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Relationships between agro-ecological factors and population densities of *Meloidogyne exigua* and *Pratylenchus coffeae sensu lato* in coffee roots, in Costa Rica

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ABSTRACT

Controlling coffee nematodes using fewer pesticides is a priority for most producing countries. The aim of this study was to identify ecological and agricultural factors associated with Meloidogyne exigua and Pratylenchus coffeae sensu lato population densities in coffee roots. The influence of these factors was studied in a two-year survey by characterizing 57 coffee plots in five major producing regions in Costa Rica, where coffee is generally cultivated in intensified systems. Correspondence analyses and Chisquared automatic interaction detector (CHAID) classification trees were used to identify and classify the ecological and agricultural factors associated with nematode population densities. M. exigua and P. coffeae population densities were opposites with respect to soil characteristics. Low M. exigua population densities were especially found in soils with low sand and high Zn and K contents. The opposite was seen for P. coffeae. In addition, M. exigua and P. coffeae seemed to have different climatic requirements: M. exigua was mainly found at lower altitudes than P. coffeae. Consequently and through possible competition phenomena between P. coffeae and M. exigua, a negative correlation was found between the two nematode population densities on a plot scale. We found only one cropping practice that seemed to affect both nematodes similarly: a within-row distance between coffee trees of less than 0.9 m was associated with large M. exigua and P. coffeae population densities. The results of this study provide further evidence of the greater intensity of pest and disease attacks in intensified coffee systems.

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1. Introduction

Parasitic nematodes on coffee tree roots are a major limiting factor, especially for *Coffea arabica*, the only coffee species cultivated in Costa Rica. There have been numerous reports of nematode attacks on coffee in that country (Salas and Echandi, 1961; López and Salazar, 1989; Flores and López, 1989; Bertrand et al., 1998, 2002; Hernández et al., 2004a,b; Hervé et al., 2005; Alpízar et al., 2007; Villain et al., 2008). The major parasitic nematodes of coffee are root-lesion nematodes of the genus *Pratylenchus* and root-knot nematodes of the genus *Meloidogyne*. They cause serious damage throughout the world, and especially in Latin America (Campos et al., 1990; Villain et al., 2008; Souza and Bressan-Smith, 2008; Villain, 2008; Villain et al., 2008), where they are often found together (Bertrand et al., 1998; Hervé et al., 2005). In Costa Rica, the main two species on *C. arabica* are *M. exigua* and *P. coffeae* (Flores

and López, 1989; Hernández et al., 2004a; Hervé et al., 2005; Villain et al., 2008). However, knowledge on the abundance of these species under different ago-ecological conditions remains limited.

Genetic resistance to nematodes has been identified in C. canephora trees. Hypocotyledonary grafting of C. arabica onto C. canephora enables some control of both M. exigua and P. coffeae (Villain et al., 2000; Bertrand et al., 2000, 2002; Villain, 2008; Villain et al., 2008). However, this technique, which was developed and is widely used in Guatemala is not so common elsewhere, where the practice is less well known. Moreover, coffee is a perennial crop and the substitution of susceptible trees with resistant ones means substantial investment. Consequently, when necessary, Costa Rican coffee growers have generally tried to manage nematodes by other methods, primarily nematicides, despite their poor efficiency and their harmful side-effects (Villain et al., 2000, 2008; Villain, 2008). Nevertheless, over the past decade, low coffee prices and the emergence of certification for environmentally friendly practices have led to reduced reliance on pesticides in Costa Rica.

This study aimed to identify factors conducive to the development of the two main coffee nematode pests, *M. exigua* and *P.*

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coffeae sensu lato. The study focused on environmental factors, particularly soil, and on cropping systems which could be modified by growers depending on parasite risks. Data from 57 coffee plots that were being characterized for a survey on American leaf spot disease in 2002 and 2003, were additionally used for the current study (Avelino et al., 2007).

2. Materials and methods

The study area, the plot sampling methods and the plot descriptors have already been described in Avelino et al. (2007). In this article, we particularly describe nematode sampling, population density assessments, and the processing of data specific to the study.

2.1. Description of the study area and cropping systems

The study took place in five major coffee producing regions of Costa Rica: Western Valley, Central Valley, Coto Brus, Tarrazú, and Turrialba (Fig. 1), which differed in altitude. Tarrazú is the highest region, with a large proportion of coffee plots located close to the upper limit of coffee cultivation in Costa Rica (around 1800 m), while Turrialba and Coto Brus are regarded as low to medium altitude (mainly between 700 and 1100 m), and Western Valley and Central Valley as medium to high altitude regions (mainly between 1000 and 1500 m). The regions also differ with respect to their rainfall volumes and distributions, due to various oceanic influences (strong Pacific influence in the West with a very marked dry season, strong Caribbean influence in the East with a short dry season) (Coen, 1983). The soils are also varied in terms of chemical composition and texture, which mainly depend on geological origin, climate and cropping systems (Bornemisza et al., 1999).

Coffee growing in Costa Rica has produced some of the highest yields in the world (De Graaff, 1986; Fernández and Muschler, 1999). The use of dwarf cultivars, primarily cv. Caturra and cv. Catuai, and of high planting densities is widespread (Fernández and Muschler, 1999). By replacing tall with dwarf cultivars in the 1960s, it became possible to apply intensified cropping practices, which now typify Costa Rican coffee growing, such as reducing or removing shade trees, planting two or three coffee trees per



Fig. 1. Location of Costa Rican coffee plots sampled (number per region) during the survey of the nematodes, *Meloidogyne exigua* and *Pratylenchus coffeae sensu lato*, in 2002 and 2003.

planting hole, intensive use of inputs, especially fertilizers and herbicides, and the introduction of elaborate pruning systems. However, cropping systems have always been quite varied, as the techniques have often been only partially applied by growers depending on their knowledge and investment capacity. With the global slump in coffee prices at the end of the 1990s, there was some return to moderate shading, usually providing suitable conditions for quality coffee production and prolonging the life span of the coffee trees (Fernández and Muschler, 1999).

Given the diversity of environments and cropping systems, there is considerable diversity in coffee production situations (De Wit, 1982) in Costa Rica.

2.2. Plot sampling

Our study was based on the characterization of 91 samples, taken from 48 plots located in 4 producing regions (Western Valley, Central Valley, Coto Brus, Tarrazú) in 2002, and from 43 plots in 2003: 34 already observed in 2002 and nine new plots, with three from a 5th producing region—Turrialba (Fig. 1) (Avelino et al., 2007). Sampling was carried out in such a way as to cover a wide range of production situations (De Wit, 1982). Each plot comprised 10 rows of 10 coffee trees.

2.3. Plot descriptors

The plot descriptors assessed during the survey involved environmental factors (Table 1), crop management (Table 2), and certain tree characteristics (Table 3) (Avelino et al., 2007).

