g of fresh roots in banana (*Musa AAA*) root samples processed from 2000-2007. Values in ( ) are the frequencies.



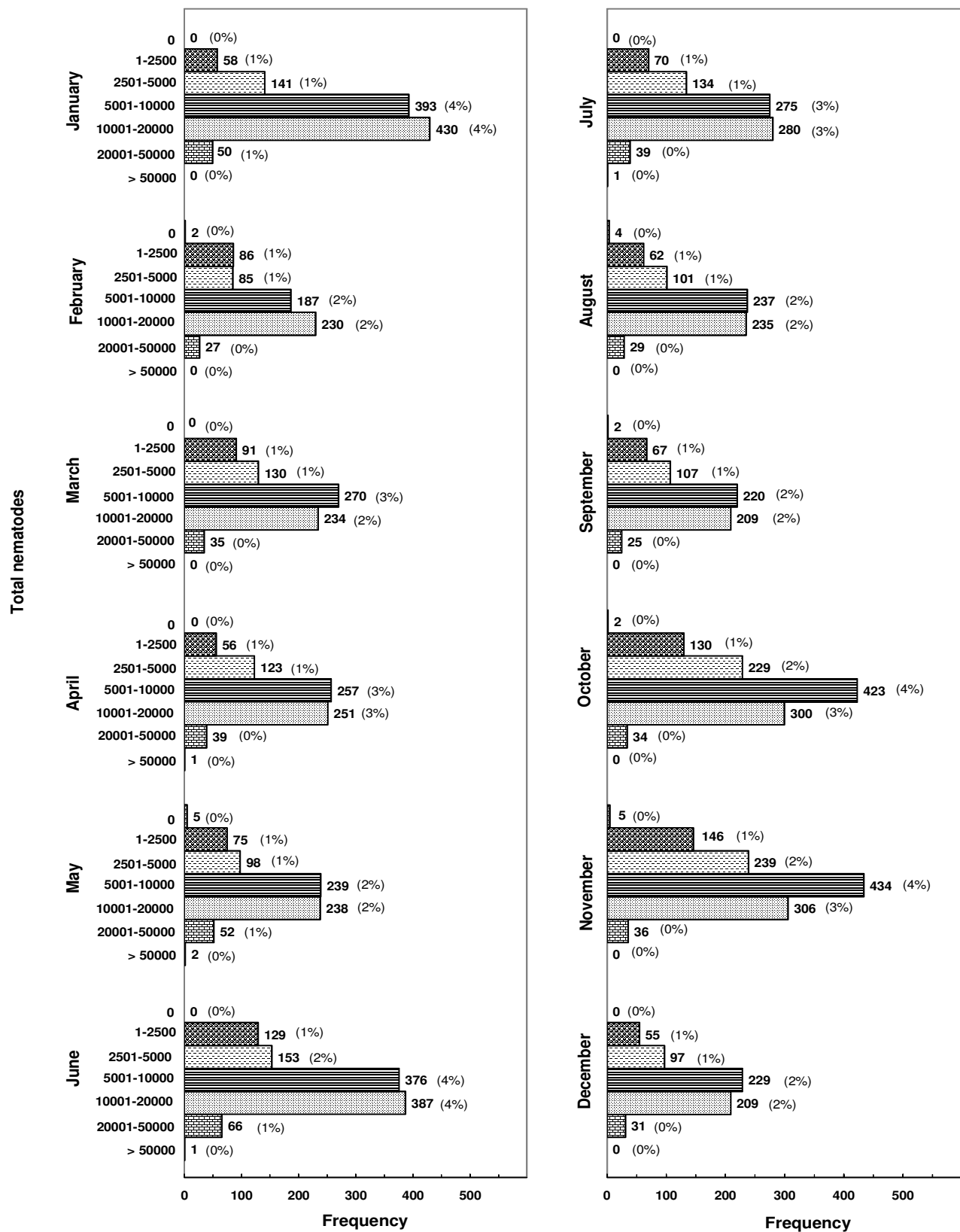


Figure 4: Distribution of the total nematode (sum of *Radopholus similis* + *Helicotylenchus* spp. + *Pratylenchus* spp. + *Meloidogyne* spp.) counts per 100 g of fresh banana (*Musa* AAA) roots as a monthly mean for the years 2000-2007.

