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FIELD ASSESSMENT OF DIFFERENT SAMPLING STRATEGIES FOR COFFEE PLANTATIONS PARASITIZED BY *MELOIDOGYNE EXIGUA*

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ABSTRACT

Souza, R. M., A. R. Volpato, and A. P. Viana. 2007. Field assessment of different sampling strategies for coffee plantations parasitized by *Meloidogyne exigua*. Nematropica 37:345-355.

To this date, there has been no assessment of strategies for quantitative sampling of *Meloidogyne exigua*. This imposes a major obstacle for realistic estimation of nematode population levels, establishment of damage thresholds, and improvement of the decision process for nematode management in coffee plantations. In this work, a commercial coffee plantation naturally infested by *M. exigua* was sampled for two years for comparison of different sampling core locations and J2- and root galling-related epidemiological variables. The sampling strategy primarily used by nematologists and agricultural workers—sampling near the edge of the coffee canopy to quantify second-stage juveniles in the soil—was revealed as inadequate for assessment of *M. exigua* populations. The best strategy for this nematode is to quantify the number of root galls/5 g of roots obtained from sampling cores located under the canopy, at the depth of 0-25 cm.

Key words: Coffea arabica, coffee root-knot nematode, epidemiology, population assessment, sampling pattern.

RESUMEN

Souza, R. M., A. R. Volpato, y A. P. Viana. 2007. Evaluación de campo de differentes estrategias de muestreo en plantaciones de café con *Meloidogyne exigua*. Nematropica 37:345-355.

Hasta la fecha, no se han evaluado las estrategias para el muestreo cuantitativo de *Meloidogyne exigua*. Esto constituye un obstáculo para la estimación real de las densidades de población, el establecimiento de umbrales de daño, y el mejoramiento de los procesos de decisión para el manejo de nematodos en plantaciones de café. En este estudio, se muestreó una plantación comercial de café naturalmente infestada con *M. exigua* durante dos años y se compararon diferentes sitios de muestreo y variables epidemiológicas de juveniles y agallas. Se demostró que la estrategia de muestreo más usada por nematólogos y agricultores—el muestreo cerca al borde del dosel para cuantificar los juveniles de segundo estadio en el suelo—es inadecuada para evaluar las poblaciones de *M. exigua*. La mejor estrategia de muestreo es determinar el número de agallas/5 g de raíces obtenidas de muestras localizadas bajo el dosel, a una profundidad de 0-25 cm.

Palabras clave: Coffea arabica, café, nematodo agallador, epidemiología, cuantificación de poblaciones, patrones de muestreo.

INTRODUCTION

Meloidogyne exigua Goeldi, 1887 is the most important root-knot nematode affecting arabica coffee (*Coffea arabica* L.) in the Americas. Besides being the most widespread species in the continent (Campos and Villain, 2005), it may cause large yield losses depending on the cultivar or variety involved, the edaphic and climatic conditions and cul-

tural practices (Arruda and Reis, 1962; Gonçalves, 1997; Barbosa *et al.*, 2004).

To this day, all studies dealing with yield losses due to, and epidemiology of *M. exigua* have monitored the nematode population by quantifying second-stage juveniles (J_2) in the soil or roots, females or eggs in the roots, or root galls (Huang *et al.*, 1984; Almeida *et al.*, 1987; Maximiano *et al.*, 2001; Barbosa *et al.*, 2004). In most of these studies, the sampling cores were randomly located around the coffee trees, and were collected from the soil zone of 0-20 cm. These sampling strategies have never been assessed for precision or accuracy, although J2 are reported to be more abundant at 20-40 cm deep and at 40 cm from the trunk in non-irrigated plantations (Almeida *et al.*, 1987). In contrast, Zhang and Schmitt (1995) found *M. konaensis* to be highly concentrated along the irrigation dripline and at a depth of 16-45 cm.

As stated by McSorley (1987) and several other authors, reliable methods for sampling and extraction of nematodes are essential for proper assessment of nematode densities in the field, which in turn, should be related to yield losses and environmental factors to generate damage thresholds for optimum control of plant-parasitic nematodes. In a first effort to develop an accurate strategy for quantitative sampling of *M. exigua*, this study compared different sampling core locations around the plant, and different J2- and root galling-related epidemiological variables in a naturally infested coffee plantation.

MATERIALS AND METHODS

This study was established in a 1.5 ha commercial, six year-old arabica coffee plantation (cultivar 'Catuaí') in the municipality of Varre-Sai, State of Rio de Janeiro, Brazil. The plantation contained about 9,000 plants spaced 1 m between plants and 1.8 m between rows, with no irrigation. The area presented a red-yellow latosol (oxisol in the USDA system), with declivity of 30% and infestation of no other *Meloidogyne* species besides *M. exigua*.