The environmental variables were soil, rainfall, and topography (Table 1). The soil analyses performed by Avelino et al. (2007) for the American leaf spot disease study were supplemented for the nematode study with Al, P, Zn, Cu, Mn, and Fe. The methods of determination were those listed by Miranda Arauz (1989) for Central American coffee soils. Rainfall data were collected from 21 rain gauges installed close to the studied coffee plots (one rain gauge for three coffee plots on average).

Crop management variables included practices related to plot organization, which were generally stable over time, and other practices, which could change from one year to another (Table 2).

Table 1

Environmental descriptors of the plots monitored in the survey on *Meloidogyne* exigua and *Pratylenchus coffeae sensu lato*.

Variable group	Variable	Code
Soil	рН	-
	Al	-
	K	-
	Ca	-
	Mg	-
	Р	-
	Zn	-
	Cu	-
	Mn	-
	Fe	-
	Organic matter	OM
	Sand	-
	Clay	-
Rainfall	Total annual rainfall (March to December)	TRain
	Beginning of the wet season (March to the	MJRain
	first half of June)	-
	Middle of the wet season (second half of	JARain
	June to August)	
	End of the wet season (September to December)	SDRain
Topography	Altitude	Alt
1 0 1-5	Slope percentage	SloP
	Slope aspect	SloA

Table 2

Crop management descriptors of the plots monitored in the survey on *Meloidogyne* exigua and *Pratylenchus coffeae sensu lato*.

Variable group	Variable	Code
Plot organization	Cultivar	-
-	Distance between coffee tree rows	DRow
	Distance between coffee trees in the row	DCTr
	Planting density	PlD
	No. coffee trees per hole	CTr/H
	Shade type	ShT
	Shade percentage	ShP
Other practices	Pruning system	PrS
	Total no. weedings (chemical and mechanical)	TWee
	No. chemical weedings	CWee
	No. mechanical weedings	MWee
	Total no. fertilizer applications (chemical,	TFert
	non-chemical and liming)	
	No. chemical foliar fertilizer applications	CFFert
	No. chemical soil fertilizer applications	CSFert

Data on the cultivar, number of weeding rounds (chemical or mechanical), number of fertilizer applications, and number of amendments were provided by farmers. Other variables were assessed using five trees randomly selected in a zigzag manner in each plot: the distance between coffee trees in the planting row, the distance between rows, the number of coffee plants per planting hole, and the shade percentage, which was quantified using a spherical densiometer (Lemmon, 1957). In addition, the pruning system and the type of shade used were described. No nematicides were used in the surveyed plots.

The same five coffee trees were also characterized by their age, trunk circumference at ground level, which is a sign of vigour, and height (Table 3). Their potential yield was quantified by counting the number of fruiting nodes just before the harvest (Upreti et al., 1992). In addition, as another measurement of coffee tree vigour, the number of young leaves per branch at the beginning of the wet season was assessed by sampling three branches on each of the five coffee trees.

2.4. Nematode sampling and population density assessments

In each coffee plot and for each year, a composite coffee root sample was collected in November, towards the end of the wet season. Each sample comprised of 20 sub-samples: four subsamples for each of the five coffee trees mentioned above, two taken from the neighbouring coffee trees in the same row, and two taken from the neighbouring coffee trees in the adjacent rows. Nematode extraction from plant tissue was undertaken using the macerationsieving method (Taylor and Loegering, 1953) modified according to Alvarado-Soto and López-Chávez (1981). Nematodes and eggs were rinsed through and collected on nested 100 and 500 mesh sieves and their densities estimated under a stereomicroscope. *M. exigua* individuals were identified morphologically using perineal patterns (Franklin, 1962; Taylor and Netscher, 1974). Morphologically, all the *Pratylenchus* that were encountered belonged to *P. coffeae sensu lato* or a closely related species (Duncan et al., 1999).

Table 3

Coffee tree descriptors of the plots monitored in the survey on *Meloidogyne exigua* and *Pratylenchus coffeae sensu lato*.

Variable	Code	
Age of the coffee trees	Age	
Coffee tree height	CTrHe	
Coffee tree trunk circumference at soil level	CTrCi	
Number of young leaves per branch at the		
beginning of the rainy season		
Number of fruiting nodes per coffee tree	FrNo/CTr	

2.5. Statistical methods

Most of the variables assessed in characterization studies are often linked (Savary et al., 1995; Avelino et al., 2006). This is frequently the case with variables characterizing soils and crop management. For example, soil pH is associated with base contents, and weeding in coffee cultivation with the shade percentage. Consequently, it did not seem advisable, at first, to study isolated factors, without taking into account their context. Therefore, correspondence analysis (CA) was used to study the relationships between nematode population densities, and also: (i) soil descriptors, (ii) topography and rainfall, (iii) crop management and (iv) coffee tree characteristics. Classification trees were then developed with all the variables in order to explain the nematode population densities as a function of some selected and hierarchized plot descriptors. In addition, a regression analysis was performed to study bivariate relationships between the densities of the two nematode species.

2.5.1. Correspondence analyses

CA is a descriptive technique designed to explore the relationships between two or more categorical variables using χ^2 -metrics (Benzécri, 1973; Greenacre, 1984; Savary et al., 1995). It is usually performed on one or more contingency tables. CA provides graphical representations of the contingency tables, and especially of the relationships between rows and columns highlighted by previous χ^2 tests of independence. When interpreting graphical representations, three main parameters have to be considered (i) the proportion of total inertia explained by each axis, (ii) the contribution of the modalities to the inertia explained by the axes, which helps in understanding what the axes mean, (iii) the orthogonal distance from the modalities to the axes, or the \cos^2 of the angle between the line origin-modality and the axes: \cos^2 close to 1 indicates a very good representation of the modality on the axis.

The plot descriptors were heterogeneous. Some were quantitative, and others were qualitative. The quantitative variables were therefore categorized to enable combined analysis of the information (Savary et al., 1995; Avelino et al., 2006). Generally, three categories were established per plot descriptor. The category limits were chosen in order to ensure sufficient content in each class for performing χ^2 tests of independence by crossing plot descriptor categories with nematode population density categories. Three categories were established for nematode population densities (per 100 g of coffee roots): null values, 0–20,000, 20,000– 69,760 for *M. exigua* (respectively Mel1, Mel2 and Mel3); null values, 0–5000, 5000–18,960 for *P. coffeae* (respectively Prat1, Prat2 and Prat3). Plot descriptors, for which both χ^2 tests were not significant (*P* > 0.05), were not used in the subsequent CA.

In our analysis, four CA were performed on contingency tables in which the nematode categories corresponded to columns and plot descriptor categories to rows. In each CA, the sampled coffee regions were displayed as supplementary variables. Only the graphical representations of the first two axes were given, as they accounted for the greatest inertia. In addition, only the plot descriptor categories that were well correlated to these two axes (sum of $\cos^2 > 0.5$) were presented.

