OCCURRENCE AND POPULATION DENSITIES OF NEMATODE PARASITES OF BANANA (*MUSA* AAA) ROOTS IN COSTA RICA

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ABSTRACT

Araya, M., D. De Waele, and R. Vargas. 2002. Occurrence and population densities of nematode parasites of banana (*Musa* AAA) roots in Costa Rica. Nematropica 32:21-33.

An analysis was conducted of plant parasitic nematodes occurring in banana (*Musa* AAA) plantations in Costa Rica from 1995 to 1999. Nematodes were recovered on 0.038-mm-pore sieves after macerating 25 g of fresh roots in a blender. Data were subjected to frequency analysis and the absolute frequency was calculated for each genus. Four genera of plant parasitic nematodes were detected. *Radopholus similis* was the most abundant nematode accounting for 82 to 86% of the overall root population throughout the survey. From a total of 60,032 root samples, 58,297 (97%) contained *R. similis*, 33,504 (56%) *Helicotylenchus* spp., 30,227 (50%) *Meloidogyne* spp., and 8,674 (13%) *Pratylenchus* spp. Only on 355 (0.6%) samples, were any of those nematodes undetected. High population densities of *R. similis* were found in all years, months, and counties. Additional studies are necessary to verify if this relative importance coincides with root damage and economic threshold.

Key words: Helicotylenchus spp., *Meloidogyne* spp., *Musa* AAA, occurrence, population densities, *Pratylenchus* spp., *Radopholus similis*, root samples.

RESUMEN

Araya, M., D. De Waele y R. Vargas. 2002. Ocurrencia y densidades poblacionales de los nematodos parásitos de las raíces del banano (*Musa* AAA) en Costa Rica. Nematrópica 32:21-33.

Se realizó un análisis de los nematodos parásitos que ocurren en las plantaciones bananeras (*Musa* AAA) de Costa Rica de 1995 a 1999. Los nematodos fueron recobrados en cribas de 0.038 mm de diámetro de apertura después de macerar 25 g de raíces frescas en una licuadora de cocina. Los datos se sometieron a un análisis de frecuencias en PC-SAS y se calculó la frecuencia absoluta de cada género. Cuatro géneros de nematodos fueron detectados, y en base a sus frecuencias y densidades poblacionales se estableció que la importancia relativa fue en orden decreciente: *R. similis* > *Helicotylenchus* spp. > *Meloidogyne* spp. > *Pratylenchus* spp. *Radopholus similis* fue el nematodo más abundante, alcanzando entre un 82 y 86% de la población total, a través del estudio. Del total de 60 032 muestras, 58 297 (97%) contenían *R. similis*, 33 504 (56%) *Helicotylenchus* spp., 30 227 (50%) *Meloidogyne* spp. y 8 674 (13%) *Pratylenchus* spp. Solamente en 355 (0,6%) de las muestras no se detectó ninguno de los nematodos. Altas poblaciones de *R. similis* fueron encontradas en todos los años, meses y cantones. Estudios adicionales de patogenicidad individual y en concomitancia son necesarios para verificar si la importancia relativa corresponde con el daño en las raíes y el umbral económico.

Palabras claves: densidades poblacionales, *Helicotylenchus* spp., *Meloidogyne* spp., *Musa* AAA, ocurrencia, *Pratylenchus* spp., *Radopholus similis.*

INTRODUCTION

Banana (*Musa* AAA, L. Simmonds, 1962) is the most important crop in Costa Rica accounting for almost 30% of the agricultural gross national product. Exported bananas in 1999 were valued at US $649 million and were produced on 49 000 ha

(Sánchez and Zuñiga, 2000). In addition to the constraints of banana market requirements and demands, there are other limiting factors. Among the biotic factors affecting yield, plant parasitic nematodes rank second after black Sigatoka disease (*Mycosphaerella fijiensis*), reducing bunch weight and longevity, and increasing the crop cycle duration.

Polyspecific nematode communities usually occur in local plantations and consist of mixtures of *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), *Pratylenchus* spp. and rarely *Rotylenchulus reniformis* (Linford and Oliveira, 1940). The only nematode management strategy currently available, that growers accept as economically feasible, is the regular application of non-fumigant nematicides. However, economic and environmental constraints dictate rational use of nematicides at minimum dosages. To anticipate and prevent nematode population increase, more research is needed to evaluate nematicide application systems, natural biocontrol agents, and the effects of cultural practices.