The experimental plot was composed of five contiguous planting rows of 34 plants each, totaling 170 plants. Individual coffee trees were randomly selected and tagged so that 10 plants (two per planting row, in random positions) were sampled every three weeks from October/2002 through October/2004 (32 sampling dates). During this period of time, every plant was resampled once, 12 months after being first sampled.

The sampling was performed with an 8 cm-diam, 16 cm-length soil bucket auger (approximately 800 cm^3 of soil). Horizontally, the sampling cores were located at 20 and 40 cm from the trunk (under the canopy) and at approximately 80 cm from the trunk (at the edge of the canopy, the location usually chosen for routine samplings). Vertically, the cores were located at the depths of 0-25 and 25-50 cm. Hereafter, these locations will be referred to as locations 1 (20 cm from the trunk/0-25 cm soil profile zone), 2 (20/25-50), 3 (40/0-25), 4 (40/25-50), and 5 (80/0-25). No samples were collected at the 80/25-50 cm position because few roots occur at this location.

At every sampling date, newly-grown feeder roots present in each of the 50 samples collected were removed from soil and weighed; old, thick, brownish root segments that were not infected by *M. exigua* J2 were not considered. The *M. exigua*-induced galls were counted, and expressed as number of root galls/5 g of roots, and as number of root galls/sampling (800 cm^3 of soil).

For each sample, the roots were then mixed for 10 seconds in a commercial blender, poured onto a 500 mesh screen and washed in tap water. The resulting suspension was kept at 4°C until it was thoroughly examined with the aid of a

dissecting microscope. The J2 population was expressed as number of J2/5 g of roots.

Finally, for every sample the soil was thoroughly and gently mixed and J2 extracted by flotation-centrifugation from a subsample of 100 ml of soil (Jenkins, 1964). The resulting suspension was kept at 4°C until it was thoroughly examined with the aid of a dissecting microscope. Juveniles were counted and expressed as number of J2/100 ml of soil. All these sampling, processing, and counting procedures were carried out by the same operator.

Therefore, four epidemiological variables (number of J2/100 ml of soil, number of J2 and root galls/5 g of roots, and number of root galls/sampling) were assessed in locations 1 through 4. These combinations were compared to the routine sampling method (number of J2/100 ml of soil in location 5).

The soil temperature was monitored with Watchdog® (Spectrum Technologies, Inc.) sensors placed at depths of 12.5 and 38.5 cm. For each sample, the soil moisture content was gravimetrically calculated after drying at 105°C for 72 h. The ambient temperature and rainfall were monitored with a WatchDog® weather station located 15 km from the experimental site.

In accordance with the type of data collected, the statistical methods employed in this study involved regression analysis, descriptive comparison of variance-to-mean ratios, ANOVA or Tukey tests. No logarithmic transformation of the data was used in this work.

RESULTS AND DISCUSSION

As conceived, this study allows for comparisons of the epidemiological variables and sampling locations assessed over time, in relation to the host signal and the environmental variables monitored. It also allows for non-temporal, statistical comparisons based on variance-to-mean ratios and frequency distributions.

Host Signal and Environmental Variables Monitored

The root system of coffee plants in Brazil grows primarily during the rainy season (October through March) (Barros and Maestri, 1972, 1974). These new roots are believed to stimulate J2 hatching and root infections (Huang *et al.*, 1984).

Root growth occurred throughout the seasons [Fig. 1; polynomial equations and R^2 are shown in Table 1; because most of the epidemiological variables assessed presented a strong seasonal variation (see below), polynomial equations of lower than 6^{th}-order presented lower R^2 values]. Mild winter conditions under the canopy allowed a suitable environment for root growth. A Tukey analysis at $P < 0.05$ revealed no differences among average root weights collected in locations 1, 2, and 3 throughout the sampling period (averages of 2.3, 1.62, and 1.55 g, respectively). The average weight at location 4 (1.04 g) was statistically different. In summary, no changes in the availability of roots that could act as a host signal for *M. exigua* were detected.

The ambient temperature, rainfall, and the soil moisture content (at two depths) followed a seasonal fluctuation during the two year-sampling period (Fig. 2), which correlated well with *M. exigua* epidemiological variables (see below). For the first sixty weeks of the experiment, the ambient and soil temperatures fluctuated simultaneously. An equipment malfunction thereafter halted the collection of soil temperature data.