2.5.2. Classification trees

Chi-squared automatic interaction detector (CHAID) is an exploratory method used to study the relationships between a response variable, either qualitative or quantitative, and predictors, of a heterogeneous nature, that may interact (Kass, 1980). The result, which is graphically represented as a tree, is a partitioning of the dataset, based on predictor values, into the most homogeneous groups possible with respect to the response variable. Each group can be a function of different predictors. Firstly, the initial population is divided into two or more subpopulations by splitting the values of a predictor into two or more categories. The predictor and the splitting value adopted are those which lead to the best discrimination of the response variable, which means with the smallest *P* value of a χ^2 test if the response variable is qualitative, or of an *F* test if quantitative (*P* values are adjusted to take into account multiple testing). The subpopulations obtained are subdivided again according to the same principle. As a consequence, successive subdivisions lead to a hierarchy between predictors according to their explanatory power.

In our study, classification trees were developed in order to establish the hierarchy between *M. exigua* and *P. coffeae* predictors. Here, the two nematode population densities, grouped into three categories as above, were the nominal variables to be explained. Tree construction was concluded when none of the adjusted *P* values was below 0.05 or when nodes that could be formed had fewer than nine individuals. For each nematode, two CHAID classification trees were developed, one including the region variable, and one without.

3. Results

3.1. Variable categorization, χ^2 tests of independence, and correspondence analyses

3.1.1. Relationships between soil and nematode population densities According to χ^2 tests of independence, most of the soil characteristics (Al, K, Zn, Cu, Fe, organic matter, sand, clay) were significantly linked (*P* < 0.05) to population densities of both nematodes (Table 4). No relationship with soil pH was detected. Additionally, Ca, Mg, Mn were associated only with *M. exigua*, and P only with *P. coffeae*. From the CA (Fig. 2A), it was apparent that *M. exigua* and *P. coffeae* opposed each other: axis 1 is an axis of increasing values of *M. exigua* and decreasing values of *P. coffeae*. Prat1 and Mel3 largely explain axis 1, with contributions to inertia of 26.6% and 32.1% respectively. It also appeared that Mel3 was especially associated with low levels of Al, K, Ca, Mg, Mn, Fe and high levels of Cu, whereas Prat3 – and Mel2 – were associated with high levels of K and medium Cu and Zn. Moreover, Mel1 was linked to high levels of Mn, Fe, clay and low Cu, organic matter and sand, whereas Prat1 was linked to high levels of organic matter and sand, and low P and Zn.

3.1.2. Relationships between rainfall, topography variables and nematode population densities

Only altitude and total annual rainfall were significantly linked (P < 0.05) to both species of nematode (Table 5). Additionally, slope characteristics (inclination and aspect) and rainfall at the beginning of the wet season were linked only to *P. coffeae*, while *M. exigua* was associated with rainfall at the end of the wet season. In the CA graphical representation (Fig. 2B), axis 1 is mainly an axis of decreasing values of *P. coffeae* from low to null values: the total contribution of Prat1 and Prat2 to inertia was 74.7%. Axis 2 is mainly an axis of increasing values of *M. exigua* population densities and also of *P. coffeae* population densities from low to high values only. *M. exigua* categories contributed 37.1% to inertia

Table 4

Soil variable categories and results of χ^2 tests of independence performed with Pratylenchus coffeea sensu lato and Meloidogyne exigue categories^a.

Variable ^b	Categories			χ^2 test	χ^2 test	
	1	2	3	Pratylenchus coffeae sensu lato	Meloidogyne exigua	
рН	4.3-5.0	5.0-5.4	5.4-6.6	3.9	9.4	
$\operatorname{Al}(\operatorname{g}\operatorname{kg}^{-1})$	0.00-0.08	0.08-0.27	0.27-0.47	11.3	14.5**	
$K (g kg^{-1})$	0.04-0.13	0.13-0.18	0.18-0.47	13.6**	11.5	
$Ca (g kg^{-1})$	0.13-0.72	0.72-1.44	1.44-5.85	6.2	12.2 [*]	
$Mg (g kg^{-1})$	0.06-0.10	0.10-0.23	0.23-0.62	6.1	12.8 [°]	
$P(mg kg^{-1})$	1.3-4.3	4.3-10.0	10.0-77.0	35.3**	8.5	
$Zn (mg kg^{-1})$	0.1-1.5	1.5-2.9	2.9-14.0	32.5**	18.4**	
$Cu (mg kg^{-1})$	2.0-7.7	7.7-10.7	10.7-30.0	10.5	17.2**	
$Mn (mg kg^{-1})$	0–9	9-19	19–65	9.3	20.6**	
Fe $(g kg^{-1})$	0.03-0.11	0.11-0.23	0.23-1.01	15.9**	25.5**	
OM (% of dry weight)	3.48-8.80	8.80-11.20	11.20-16.10	14.7**	23.9**	
Sand (% of dry weight)	19.7-45.6	45.6-55.7	55.7-76.5	26.7**	36.0**	
Clay (% of dry weight)	5.5-13.0	13.0-32.0	32.0-62.4	12.2*	28.8**	

^a Pratylenchus coffeae sensu lato categories: null values, 0–5000, 5000–18,960; Meloidogyne exigua categories: null values, 0–20,000, 20,000–69,760; densities per 100 g of coffee roots.

^b See Table 1 for explanation of codes.

P < 0.05.

P < 0.01.

Table 5

Rainfall and topography variable categories and results of χ^2 tests of independence performed with Pratylenchus coffeae sensu lato and Meloidogyne exigua categories^a.

Variable ^b	Categories			χ^2 test		
	1	2	3	Pratylenchus coffeae sensu lato	Meloidogyne exigua	
TRain (mm)	1937-2500	2500-3300	3300-4587	12.2 [*]	11.9 [°]	
MJRain (mm)	418-750	750-1025	1025-1392	20.0**	2.5	
JARain (mm)	525-695	695-870	870-1142	1.7	5.2	
SDRain (mm)	904-1200	1200-1510	1510-2385	9.0	9.9*	
Alt (m)	950-1200	1200-1480	1480-1785	49.1**	15.3**	
SloP	2-12	12-22	22-70	11.0 [*]	7.7	
SloA (Degree, N = 0)	NE = 0–115	S = 115-240	NW = 240-355	20.7**	2.6	

^a Pratylenchus coffeae sensu lato categories: null values, 0–5000, 5000–18,960; Meloidogyne exigua categories: null values, 0–20,000, 20,000–69,760; densities per 100 g of coffee roots.

^b See Table 1 for explanation of codes.

* *P* < 0.05.