The objective of this study was to provide quantitative information about population densities and frequencies of the major nematode pests in the ten counties where banana is grown in Costa Rica. This information will be used to help identify locations appropriate for research on nematode management and as a basis to justify the investment.

MATERIALS AND METHODS

Data from the records of the monthly monitoring sampling program at the CORBANA (Costa Rican National Banana Corporation) Nematology Laboratory were used for this analysis. A total of 60 032 root samples were processed from January 1995 to December 1999 and nematode data were entered into a computer data base along with farm identity, county, month and year. Samples included in the analysis were from the ten counties (Atlantic region: Pococí, Guácimo, Siquirres, Matina, Talamanca, and Sarapiquí; Pacific region: Osa, Golfito and Corredores) where long-term ratoon commercial bananas are grown. The counties vary in climatic conditions, soil types and texture.

The age of the plantations ranged from 5 to 30 years with a plant density of 1 600 to 1 850 plants ha^{-1} and the cultivars used were Grand Naine, Valery or Williams. Bunching plants were supported by tying them to adjacent plants with double polypropylene twine or by aerial guying. Various banana cultural practices (fertilization, control of weeds, nematodes and black Sigatoka) were practiced and may have influenced the nematode population densities reported in this paper. In some farms volunteer cover crops, including pastures, were allowed to grow. 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.

As is usual for the banana plantations located in the Atlantic region, the total water requirement was supplied by rainfall, while in the Pacific region microsprinkler irrigation was necessary from December to March each year. Average rainfall (1995-1999) varied in the Atlantic region from 3 697 to 4 264 mm and in the Pacific from 3 726 to 4 240 mm. A complex system of primary, secondary and tertiary drains was installed to carry off excess water, lower the water table and prevent waterlogging.

Each root sample consisted of the roots of five randomly selected plants. Samples were taken in the area between the recently flowered plants (within eight days of flower emergence) and their follower suckers. A hole 15 cm long, 15 cm wide and 30 cm deep 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 insulated chests.

Root samples were registered and processed immediately or stored in a refrigerator at 6-8°C until being processed. Roots were rinsed free of soil, separated into functional (living roots, either healthy or with symptoms of nematode damage) and non-functional roots (dead, snapping, or extensively necrotic root tissue), and 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 necrotic areas were separated.

Nematodes were extracted from 25 g of fresh functional root subsamples. Roots were macerated in a kitchen blender (Taylor and Loegering, 1953) 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 0.5, 0.15, and 0.038 mm openings (35, 100, 400 mesh). The residue on the 0.5 and 0.15 mm sieves was discarded and that on the 0.038 mm sieve was washed into a 250 ml beaker. Nematodes were identified and counted as described in Araya *et al.*, (1995). Population densities of all plant-parasitic nematodes present were determined. All counts were made with an Olympus BH microscope at 4× magnification and values converted to numbers of nematodes per 100 g of fresh roots.

Data were subjected to frequency distribution analysis for each nematode by year, month, and county 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; 2 501-5 000; 5 001-10 000; 10 001-20 000; 20 001-50 000; and >50 000 individuals per 100 g of fresh root.

RESULTS

The major plant-parasitic nematodes present in the sampling areas were *R. similis, Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. The *Helicotylenchus* species consisted of *H. multicinctus* and a few *H. dihystera* with a proportion of 3:1. The perineal patterns of *Meloidogyne* spp. females indicated that *M. incognita* was common and *M. javanica* was rarely present with a proportion of 8:2.

Radopholus similis was the most abundant nematode (82-86% of the plant parasitic nematodes recovered), with *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. comprising 7.6-9.7%; 4.8-8.5% and 0.6-0.8%, respectively, of the overall root population during each of the different years (Fig. 1). The absolute frequency of the different nematodes was very stable during the different years of the survey (Table 1). The highest frequency was always that for *R. similis* (>96%), followed by *Helicotylenchus* spp. which ranged from 52 to 60%.

The four nematode genera encountered were detected in all months of the year, with little variation in their absolute frequencies (Table 2). The absolute frequencies of *R. similis* and total nematodes were also stable across counties, but those of the other genera were more variable (Table 3). The widest variation in absolute frequency was exhibited by *Helicotylenchus* spp. which varied from 30% in Sarapiquí to 100% in Corredores. None of the 10 counties was free of nematodes.