Analysis of Epidemiological Variables and Sampling Locations as Related to Environmental Variables Over Time

The number of J2/100 ml of soil had a clear seasonal fluctuation in all locations

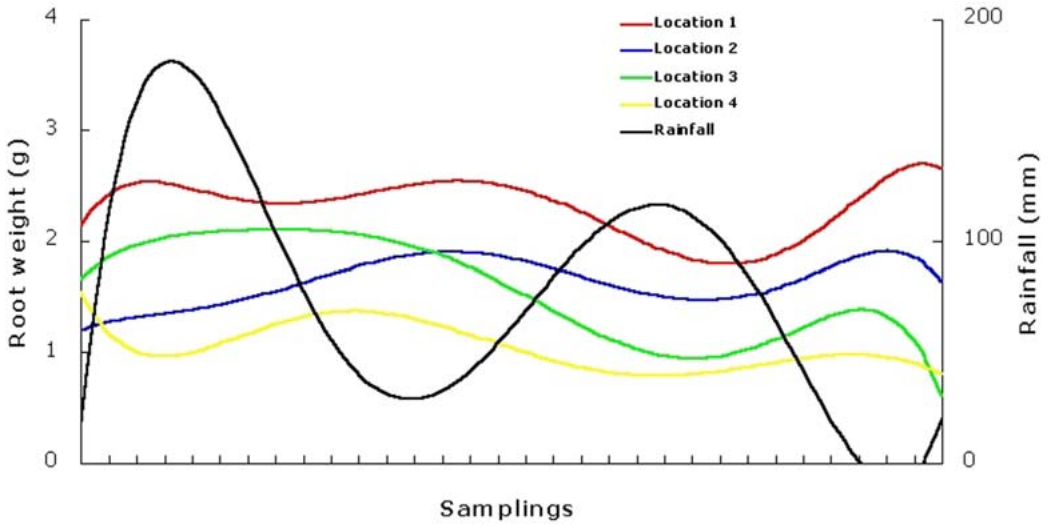

Fig. 1. Amount of coffee roots collected/auger sampling, and rainfall along 32 samplings performed every three weeks in a coffee plantation infested by *Meloidogyne exigua*. Root weights are averages of 10 replicates (plants) for each sampling core location. Rainfall amounts are cumulative for each three-week period.

(Fig. 3; for location 5, the actual data was added to the fitted curve). In comparison to regression analysis involving the whole sampling period (2002-2004) (Table 1), analysis for the periods 2002-2003 and 2003-2004 produced equations (not shown) with higher R^2 values. For number of J2/100 ml of soil, R^2 ranged from 0.37 to 0.9 for all locations.

The number of J2/5 g of roots had a clear seasonal fluctuation in locations 1 through 4 (Fig. 4). Regression analysis of this variable for the years 2002-2003 and 2003-2004 revealed R^2 values ranging from 0.41 to 0.91 for all locations. The number of galls/5 g of roots fluctuated during the seasons in locations 2 and 4 (Fig. 5). Regression analysis of this variable for the years 2002-2003 and 2003-2004 revealed R^2 values ranging from 0.37 to 0.79 for all locations. The number of root galls/sampling exhibited a seasonal fluctuation in all locations (Fig. 6). Regression analysis of this variable for the years 2002-2003 and 2003-2004 revealed R^2 values ranging from 0.35 to 0.83 for all locations. In summary, none of the epidemiological variables and sampling locations assessed stood as significantly more accurate for monitoring *M. exigua* populations when the data were analyzed in relation to environmental variables during the entire sampling period.

Non-temporal Statistical Analysis of Epidemiological Variables and Sampling Locations

As performed by Goodell and Ferris (1981), Davis (1984), Schmitt *et al.* (1990) and others for several nematode species, the mean and the variance of nematode counts were calculated for each epidemiological variable and each sampling location assessed, on four randomly chosen sampling dates (December 19, 2002; March 10, 2003; June 6, 2003; and January 28, 2004). Of the 68 combinations above, 50 had their variance values lower than the mean. When the variance was higher than the mean, the variable K reached high values, indicating a near random distribution. According to

Table 1. Descriptive statistical data for the fitted curves shown in Figures 1, and 3 through 6.