Fig. 2. Graphical representations of the first two axes of four factorial correspondence analyses performed on contingency tables where the soil (A), topography and rainfall (B), crop management (C), and coffee tree characteristics (D) are in rows and nematode categories in columns. *Meloidogyne exigua* categories: Mel1 (null values), Mel2 (0–20,000), Mel3 (20,000–69,760). *Pratylenchus coffeea sensu lato* categories: Prat1 (null values), Prat2 (0–5000), Prat3 (5000–18,960). Densities expressed per 100 g coffee roots. Only the best represented predictor categories are displayed (sum of cos² > 0.5). Abscissa axis is axis 1. Ordinate axis is axis 2. Percentages indicate the proportion of inertia explained by the axes. See Tables 1–3 for explanations of codes and Tables 4–7 for category limits. The sampled coffee regions (Fig. 1) are displayed as supplementary variables: CV (Central Valley), CB (Coto Brus), TA (Tarrazú), TU (Turrialba). Western Valley less well represented.

represented on axis 2, and Prat3 49.9%. Prat3 was mainly associated with topographical factors: slight and south-oriented slopes, altitudes between 1200 and 1480 m. Mel3 was mainly associated with rainfall characteristics: medium total annual rainfall and especially medium rainfall at the end of the wet season, when the coffee roots were sampled. Additionally, Prat1 was found at the lowest altitudes (between 950 and 1200 m), whereas Mel1 and Prat2 were associated with high altitudes (above 1480 m) and low rainfall, especially at the end of the wet season.

3.1.3. Relationships between crop management and nematode population densities

Only the distance between coffee tree rows and the annual number of fertilizer applications were significantly linked (P < 0.05) to both species of nematodes (Table 6). In addition, *P. coffeae* population densities were associated with the distance between coffee trees in the row, coffee tree planting density, the number of coffee trees per planting hole, shade percentage, pruning system and the number of mechanical weedings. On the other hand, *M. exigua* population densities were associated with shade type and the annual number of foliar and soil fertilizer applications. As a consequence, *P. coffeae* and *M. exigua* population densities appeared to be independent in the CA graphical representation (Fig. 2C). Axis 1 is an axis of increasing values of *M. exigua* population densities, whereas axis 2 is an axis of increasing values of *P. coffeae* population densities. The contribution of *M. exigua* categories to the inertia represented on axis 1 was 71.1%, whereas the contribution of *P. coffeae* categories to the inertia represented on axis 2 was 89.8%. Mel3 was especially

Table 6

Crop management variable categories and results of χ^2 tests of independence performed with *Pratylenchus coffeae sensu lato* and *Meloidogyne exigua* categories^a.

Variable ^b	Categories		χ^2 test		
	1	2	3	Pratylenchus coffeae sensu lato	Meloidogyne exigua
Cultivar	Caturra	Catuaï		4.2	4.7
DRow (m)	1.1-1.6	1.6-1.9	1.9-2.3	11.7 [*]	16.3**
DCTr (m)	0.7-0.9	0.9–1.0	1.0-1.40	14.3**	4.2
PlD (coffee trees ha^{-1})	3434-5051	5051-5723	5723-10,629	15.7**	1.2
CTr/H	1.0-1.9	1.9-4.0		7.1 [*]	1.8
ShT	Shade with no banana plants	Banana plants and other shade species	No shade	2.2	21.2**
ShP	0	0-8	8-65	13.3**	3.8
PrS	By branch, tree, or no pruning	By entire row or plot		12.9 ^{**}	0.1
Twee	0 or 1	2	3–5	6.7	7.1
CWee	0	1	2-4	6.7	0.9
MWee	0	1	2 or 3	9.8 [*]	6.6
TFert	0-2	3	4-8	12.0 [°]	12.7
CFFert	0	1-4		0.1	9.6
CSFert	0 or 1	2	3 or 4	3.3	14.8**

^a Pratylenchus coffeae sensu lato categories: null values, 0–5000, 5000–18,960; Meloidogyne exigua categories: null values, 0–20,000, 20,000–69,760; densities per 100 g of coffee roots.

^b See Table 2 for explanation of codes.

P < 0.05.

** *P* < 0.01.

associated with shading by banana plants, foliar fertilizer application, and a low or medium number of soil fertilizer applications. On the other hand, Mel1 was linked to the absence of banana plants in the coffee plot, no use of foliar fertilizer, a high number of soil fertilizer applications, and a medium total annual number of fertilizer applications. For *P. coffeae*, Prat3 was mainly linked to a high coffee tree planting density and coffee tree pruning of entire rows or plots, i.e. when stumping was carried out, a pruning system that led to the removal of a large quantity of foliage and branches over a large and compact area. Conversely, Prat1 was mainly associated with a medium coffee tree planting density, a small number of coffee trees per hole, no pruning or a conservative pruning system by tree or branch.

3.1.4. Relationships between coffee tree characteristics and nematode population densities

The number of young leaves per branch counted at the beginning of the wet season (foliage density), was significantly linked (P < 0.05) to both species of nematode (Table 7). In addition, tree height and tree trunk circumference were linked to *P. coffeae* only. In the CA graphical representation (Fig. 2D), axis 1 is an axis of decreasing values of *P. coffeae* population densities, whereas axis 2 is an axis of increasing values of *M. exigua* population densities, as well as an axis of decreasing values. Actually, *P. coffeae* population densities from low to null values. Actually, *P. coffeae* categories contributed 92.0% to the inertia represented on axis 1, whereas *M. exigua* categories contributed 48.9% to inertia represented on axis 2. Prat3 was associated with short trees, with trunks of medium

thickness, whereas Mel3 and Prat1 were mainly linked to coffee trees with a high foliage density.

3.1.5. Nematode distribution and relationships between M. exigua and P. coffeae

P. coffeae and *M. exigua* were not universally present across the regions sampled. *P. coffeae* was absent from Turrialba and Coto Brus, the lowest altitude regions, and *M. exigua* was mostly absent from Tarrazú, the highest region (Fig. 2). The absence of one nematode species seemed to be associated with the development of the other. The highest *P. coffeae* or *M. exigua* population densities were actually observed where only one species was present and a significant negative correlation (r = -0.29, P < 0.01) was found between the densities of the two species (Fig. 3). However, considering the low *r* value, this relationship is likely affected by other factors.

3.2. CHAID analyses

3.2.1. Main predictors of M. exigua population densities

Soil characteristics were among the main predictors of *M. exigua* population densities. Sand and soil Ca contents remained, respectively, on the first and third level of the classification tree when not considering the region (Fig. 4A). When the region variable was included in the CHAID analysis (Fig. 4B), organic matter and P appeared on the second level of the classification tree. Altitude was another important predictor. It appeared in both classification trees, on the third level, with the region variable as a

Table 7

Coffee tree variable categories and results of χ^2 tests of independence performed with Pratylenchus coffee sensu lato and Meloidogyne exigua categories^a.

Variable ^b	Categories			χ^2 test	
	1	2	3	Pratylenchus coffeae sensu lato	Meloidogyne exigua
Age (years)	2.0-7.5	7.5-11.5	11.5-23.0	2.7	4.9
CTrHe (m)	1.2-1.6	1.6-1.9	1.9-2.8	13.1*	5.9
CTrCi (cm)	9.5-19.2	19.2-22.2	22.2-29.8	11.7*	1.9
Yl/Br	2.7-7.4	7.4-8.8	8.8-11.7	9.9*	11.9*
FrNo/CTr	53-447	447-633	633-1796	4.1	6.0

^a Pratylenchus coffeae sensu lato categories: null values, 0–5000, 5000–18,960; Meloidogyne exigua categories: null values, 0–20,000, 20,000–69,760; densities per 100 g of coffee roots.