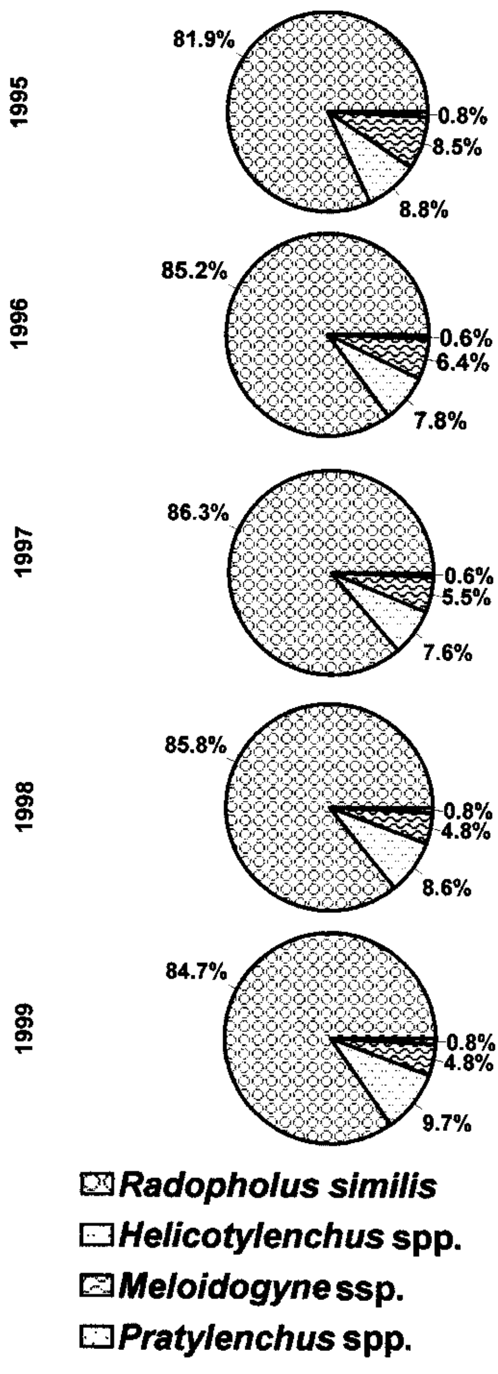

Fig. 1. Percentage of the total plant parasitic nematode population recovered from banana (*Musa* AAA) root samples for each genus during each year of a survey in 10 counties in Costa Rica.

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 60 032 root samples, no *R. similis* were detected on only 1 735 (2.9%) and 32 758 (54%) contained >10 000 *R. similis*/100 g of roots (Fig. 2). For *Helicotylenchus* spp., 26 528 (44%) of the samples were negative and only 1 486 (2.4%) had numbers >10 000 nematodes. More than 49% (29 805) of the samples were free *Meloidogyne* spp. and 1.4% (858) contained numbers >10 000 nematodes. *Pratylenchus* spp. were present in 13% (8 674) of the samples, with only 30 (0.05%) above 10 000 nematodes. When all nematodes were pooled (total nematodes), nematodes were not detected in only 355 (0.6%) samples and 37 875 (63%) samples contained >10 000 nematodes/100 g of roots.

Because *R. similis* comprised more than 82% of the overall nematode population it strongly influenced the total nematode density ratios distribution by years, months and counties. A stable trend in the number of samples (60-65%) with >10 000 plant parasitic nematodes was evident among the different years analyzed (Fig. 3). In each month of the year, between 57-68% of the samples had >10 000 plant parasitic nematodes and nematodes were undetected in fewer than 1% of the samples (Fig. 4). A similar level of consistency for plant parasitic nematode occurrence and abundance was observed among all counties (Fig. 5).

DISCUSSION

The four nematode genera detected in this survey are well known pathogens of banana roots (De Waele and Davide, 1998; Marin *et al.*, 1998; Bridge *et al.*, 1997; Sarah *et al.*, 1996; Gowen, 1995; Sarah, 1989;

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

Nematode	Years 95	96	97	98	99	Mean
Number of samples	10 618	11 253	10 929	12 707	14 525	
Radopholus similis	96	96	99	98	97	97
Helicotylenchus spp.	56	52	54	56	60	56
Meloidogyne spp.	59	51	57	48	47	50
Pratylenchus spp.	13	11	13	13	14	13
Total nematodes[z]	100	99	100	99	99	99

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

McSorley and Parrado, 1986). Nematode genera encountered in this study are consistent with those found in Costa Rica by Flores and Salazar (1987), Figueroa (1987), and Araya *et al.* (1995).