Locations (cm)	Polynomial equations[z]	R^2
	Root weight (Fig. 1)	
20/0-25	$y = -4E-07x^6 + 4E-05x^5 - 0.0015x^4 + 0.0252x^3 - 0.2096x^2 + 0.7571x + 1.569$	0.3099
20/25-50	$y = -2E-07x^6 + 2E-05x^5 - 0.0006x^4 + 0.0088x^3 - 0.0558x^2 + 0.1882x + 1.0637$	0.3099
40/0-25	$y = -3E-07x^6 + 2E-05x^5 - 0.0007x^4 + 0.0115x^3 - 0.0937x^2 + 0.408x + 1.3361$	0.6261
40/25-50	$y = 1E-07x^6 - 2E-05x^5 + 0.0008x^4 - 0.017x^3 + 0.1806x^2 - 0.7977x + 2.1738$	0.3656
	Number of J2/100 ml soil (Fig. 3)	
20/0-25	$y = -5E-05x^6 + 0.004x^5 - 0.1274x^4 + 1.7903x^3 - 10.156x^2 + 13.678x + 76.205$	0.3038
20/25-50	$y = -2E-05x^6 + 0.0015x^5 - 0.029x^4 - 0.1324x^3 + 8.8004x^2 - 69.729x + 190.98$	0.2361
40/0-25	$y = -7E-05x^6 + 0.0066x^5 - 0.2107x^4 + 2.9454x^3 - 16.115x^2 + 16.493x + 81.875$	0.2438
40/25-50	$y = -3E-05x^6 + 0.0023x^5 - 0.0646x^4 + 0.6194x^3 + 0.6692x^2 - 30.396x + 109.62$	0.3630
80/0-25	$y = -1E-05x^6 + 0.0011x^5 - 0.0287x^4 + 0.1987x^3 + 2.0934x^2 - 28.578x + 97.585$	0.4812
	Number of J2/5 g of roots (Fig. 4)	
20/0-25	$y = -2E-05x^6 + 0.0003x^5 + 0.0746x^4 - 3.2842x^3 + 47.99x^2 - 240.27x + 450.7$	0.3557
20/25-50	$y = -1E-06x^6 - 0.0021x^5 + 0.1928x^4 - 6.2161x^3 + 85.459x^2 - 463.04x + 865.27$	0.5080
40/0-25	$y = -7E-05x^6 + 0.0043x^5 - 0.0314x^4 - 2.7665x^3 + 63.631x^2 - 423.05x + 870.75$	0.3572
40/25-50	$y = -2E-05x^6 + 0.0004x^5 + 0.0812x^4 - 3.7436x^3 + 56.754x^2 - 303.73x + 562.82$	0.4546
	Number of galls/5 g of roots (Fig. 5)	
20/0-25	$y = 2E-05x^6 - 0.0026x^5 + 0.1071x^4 - 2.0871x^3 + 19.445x^2 - 71.58x + 208.76$	0.3631
20/25-50	$y = -5E-06x^6 - 0.0005x^5 + 0.0662x^4 - 2.1899x^3 + 29.427x^2 - 154.17x + 399.36$	0.7009
40/0-25	$y = 2E-05x^6 - 0.0023x^5 + 0.1054x^4 - 2.3338x^3 + 25.7x^2 - 124.1x + 343.69$	0.2451
40/25-50	$y = -1E-05x^6 + 0.0008x^5 - 0.0166x^4 + 0.0838x^3 + 0.3724x^2 + 2.1922x + 135.53$	0.5412
	Number of root galls/sampling (Fig. 6)	
20/0-25	$y = 3E-06x^6 - 0.0004x^5 + 0.0185x^4 - 0.4369x^3 + 4.5031x^2 - 13.835x + 67.275$	0.5204
20/25-50	$y = -1E-05x^6 + 0.0006x^5 + 0.0004x^4 - 0.4984x^3 + 9.1525x^2 - 50.399x + 115.78$	0.6623
40/0-25	$y = -2E-05x^6 + 0.0012x^5 - 0.0308x^4 + 0.2608x^3 + 0.4265x^2 - 9.2051x + 76.189$	0.2320
40/25-50	$y = 4E-06x^6 - 0.0006x^5 + 0.0297x^4 - 0.7124x^3 + 7.7123x^2 - 32.203x + 69.32$	0.3037

[z]In all equations, "x" is sampling time and "y" has different values for each figure and sampling location.

Barker (1985) and McSorley (1987), this indicates that *M. exigua* presents a random to regular horizontal and vertical distribution in the soil of mature coffee plantations. Probably, the traffic of workers in the plantations performing cultural practices helps to disseminate the nematode in the field. Also, as a perennial crop densely cultivated with minimal soil disturbance, coffee fields may offer conditions for *M. exigua* populations to reach a relatively uniform distribution in the soil.