^b See Table 3 for explanation of codes.

* *P* < 0.05. ** *P* < 0.01.



Fig. 3. Association between *Meloidogyne exigua* and *Pratylenchus coffeae sensu lato* population densities in the surveyed plots. Densities expressed per 100 g coffee roots. \times : Western Valley, \odot : Central Valley, \bigcirc : Coto Brus, \square : Tarrazú, \blacktriangle : Turrialba. Regression was performed excluding the cases where nematodes were not detected.

predictor, and on the second level when that variable was not included. Finally, some cropping practices were also present in the classification trees. The pruning system and the distance between coffee trees in the row were retained on the second and fourth level respectively when the region variable was included in the analysis, whereas the shade type was retained on the second level when the region variable was not included. Including the region variable in the analysis led to sand being replaced by that variable on the first level of the classification tree, and to profound changes on the subsequent levels. Only altitude was retained as a predictor in both classification trees.

3.2.2. Main predictors of P. coffeae population densities

Soil characteristics were among the most important predictors of *P. coffeae.* especially sand and soil Zn contents, which remained. respectively, on the first and second level of the classification tree when not considering the region (Fig. 5A). In addition, Mn content appeared on the third level of the same classification tree. When including the region variable (Fig. 5B), only Mn content was retained as a soil predictor (on the second level). The rainfall and topography variables also looked important. Slope aspect and total annual rainfall appeared respectively on the second and third level of the classification tree with the region variable as a predictor. When that variable was not used in the analysis, only altitude remained (on the third level). Moreover, the annual number of mechanical weedings appeared in both classification trees (on the third level). As for M. exigua, including the region variable in the analysis led to the replacement of soil sand content by this variable on the first level of the classification tree, and to changes on the successive levels. In this case, only Mn and the annual number of mechanical weedings remained as predictors in both classification trees.

4. Discussion

4.1. Null values of nematode population densities

As nematode spread in coffee plantations is favoured by human activities, principally by setting up plantations with infested



Fig. 4. Classification trees, obtained by the CHAID method, explaining *Meloidogyne exigua* population densities, without the region variable (A) or included (B) in the analysis. The retained predictors are: sand, Ca, P and organic matter (OM) soil contents, shade type (ShT), pruning system (PrS), distance between coffee trees in the row (DCTr), altitude (Alt) and region. See details on predictors in Tables 4–6. WV: Western Valley, CV: Central Valley, CB: Coto Brus, TA: Tarrazú, TU: Turrialba. The histograms show the percentage of the individuals in three density categories (expressed per 100 g roots)– \Box : Null values, Ξ : 0,000–69,760. *P*: adjusted probability of the χ^2 tests, *n*: number of plot-year individuals.



Fig. 5. Classification trees, obtained by the CHAID method, explaining *Pratylenchus coffeae sensu lato* population densities, without the region variable (A) or included (B) in the analysis. The retained predictors are: sand, Zn and Mn soil contents, annual number of mechanical weedings (MWee), altitude (Alt), slope aspect (SloA), total annual rainfall (Train) and region. See details on predictors in Tables 4–6. WV: Western Valley, CV: Central Valley, CB: Coto Brus, TA: Tarrazú, TU: Turrialba. The histograms show the percentage of the individuals in three density categories (expressed per 100 g roots)– \Box : Null values, \Box : 0–5000, \blacksquare : 5000–18,960. *P*: adjusted probability of the χ^2 tests, *n*: number of plot-year individuals.

nursery seedlings (Inomoto and Oliveira, 2008; Villain et al., 2008), most of the relationships observed between plot characteristics and an absence of *M. exigua* or *P. coffeae* probably resulted from an absence of infestation, and not necessarily from an effect of those characteristics on nematode development. For example, according to the CHAID analysis performed without the region variable, only null population densities of *P. coffeae* were found in plots with a very high sand content (above 63.1%). That relationship was probably due to the association of soil texture characteristics with the region variable, which was confirmed by automatic removal of sand from the classification trees when including regions in the CHAID analysis, and to the absence of *P. coffeae* in all the plots sampled in Turrialba and Coto Brus. A total absence of *P. coffeae* on a regional scale supported the hypothesis that this nematode had never been introduced.

4.2. Relationships between soil and nematode population densities

M. exigua and *P. coffeae* population densities were closely linked to soil chemical and physical characteristics, according to the χ^2 tests of independence, CA and CHAID analyses, which is not uncommon for plant parasitic nematodes (Prot and Van Gundy, 1981; Quénéhervé, 1988; Norton, 1989; Robinson et al., 1987; Korthals et al., 1996; Cadet and Thioulouse, 1998; Siddiqui and Mahmood, 1998; Kandji et al., 2001; Siddiqui et al., 2002; Cadet et al., 2004, 2005; Dabiré et al., 2007). However, to our knowledge, this is the first time that the links between soil chemical and physical characteristics and *M. exigua* and *P. coffeae* on coffee have been formally described.

According to the results of the CHAID analysis performed without the region variable, soil sand content was one of the most important predictors of *M. exigua* populations on coffee roots. High levels of sand (above 46.7%) were associated with large *M. exigua* population densities. This has previously been observed for other *Meloidogyne* species, such as *M. incognita* (Prot and Van Gundy, 1981; Robinson et al., 1987; Quénéhervé, 1988; Cadet and Thioulouse, 1998), *M. javanica* (Siddiqui and Mahmood, 1998; Dabiré et al., 2007) and *M. arenaria* (Cadet and Thioulouse, 1998).

In fact, light, sandy soils provide adequate moisture for nematode survival and reproduction (Siddigui and Mahmood, 1998; Dabiré et al., 2007) and are propitious to the migration of *Meloidogyne* spp. juveniles (Prot and Van Gundy, 1981). In addition, soil chemical factors, and especially Zn content, appeared important for both M. *exigua* and *P. coffeae*. According to the χ^2 tests of independence and CA, M. exigua population densities were low when soil Zn content rose above 1.5 mg kg⁻¹. Similar observations have been described for M. javanica on tomato. Soil amendment with Zn reduced nematode penetration in tomato roots and enhanced biological control by Pseudomonas aeruginosa (Siddiqui et al., 2002). For P. coffeae, Zn appeared on the second level of the classification tree when the region variable was not included in the analysis. High P. coffeae population densities were found only in soils with medium to high Zn content (\geq 1.4 mg kg⁻¹). Apparently, *Pratylenchus* spp. have a higher tolerance for Zn compared to other nematode groups, as reported by Korthals et al. (1996) who found Pratylenchus in significantly higher relative abundance after addition of large quantities of Zn. Soil K and Ca also appear important for the presence of M. exigua in particular: large population densities of M. *exigua* were associated with low soil K (≤ 0.13 g kg⁻¹ according to CA) and Ca contents (\leq 0.72 g kg $^{-1}$ or <0.59 g kg $^{-1}$ according to CA or CHAID analysis respectively). Our results however, seem to contradict other reports of a strong positive correlation between soil K content and soil population densities of *Meloidogyne* spp. in Kenya (Kandji et al., 2001), and M. javanica abundance on an African egg-plant in Senegal (Dabiré et al., 2007). Conversely, a similar relationship between soil K and Ca contents and Meloidogyne spp. in soil and tomato roots was recorded by Cadet and Thioulouse (1998) in the French West Indies. For P. coffeae population densities, a positive relationship with soil K content was observed in our study, whereas Cadet et al. (2004) observed the opposite for P. zeae densities in soil and sugarcane roots in South Africa. It appears that, despite the fact that inorganic ions, such as K^+ , are known to be potentially repellent to nematodes, as demonstrated for *M. incognita* (Castro et al., 1990), their effect may vary between sites, likely as a consequence of a combination of factors, such as soil physical or even biological characteristics, and