Nematodes were present in all the counties and months because of the favorable edaphic and climatic conditions. Low variation in nematode population densities and frequencies may be due in part to the absence of a dry season and low variation in temperature. 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* > *Helicotylenchus* spp. > *Meloidogyne* spp. > *Pratylenchus* spp. The relative importance of *R. similis* as a major pathogen of banana is supported by the observations of Speijer and Fogain (1999), Kashaija *et al.* (1999), and Fargette and Quénéhervé (1988) in African coun-

Table 2. Mean percentage per month (1995-1999) of banana (*Musa* AAA) root samples from which various plant parasitic nematodes were recovered.

Nematode	Months J	F	M	A	M	J	J	A	S	O	N	D
Number of samples	5 142	4 732	4 842	4 948	5 173	4 843	4 976	5 153	5 140	4 793	5 242	5 048
Radopholus similis	96	96	97	97	97	98	97	97	98	97	97	97
Helicotylenchus spp.	55	56	53	55	55	56	57	55	56	58	58	55
Meloidogyne spp.	56	56	50	51	49	50	49	48	51	48	48	49
Pratylenchus spp.	14	13	12	12	12	13	14	12	14	14	13	13
Total nematodes[z]	99	99	99	99	99	100	100	99	100	100	99	99

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

Table 3. Mean percentage per county (1995-1999) of banana (*Musa* AAA) root samples from which various plant parasitic nematodes were recovered.

County	No. samples	Nematodes				Total nematodes[z]
		R. similis	*Helicotylenchus* spp.	*Meloidogyne* spp.	*Pratylenchus* spp.	
Corredores	234	100	100	53	6	100
Guácimo	7 564	97	60	48	12	100
Golfito	355	99	49	60	3	100
Limón	205	100	97	47	39	100
Matina	12 957	97	59	57	13	99
Osa	1 774	98	69	64	29	100
Pococí	13 009	96	35	39	8	99
Sarapiquí	5 054	92	30	46	7	99
Siquirres	13 152	99	69	54	15	100
Talamanca	5 728	98	76	54	22	99
Total	60 032	97	56	50	13	99

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

tries, 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.* (1995) in Costa Rica. Nevertheless, different parasitic habits of the nematode genera present—migratory endoparasitic (*Radopholus* and *Pratylenchus*), sedentary endoparasitic (*Meloidogyne*) and ectoparasitic, (*Helicotylenchus*)—are likely to exacerbate root damage, because lesions can develop at feeding sites throughout the root tissue.

The high population densities and frequencies found for *R. similis* are fostered by the lengthy banana monoculture and agree with results of other local studies (Araya *et al.*, 1995; Jiménez, 1972) and studies from Philippines (Boncato and Davide, 1992), Colombia (Gómez, 1980) and Ecuador (Gómez, 1997). The ubiquity of *R. similis* in Costa Rica banana plantations could also be a consequence of the affinity between this nematode and the commercial banana (*Musa* AAA), its type host (Orton and Siddiqi, 1973). The abundance of *R. similis* in banana roots in this study agrees with the high reproductive fitness found for *R. similis* on banana plants cultivated under controlled conditions (Stoffelen *et al.*, 1999a; Binks and Gowen, 1997; Fallas *et al.*, 1995) and *in vitro* on carrot disc cultures (Stoffelen *et al.*, 1999b; Fallas and Sarah, 1995).

Fewer numbers of *Helicotylenchus* spp. compared to *R. similis* is in agreement with other results (Gómez, 1980; Araya *et al.*, 1995) and may be due to differences in host suitability of banana and differences in life cycles. The low frequency and population density of *Meloidogyne* spp. could be due to the feeding behavior of *R. similis.* Santor and Davide (1992) found that the presence of *R. similis* in root-knot galls caused deterioration and disintegration of the giant cells, which affected the develop-