According to Noe (1985) and McSorley (1987), population counts of soil nematodes usually do not present normal frequency distributions. In these cases, nematode counts are highly skewed, and typically have large variance-to-mean

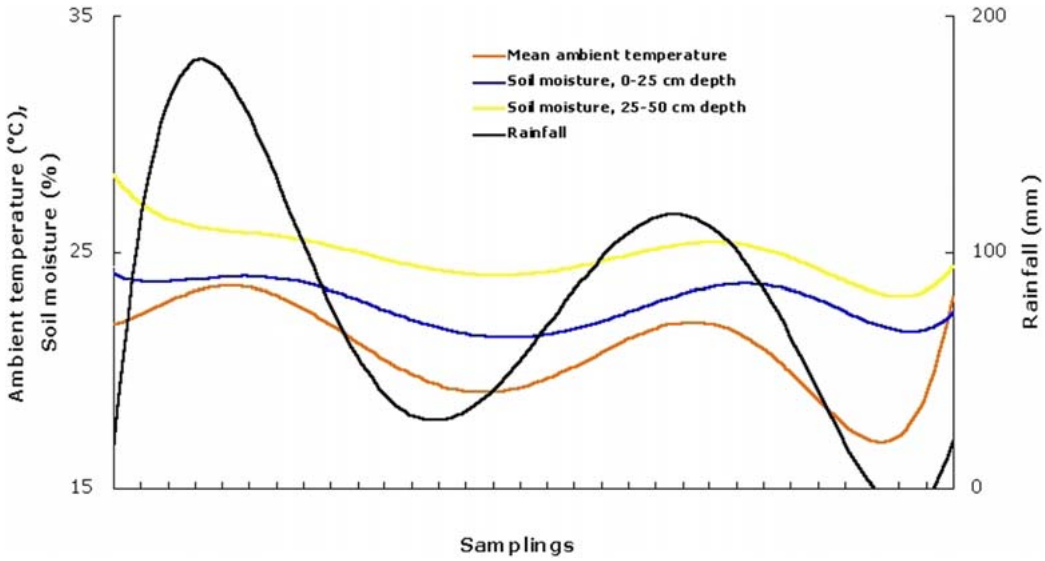

Fig. 2. Environmental variables during a period of 32 samplings performed every three weeks in a coffee plantation infested by *Meloidogyne exigua*. Rainfall amounts are cumulative for each three-week period. Mean ambient temperatures are averages of daily records for each three-week period.

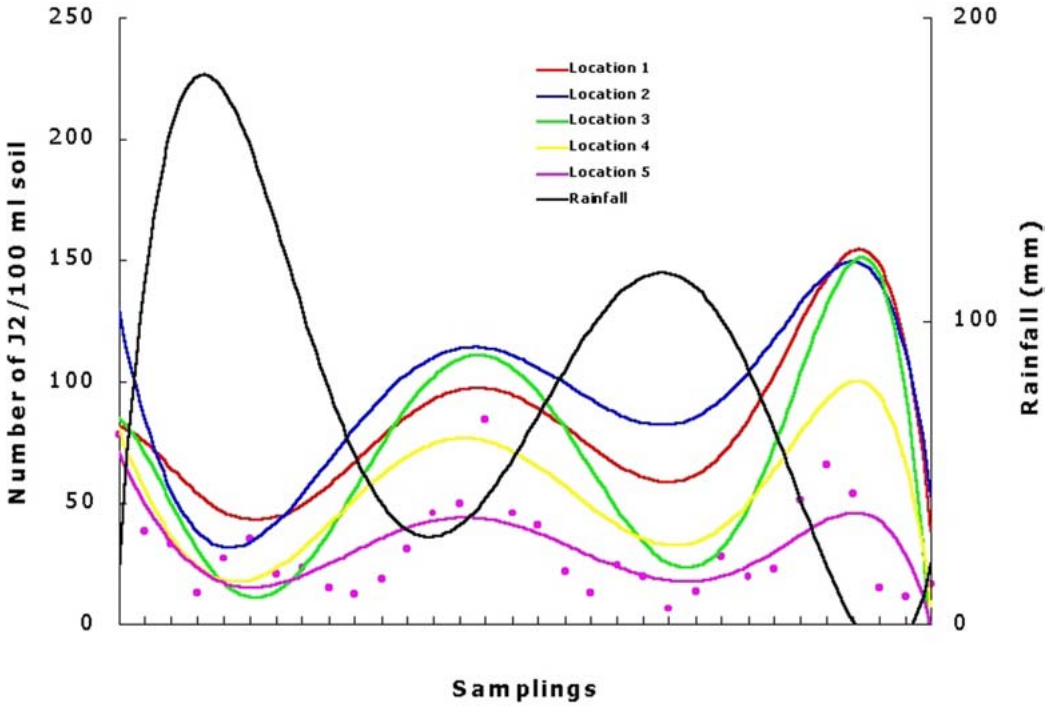

Fig. 3. Density of second-stage juveniles (J2) of *Meloidogyne exigua*, and rainfall during 32 samplings performed every three weeks in a coffee plantation. Nematode counts are averages of 10 replicates (plants) for each sampling core location. Rainfall amounts are cumulative for each three-week period. Original counts are plotted for location 5.