especially the composition of nematode communities (Villenave and Cadet, 1999). The soil organic matter content seemed to be another important variable. According to the classification tree developed with the region variable, in the Central Valley, Coto Brus and Turrialba regions, mainly large *M. exigua* population densities occurred in soils with less than 12.6% of organic matter. This may be explained by the effects of organic matter on soil characteristics and especially on its biological properties, by promoting beneficial organisms and saprophytic nematodes at the expense of plant parasitic nematodes (Widmer et al., 2002; Briar et al., 2007). However, despite the known effects of organic matter on nematodes, other relationships have also been reported depending on site, crop, and nematode communities. Kandji et al. (2001), for example, described a positive correlation between *Meloidogyne* spp. and organic matter in Kenyan soils.

4.3. Relationships between rainfall, topography and nematode population densities

According to the χ^2 tests of independence, CA and CHAID analyses, M. exigua and P. coffeae population densities were dependent on topography and rainfall. Altitude seemed to be an important limiting factor for M. exigua. From CA and CHAID analyses, it can be deduced that *M. exigua* is favoured at the lowest altitudes (below 1320 m) and mostly absent above 1500 m. For P. coffeae, according to the CA, large population densities mainly occurred at higher altitudes (between 1200 and 1480 m), and low densities above this. These results are most likely explained by the temperature requirements for *M. exigua* and P. coffege. The latter seems adapted to a wider range of temperatures than M. exigua (Pinochet et al., 1995; Inomoto and Oliveira, 2008), which is less tolerant of low temperatures (D'Arc de Lima and Ferraz, 1985; Souza and Bressan-Smith, 2008). Slope aspect seems to be another important topographical predictor of *P. coffeae*. According to the χ^2 tests of independence, CA and CHAID analyses, south-oriented slopes were linked to large populations of this nematode. To our knowledge, this is the first such observation. It could be related to soil temperature, which is likely higher with this slope orientation, due to better exposure to the sun. This could be particularly important for plots located near the upper altitude limit for effective P. coffeae development. P. coffeae and M. exigua population densities also seem well explained by rainfall. According to the CA results, low rainfall was associated with low P. coffeae population densities and null or low population densities of M. exigua. In addition, the CHAID analysis highlighted a relationship between large P. coffeae population densities and high annual rainfall. These relationships were highlighted due to the substantial rainfall differences between regions and also between the two study years: the total annual rainfall was 26% higher in 2003 than in 2002 (average of 17 rain gauges). Our results do not seem to corroborate the generally accepted negative relationship between rainfall and nematode population growth. P. coffeae populations are understood to increase during the dry season and to decrease during the wet season (Quénéhervé, 1989; Inomoto and Oliveira, 2008; Villain, 2008), which also appears the case for M. exigua (Almeida et al., 1987; Souza and Bressan-Smith, 2008; Souza et al., 2008). This behaviour has principally been explained by root growth dynamics and also by root death caused by soil-borne pathogens when soil moisture is excessive (Inomoto and Oliveira, 2008; Villain, 2008). However, that negative effect must depend on the soil texture. It is probably enhanced in heavy clay soils, and, conversely, reduced in sandy soils. In our study, 70% of the sampled soils contained more than 45% of sand and could be considered as well drained sandy soils.

4.4. Relationships between cropping practices and nematode population densities

Despite finding links between the annual number of fertilizer applications and M. exigua and P. coffeae population densities (see χ^2 tests of independence and CA), the relationships remain unclear. Fertilizer application, organic or mineral, has commonly been considered as a potential nematode management option (Siddigui et al., 2001: Covne et al., 2004: Souza and Bressan-Smith, 2008), but the nature of the fertilizer is important. In our study, fertilizer use was diverse, which probably affected the relationships observed. As a consequence, fertilizer variables were not retained as predictors in the classification trees, which tended to confirm its poor explanatory power. Other crop management characteristics looked much more relevant. For example, small distances between rows and between coffee trees in the row were well linked to large M. exigua and P. coffeae population densities. A 0.9 m distance between coffee trees in the row appeared to be the threshold. Below this distance, which is common in intensified Costa Rican coffee plantations, the increased contacts between roots likely facilitate nematode spread. To our knowledge, this is one of the first times this frequently mentioned relationship (Villain, 2008) has been documented. Increased pest and disease attack intensities in intensified coffee systems have already been documented for coffee rust (Hemileia vastatrix) (Avelino et al., 2006) and American leaf spot disease (Avelino et al., 2007). For the latter disease, high planting densities, and especially short distances between rows, were conducive to its development (Avelino et al., 2007). Another important cropping practice was the pruning system. It appeared that pruning entire rows or plots, mainly by stumping coffee trees, was associated with small M. exigua but large P. coffeae population densities. Stumping is a drastic pruning technique, causing the death of a large proportion of absorbent roots, which, no doubt, affected the M. exigua populations in our study, as previously reported (Souza and Bressan-Smith, 2008). P. coffeae, which survives better than M. exigua in the absence of living roots (Inomoto and Oliveira, 2008; Souza and Bressan-Smith, 2008), was thus probably favoured by the elimination of this competitor for resources (Bertrand et al., 1998; Hervé et al., 2005; Villain, 2008). Two other cropping practices seemed to be key factors for nematode population growth according to the χ^2 tests of independence, CA and CHAID analyses: large M. exigua population densities were mainly found in coffee plantations with banana as the intercrop and large P. coffeae population densities were mainly found where mechanical weeding was carried out. The possible mechanisms underlying these relationships are unclear. P. coffeae could be favoured by changes in soil biota following mechanical weeding which can generate large amounts of organic material, affect the entire soil food-web and favour certain functional groups (Wardle et al., 1995). As for the remaining relationship, we assume banana plants play a role in the spread of *M. exigua*. This nematode was detected in all the surveyed coffee plots in three regions (Central Valley, Coto Brus and Tarrazú), where banana plants were used as the intercrop, while in the surveyed plots with no banana plants from the same regions, M. exigua was absent in 36% of plots. Banana suckers used in coffee producing regions generally originate from coffee fields. Although the genus Musa is not known to be a good host for *M. exigua*, if banana suckers, or the soil attached to planting material, come from infested coffee fields, they might contain coffee-parasitic nematodes, especially M. exigua, whose egg masses are quite resistant to adverse conditions. Planting of banana plants, which have not been cleaned and disinfested into coffee fields may thereby be contributing to the spread of *M. exigua*.