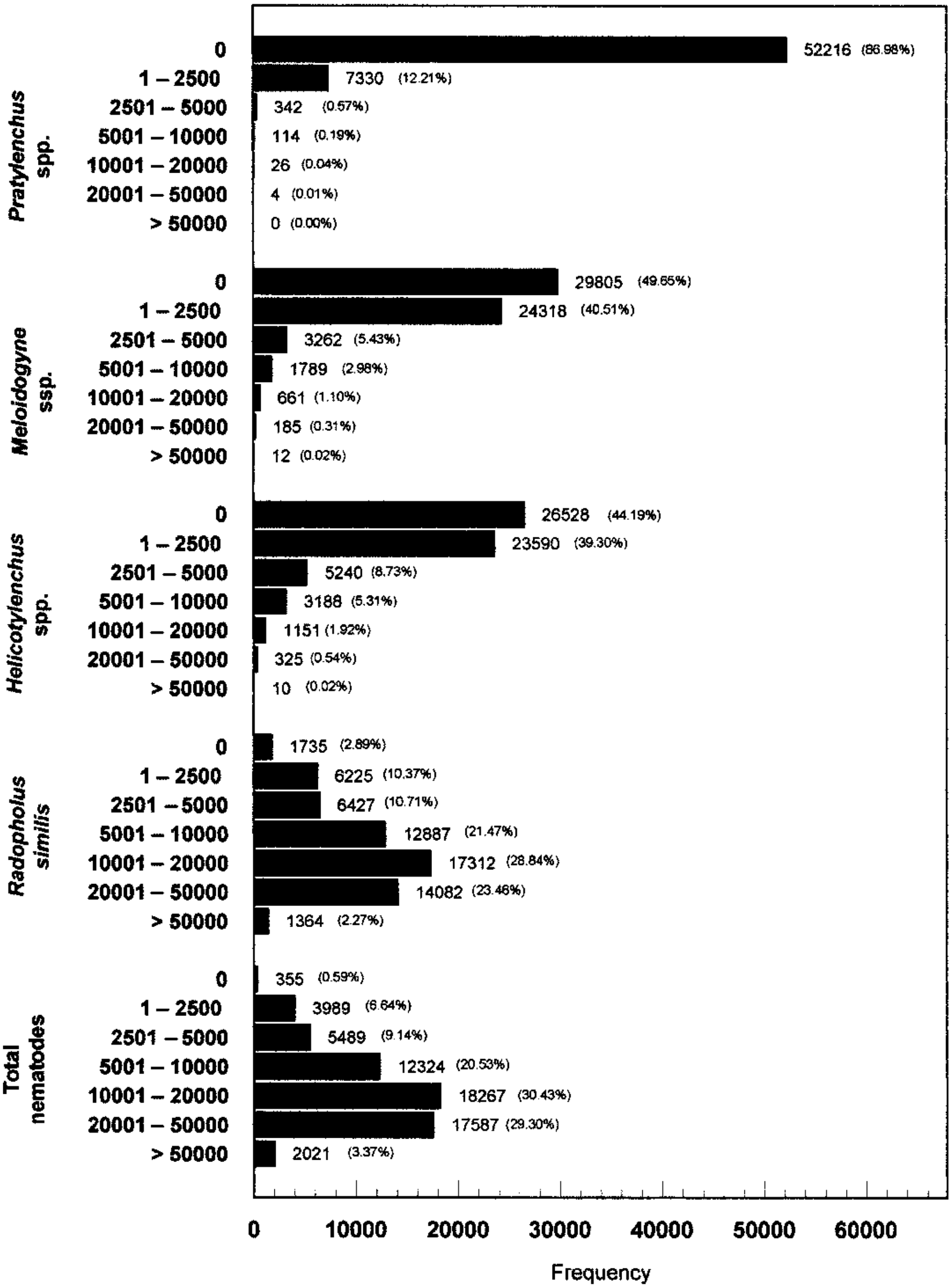

Fig. 2. Distribution of nematode counts from 100 g of fresh roots in 60 032 banana (*Musa* AAA) root samples processed from 1995 to 1999. Total nematodes = *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.