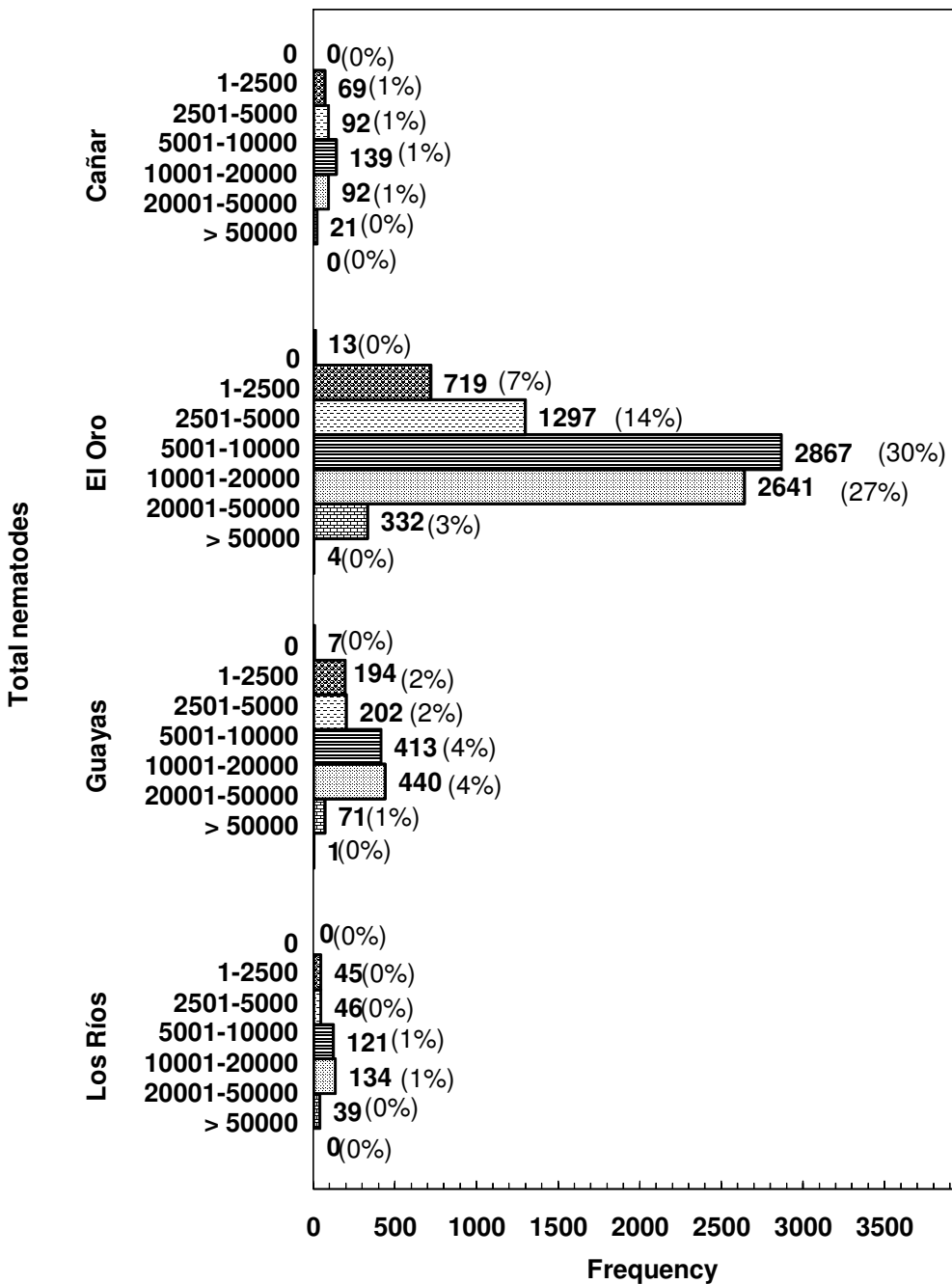


Figure 5: Distribution of the total nematode (sum of *Radopholus similis* + *Helicotylenchus* spp. + *Pratylenchus* spp. + *Meloidogyne* spp.) counts per 100 g of fresh banana (*Musa* AAA) roots as a mean per province during 2000-2007.

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