4.5. Relationships between coffee tree characteristics and nematode population densities

The relationships we found between coffee tree characteristics and nematode population densities were not particularly strong, as no coffee tree characteristic was retained in the classification trees. However, it is interesting to see that *M. exigua* grew better on coffee trees which had good aerial growth, which probably related to a good root system too. This observation indicates that *M. exigua* probably does not cause great losses in Central America, as previously mentioned by different authors (Bertrand et al., 2002; Hernández et al., 2004b; Villain et al., 2008). On the other hand, smaller coffee trees were associated with larger *P. coffeae* population densities, indicating a more negative effect on coffee, than *M. exigua* for example, confirming previous reports from Central America (Villain et al., 2000, 2008; Villain, 2008).

4.6. Relationships between M. exigua and P. coffeae

Our results indicate that *M. exigua* and *P. coffeae* have some different soil and topography requirements. Within the limits of our data, *M. exigua* population densities reached higher levels in lower altitudes, with lower Zn and K soil contents. For *P. coffeae*, the opposite was true. In addition, the largest *M. exigua* population densities were associated with sandy soils and the contrary for *P. coffeae*. As a consequence of these contrary requirements, the two nematode species appear to complement each other quite well in regard to ecological niches for coffee. Similar oppositions between different *Meloidogyne* and *Pratylenchus* species have been observed before (Cadet and Thioulouse, 1998; Kandji et al., 2001).

Moreover, the fact that the absence of one nematode species was associated with the largest population densities of the other indicates some element of competition between *M. exigua* and *P. coffeae*. This hypothesis is supported by the results of Umesh et al. (1994) who reported competition between *M. chitwoodi* and *P. neglectus* in barley. These authors found that the species that parasitized the roots first inhibited penetration by the other. In addition, Hervé et al. (2005) reported similar relationships to those we obtained on a plot scale, but on a plant scale, between *P. coffeae* and *M. paranaensis* or *M. exigua*, in Guatemala and Costa Rica respectively, and deduced that active competition existed between the two genera. This competition was confirmed by Bertrand et al. (1998) who found that planting coffee trees resistant to *M. exigua* favoured *P. coffeae* populations.

5. Conclusions

Potential direct and indirect effects of the environment and cropping practices on nematode populations have been identified, suggesting that different mechanisms could be brought into play at the same time, in order to reduce coffee nematode populations. Large P. coffeae population densities were quantified in coffee fields with soil, climatic and topographical characteristics that differed from those where large M. exigua population densities were observed. These different characteristics can be considered as different nematode risk domains, for which risk-adapted cropping practices could be suggested. Due to probable competition phenomena existing between M. exigua and P. coffeae, cropping practices that deter both should be recommended. The only cropping practice we found to be similarly associated with M. exigua and P. coffeae population densities was the distance between coffee trees in the row. Coffee tree spacing is difficult to modify in existing plantations, but could be easily defined in accordance with nematode risks for new coffee plots.

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References

- Almeida, V.F. de, Campos, V.P., Lima, R.D. de, 1987. Flutuação populacional de Meloidogyne exigua na rizosfera do cafeeiro. Nematologia Brasileira 11, 159– 175.
- Alpízar, E., Etienne, H., Bertrand, B., 2007. Intermediate resistance to Meloidogyne exigua root-knot nematode in Coffea arabica. Crop Prot. 26, 903–910.
- Alvarado-Soto, M., López-Chávez, R., 1981. Eficacia de dos métodos y sus modificaciones en la extracción de nematodos endoparásitos migratorios de raíces de piña y plátano. Nematropica 11, 129–136.
- Avelino, J., Cabut, S., Barboza, B., Barquero, M., Alfaro, R., Esquivel, C., Durand, J.F., Cilas, C., 2007. Topography and crop management are key factors for the development of American leaf spot epidemics on coffee in Costa Rica. Phytopathology 97, 1532–1542.
- Avelino, J., Želaya, H., Merlo, A., Pineda, A., Ordoñez, M., Savary, S., 2006. The intensity of a coffee rust epidemic is dependent on production situations. Ecol. Model. 197, 431–447.
- Barbosa, D.H.S.G., Vieira, H.D., Souza, R.M., Viana, A.P., Silva, C.P., 2004. Field estimates of coffee yield losses and damage threshold by *Meloidogyne exigua*. Nematologia Brasileira 28, 49–54.
- Benzécri, J.P., 1973. L'Analyse des Données, vol. 2, L'Analyse des Correspondances. Dunod, Paris, 632 pp.
- Bertrand, B., Cilas, C., Hervé, G., Anthony, F., Etienne, H., Villain, L., 1998. Relations entre les populations des nématodes Meloidogyne exigua et Pratylenchus sp., dans les racines de Coffea arabica au Costa Rica, Plantations, Recherche, Développement 5, 279–286.
- Bertrand, B., Peña Durán, M.X., Anzueto, F., Cilas, C., Etienne, H., Anthony, F., Eskes, A.B., 2000. Genetic study of *Coffea canephora* coffee tree resistance to *Meloido-gyne incognita* nematodes in Guatemala and *Meloidogyne* sp. nematodes in El Salvador for selection of rootstock varieties in Central America. Euphytica 113, 79–86.
- Bertrand, B., Ramírez, G., Topart, P., Anthony, F., 2002. Resistance of cultivated coffee (Coffea arabica and C. canephora) trees to corky-root caused by Meloidogyne arabicida and Fusarium oxysporum, under controlled and field conditions. Crop Prot. 21, 713–719.
- Bornemisza, E., Collinet, J., Segura, A., 1999. Los suelos cafetaleros de América Central y su fertilización. In: Bertrand, B., Rapidel, B. (Eds.), Desafíos de la Caficultura en Centroamérica. IICA, San José, Costa Rica, pp. 97–137.
- Briar, S.S., Grewal, P.S., Somasekhar, N., Stinner, D., Miller, S.A., 2007. Soil nematode community, organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from conventional to organic management. Appl. Soil Ecol. 37, 256–266.
- Cadet, P., Thioulouse, J., 1998. Identification of soil factors that relate to plant parasitic nematode communities on tomato and yam in the French West Indies. Appl. Soil Ecol. 8, 77–84.
- Cadet, P., Berry, S., Spaull, V., 2004. Mapping of interactions between soil factors and nematodes. Eur. J. Soil Biol. 40, 77–86.
- Cadet, P., Masse, D., Thioulouse, J., 2005. Relationships between plant-parasitic nematode community, fallow duration and soil factors in the Sudano-Sahelian area of Senegal. Agric. Ecosyst. Environ. 108, 302–317.
- Campos, P.V., Srivapalan, P., Gnanapragasam, N.C., 1990. Nematode parasites of coffee, cocoa and tea. In: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK, pp. 387–430.
- Castro, C.E., Belser, N.O., McKinney, H.E., Thomason, I.J., 1990. Strong repellency of the root knot nematode, *Meloidigyne incognita* by specific inorganic ions. J. Chem. Ecol. 16, 1199–1205.
- Coen, E., 1983. Climate. In: Janzen, D.H. (Ed.), Costa Rican Natural History. The University of Chicago Press, Chicago, pp. 35–46.
- Coyne, D.L., Sahrawat, K.L., Plowright, R.A., 2004. The influence of mineral fertilizer application and plant nutrition on plant-parasitic nematodes in upland and lowland rice in Côte d'Ivoire and its implications in long term agricultural research trials. Exp. Agric. 40, 245–256.
- Dabiré, R.K., Ndiaye, S., Mounport, D., Mateille, T., 2007. Relationships between abiotic soil factors and epidemiology of the biocontrol bacterium *Pasteuria penetrans* in a root-knot nematode *Meloidogyne javanica*-infested field. Biol. Control 40, 22–29.
- D'Arc de Lima, R., Ferraz, S., 1985. Biologia de *Meloidogyne exigua* (Nematoda: Meloidogynidae). I. Desenvolvimento embriogênico e efeito da temperatura na embriogênese. Revista Ceres 32, 339–348.
- De Graaff, J., 1986. The Economics of Coffee. Pudoc, Wageningen, The Netherlands, 294 pp.