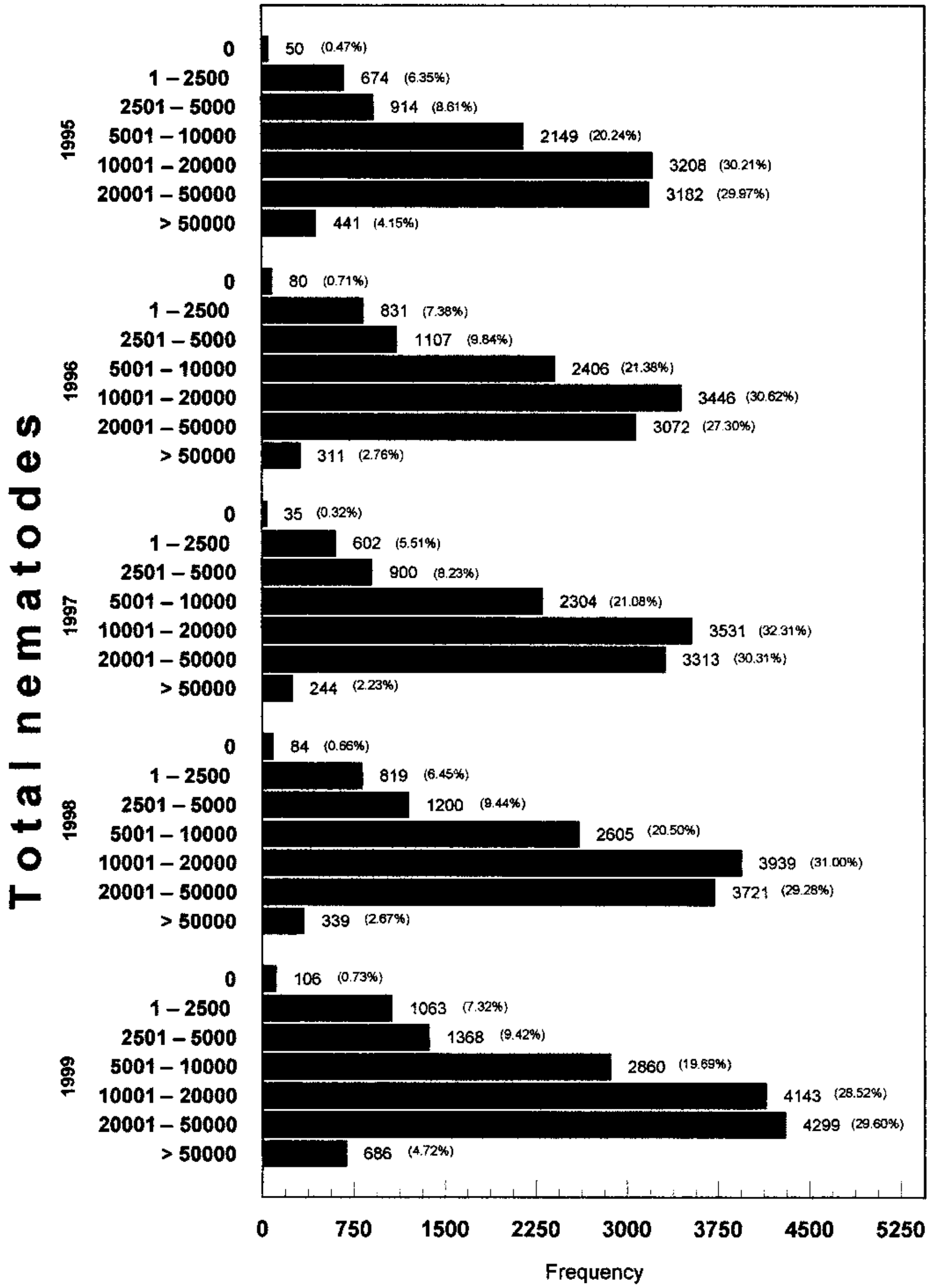

Fig. 3. Distribution of the total nematode (Sum of *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) counts per 100 g of fresh banana (*Musa* AAA) roots during 1995-1999.