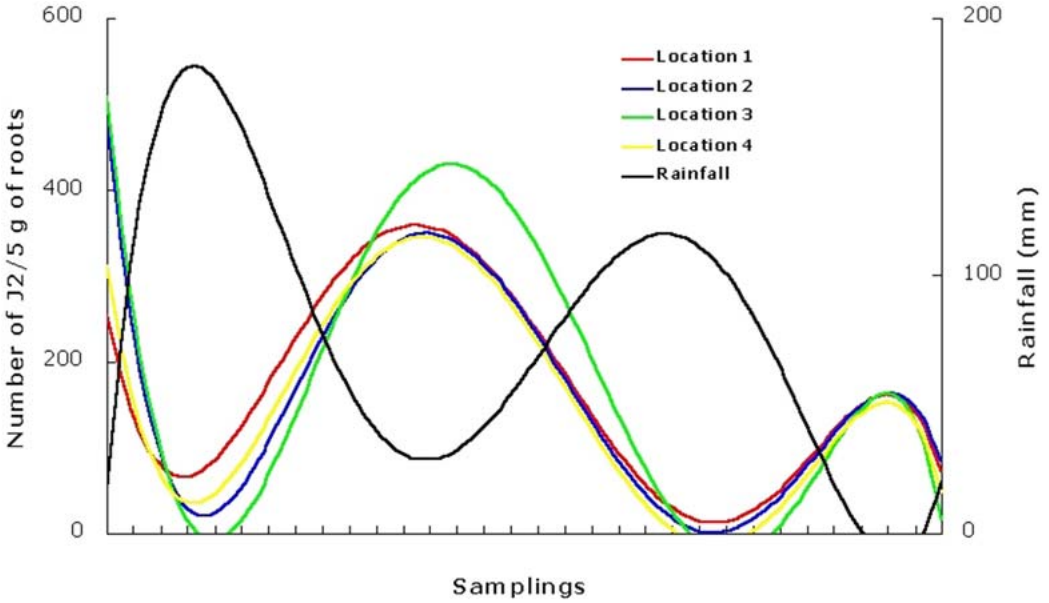

Fig. 4. Density of second-stage juveniles (J2) of *Meloidogyne exigua*, and rainfall during 32 samplings performed every three weeks in a coffee plantation. Nematode counts are averages of 10 replicates (plants) for each sampling core location. Rainfall amounts are cumulative for each three-week period.

ratios. For any population variable or sampling location, an asymmetric distribution complicates the assessment of the nematode population for advisory purposes. For statistical analysis, the data requires prior logarithmic transformation.

In the present work, the four epidemiological variables assessed presented original (not transformed) counts with sharply different frequency distributions. Indeed, on all sampling dates cited above, the number of J2/100 g soil and number of J2/5 g roots presented counts highly skewed across the classes of the histogram. On the other hand, the number of galls/5 g of roots consistently presented a typical normal distribution, while the number of root galls/sampling was intermediate. Figure 7 represents the distributions observed on the sampling date of June 6th, 2003. Because of the normal distribution curve, the number of galls/5 g of roots consistently presented the lowest variance-to-mean ratio among the variables assessed (Table 2).

Also, because of its typical normal distribution, the number of galls/5 g of roots was the only variable to express significant differences across the different sampling core locations assessed (Table 3). The number of root galls/sampling also expressed some differences.

According to Southwood (1978) (cited by McSorley, 1987), a reasonable sampling precision for advisory purposes would only be achieved by sampling strategies that give a variance-to-mean ratio no higher than 25%. Nonetheless, this strict threshold could not be met by McSorley and Parrado (1982) while assessing sampling plans for certain nematode species in fruit groves. Also, Schmitt *et al.* (1990) accepted a general variance-to-mean ratio around 60%, obtained by 40 sampling cores/ha in five fields of annual crops.