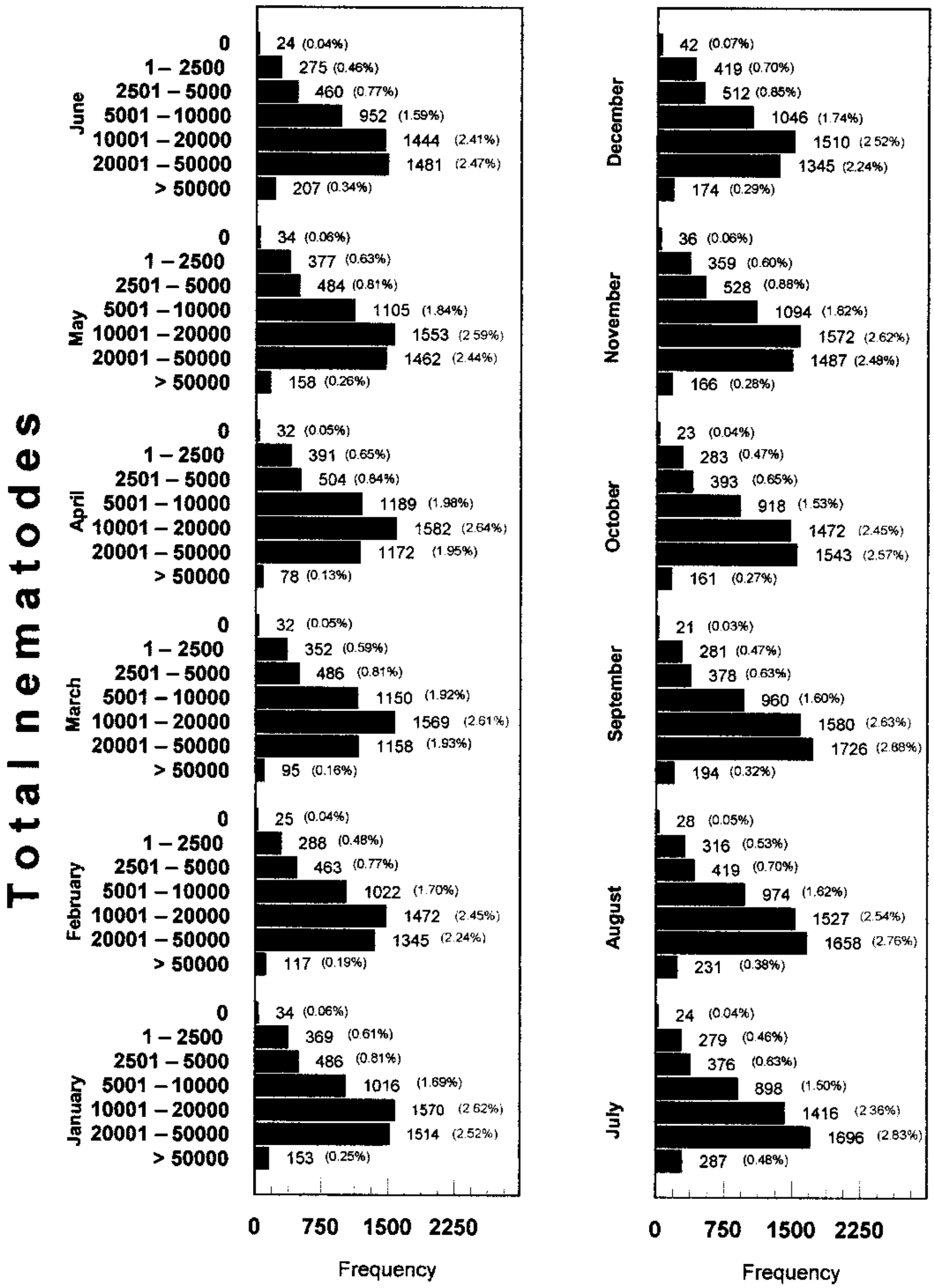

Fig. 4. Distribution of the total nematode (Sum of *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) counts per 100 g of fresh banana (*Musa* AAA) roots as a monthly mean for the years 1995-1999.

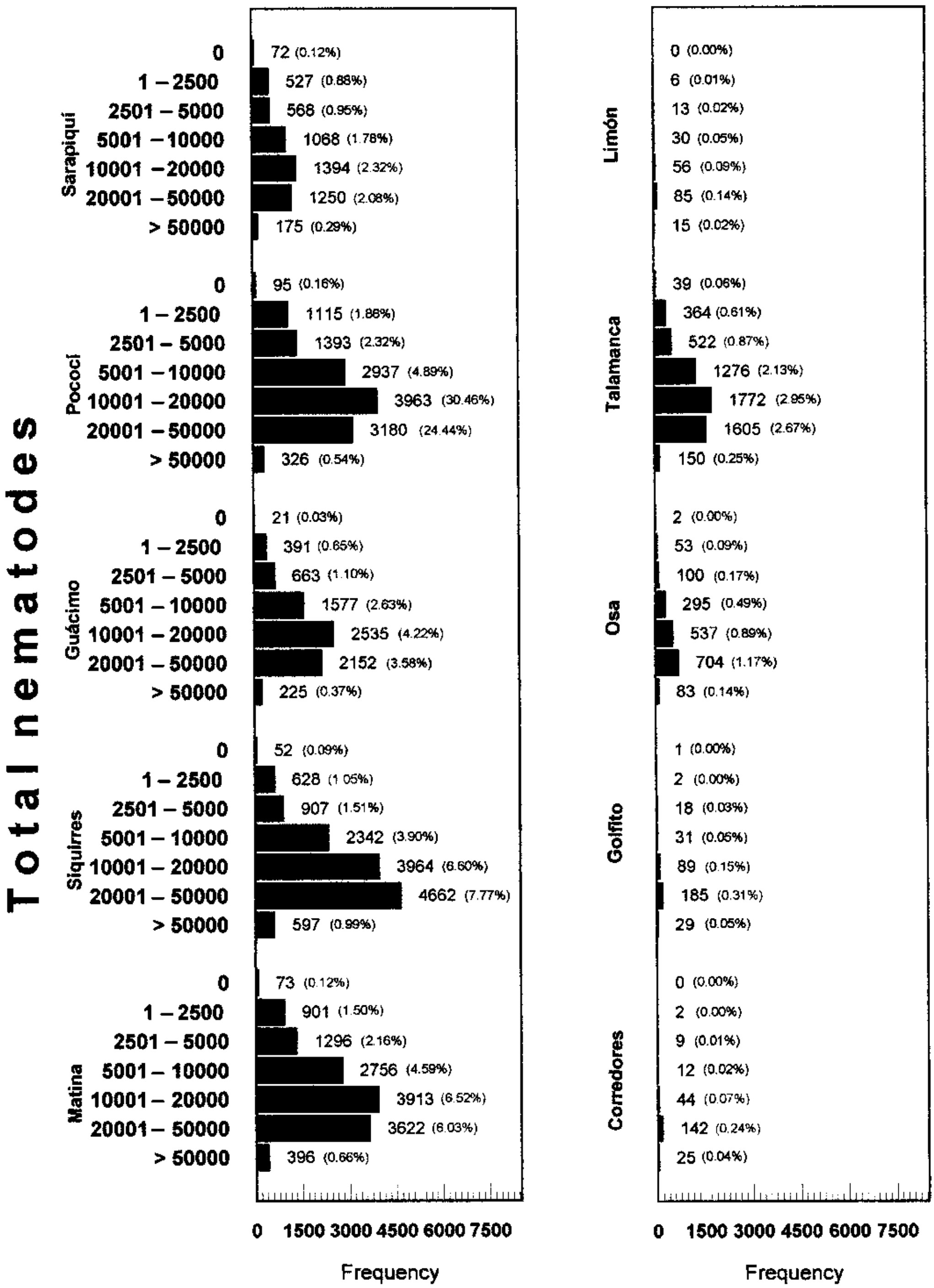

Fig. 5. Distribution of the total nematode (Sum of *R. similis* + *Helicotylenchus* spp. + *Meloidogyne* spp. + *Pratylenchus* spp.) counts per 100 g of fresh banana (*Musa* AAA) root samples as a mean per county during 1995-1999.

ment 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).

In Costa Rica, non fumigant nematicides are recommended when *R. similis* population density exceeds 10 000 nematodes/100 g of fresh roots, an action threshold suggested by Tarte and Pinochet (1981) and Gómez (1980). It has also been shown that *H. multicinctus* and *H. dihystera* can damage the banana root system (Davide, 1996; McSorley and Parrado, 1986; Chau *et al.*, 1997; Mani and Al Hinai, 1996) and reduce yields between 19% (Speijer and Fogain, 1999) and 34% (Reddy, 1994). It is well established that *Meloidogyne* spp. (Patel *et al.*, 1996; Fogain 1994; Santor and Davide, 1992; Davide and Marasigan, 1992) and *Pratylenchus* spp. (Bridge *et al.*, 1997; Rodriguez, 1990; Tarté, 1980; Pinochet, 1978) damage banana roots and reduce yield. Therefore, development of nematode management tactics requires consideration of the damage caused by the total phytonematode population. The results of this study indicate that four genera of major nematode pathogens of banana can be detected at high population densities throughout the year in all of the 10 counties surveyed. The data demonstrate that additional studies are needed to evaluate the pathogenicity of these nematodes individually and in combination to better determine their relative importance in terms of an economic threshold.

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