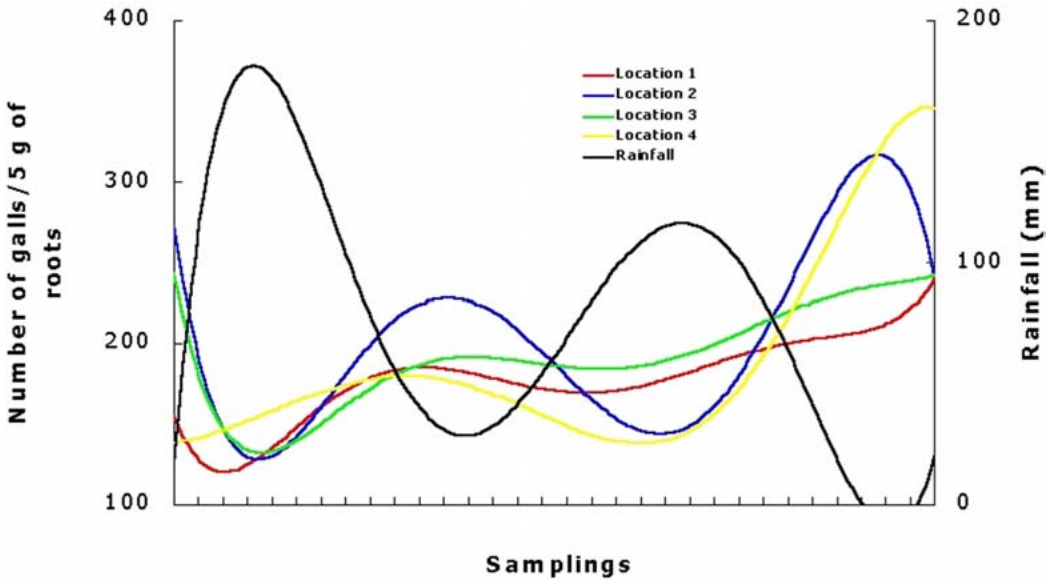

Fig. 5. Density of *Meloidogyne exigua*-induced root galls, and rainfall during 32 samplings performed every three weeks in a coffee plantation. Root gall counts are averages of 10 replicates (plants) for each sampling core location. Rainfall amounts are cumulative for each three-week period.

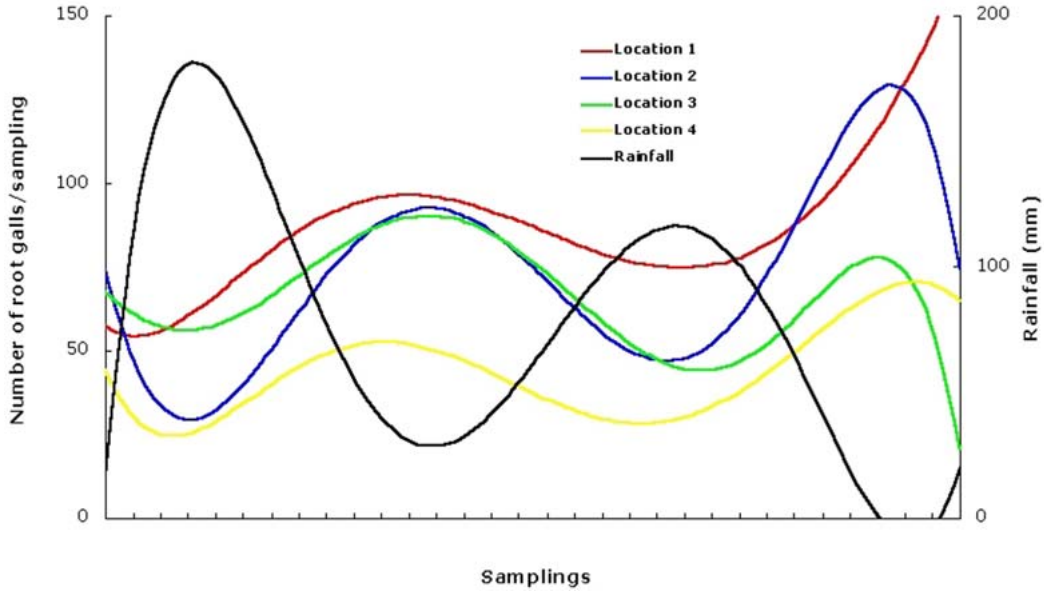

Fig. 6. Density of *Meloidogyne exigua*-induced root galls, and rainfall during 32 samplings performed every three weeks in a coffee plantation. Root gall counts are averages of 10 replicates (plants) for each sampling core location. Rainfall amounts are cumulative for each three-week period.

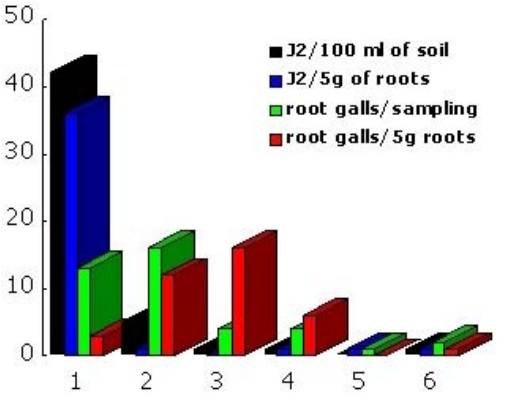

Fig. 7. Frequency distribution of original, not transformed count data of four epidemiological variables of *Meloidogyne exigua*, obtained from samplings performed on June 6, 2003. For each variable, the frequency considers all replicates (samplings cores) collected on that date. For each variable, the range of the original counts was divided in six classes.

Although the number of galls/5 g of roots presented a general variance-to-mean ratio of 45%, it seems accurate enough (and superior to the other variables assessed) to assess *M. exigua* populations, especially if one considers the random to regular distribution of *M. exigua* in the soil, and the difficulties involved in sampling compact coffee plantations in up-land fields. As this work adopted a sampling density equivalent to over 400 cores/ha, it seems difficult to imagine any sampling strategy with higher precision that would be effectively adopted by coffee growers and agricultural professionals.

This work could not detect any major differences among the four sampling locations assessed for the number of galls/5 g of roots. Indeed, the variance-to-mean ratio of counts (not transformed) in locations 1 through 4, averaged for the four sampling dates, were 44, 38, 50, and 48%, respectively. Therefore, as far as sampling core location, this work advises sampling be performed at any fixed location under the plant canopy (not at its edge). For simplicity, sampling depth should be fixed at 0-25 cm.

This is the first effort to assess different strategies for quantitative sampling of any *Meloidogyne* species in coffee plantations. The variables assessed in this work seemed the most relevant for *M. exigua*'s epidemiology. While studying the epidemiology of *M. exigua*, Huang *et al.* (1984) assessed the number of eggs/g of root, obtaining the lowest values during the rainy season. This result is counter intuitive, since these authors also found more parasitic females/g of root during that season. The number of eggs/g of root was not assessed in this

Table 2. Comparison of four epidemiological variables of *Meloidogyne exigua* based on variance-to-mean ratio of the nematode counts obtained in four sampling dates in a naturally infested plantation in Brazil.

	Variance-to-mean ratio[z]				
Variable	December 19th 2002	March 10th 2003	June 6th 2003	January 28th 2004	Average
Number of J2/100 ml of soil	68	91	97	116	93
Number of J2/5 g of roots	79	115	100	86	95
Number of galls/5 g of roots	32	50	43	54	45
Number of root galls/sampling	45	78	74	96	73

[z]Values calculated considering the original, not transformed counts from all 40 or 50 locations sampled for each variable.

Table 3. Comparison among average nematode counts of four epidemiological variables of *Meloidogyne exigua* in five sampling locations assessed in a naturally infested plantation in Brazil.

	Sampling locations				
Variable	1	2	3	4	5
Number of J2/100 ml of soil	81 a[z]	92 a	66 a	54 a	32 a
Number of J2/5 g of roots	178 a	203 a	192 a	189 a	not assessed
Number of galls/5 g of roots	166 b	161c	175 a	149 d	not assessed
Number of root galls/sampling	88 a	73 ab	66 b	43 c	not assessed

[z]Values are average of all original (not transformed) counts obtained during the two year-period of the study. Values followed by same letters in each line are not statistically different according to Tukey's test at $P < 0.05$.

work because *Meloidogyne* sp. is known to produce a high percentage of non-viable or dormant eggs, which might confound the correlation between this variable and yield loss. Also, the number of females/g of root was not assessed in this work because most routine laboratories are not equipped for enzymatic-digestion of coffee roots, and extracting females from roots using a commercial blender destroys many females.

In the future, the international coffee market is expected to demand from the growers an increasing level of rationalization of all farm practices, including the management of nematodes. Therefore, nematologists and agricultural workers involved with coffee should strive to gain insights into the damage thresholds for *Meloidogyne* spp. as well as for other important pathogenic nematodes of coffee. This information will enable growers to decide upon nematode management strategies with the best benefit-to-cost ratios.

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