- De Wit, C.T., 1982. La productivité des pâturages sahéliens. In: Penning de Vries, F.W.T., Djiteye, M.A. (Eds.), La Productivité des Pâturages Sahéliens—Une Etude des Sols, des Végétations et de l'Exploitation de cette Ressource Naturelle. Pudoc, Wageningen, The Netherlands, pp. 22–35.
- Duncan, L.W., Inserra, R.N., Thomas, W.K., Dunn, D., Mustika, I., Frisse, L.M., Mendes, M.L., Morris, K., Kaplan, D.T., 1999. Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. Nematropica 29, 61–80.
- Fernández, C.E., Muschler, R.G., 1999. Aspectos de sostenibilidad de los sistemas de cultivo de café en América central. In: Bertrand, B., Rapidel, B. (Eds.), Desafíos de la Caficultura en Centroamérica. IICA, San José, Costa Rica, pp. 69–96.
- Flores, L., López, R., 1989. Caracterización morfológica del nematodo nodulador del cafeto *Meloidogyne exigua* (Nemata: Heteroderidae). I. Hembras y huevos. Turrialba 352–360.
- Franklin, M.T., 1962. Preparation of posterior cuticular patterns of *Meloidogyne* spp. for identification. Nematologica 7, 336–337.
- Greenacre, M.J., 1984. Theory and Application of Correspondence Analysis. Academic Press, London, 364 pp.
- Hernández, A., Fargette, M., Sarah, J.L., 2004a. Characterisation of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. Nematology 6, 193–204.
- Hernández, A., Fargette, M., Sarah, J.L., 2004b. Pathogenicity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) isolates from Central America and Brazil on four genotypes of *Coffea arabica*. Nematology 6, 205–213.
- Hervé, G., Bertrand, B., Villain, L., Licardié, D., Cilas, C., 2005. Distribution analyses of Meloidogyne spp. and Pratylenchus coffeae sensu lato in coffee plots in Costa Rica and Guatemala. Plant Pathol. 54, 471–475.
- Inomoto, M.M., Oliveira, C.M.G., 2008. Coffee-associated *Pratylenchus* spp.—ecology and interactions with plants. In: Souza, R.M. (Ed.), Plant-parasitic Nematodes of Coffee. Springer, Berlin, pp. 51–64.
- Kandji, S.T., Ogol, C.K.P.O., Albrecht, A., 2001. Diversity of plant-parasitic nematodes and their relationships with some soil physico-chemical characteristics in improved fallows in western Kenya. Appl. Soil Ecol. 18, 143–157.
- Kass, G.V., 1980. An exploratory technique for investigating large quantities of categorical data. Appl. Stat. 29, 119–127.
- Korthals, G.W., van de Ende, A., van Megen, H., Lexmond, T.M., Kammenga, J.E., Bongers, T., 1996. Short-term effects of cadmium, copper, nickel and zinc on soil nematodes from different feeding and life-history strategy groups. Appl. Soil Ecol. 4, 107–117.
- Lemmon, P.E., 1957. A new instrument for measuring forest overstory density. J. For. 55, 667–668.
- López, R., Salazar, L., 1989. Meloidogyne arabicida sp. n. (Nemata: Heteroderidae) nativo de Costa Rica: un nuevo y severo patógeno del cafeto. Turrialba 39, 313– 323.
- Miranda Arauz, A., 1989. Consideraciones para uniformar los resultados de un análisis químico de suelo—separata curso regional sobre nutrición mineral del café 1988. IICA PROMECAFE, Guatemala, 33 pp.
- Norton, D.C., 1989. Abiotic soil factors and plant-parasitic mematode communities. J. Nematol. 21, 299–307.
- Pinochet, J., Fernández, C., Sarah, J.L., 1995. Influence of temperature on in vitro reproduction of *Pratylenchus coffeae*, *P. goodeyi*, and *Radopholus similis*. Fundam. Appl. Nematol. 18, 391–392.
- Prot, J.L., Van Gundy, S.D., 1981. Effect of soil texture and the clay component on migration of *Meloidogyne incognita* second-stage juveniles. J. Nematol. 13, 213– 216.

- Quénéhervé, P., 1988. Population of nematodes in soils under banana cv. Poyo in the Ivory Coast. 2. Influence of soil texture, pH and organic matter on nematode populations. Revue de Nématologie 11, 245–251.
- Quénéhervé, P., 1989. Population of nematodes in soils under banana, CV. Poyo, in the Ivory Coast. 3. Seasonal dynamics of populations in mineral soil. Revue de Nématologie 12, 149–160.
- Robinson, A.F., Heald, C.M., Flanagan, S.L., Thames, W.H., Amador, J., 1987. Geographical distributions of *Rotylenchulus reniformis*, *Meloidogyne incognita*, and *Tylenchulus semipenetrans* in the lower Rio Grande valley as related to soil texture and land use. Annals Appl. Nematol. 1, 20–25.
- Salas, L.A., Echandi, E., 1961. Parasitic nematodes in coffee plantations of Costa Rica. Coffee 3, 6–9.
- Savary, S., Madden, L.V., Zadoks, J.C., Klein-Gebbink, H.W., 1995. Use of categorical information and correspondance analysis in plant disease epidemiology. In: Andrews, J., Tommerup, I., Callow, J.A. (Eds.), Advances in Botanical Research, vol. 21. Academic Press Ltd., London, pp. 213–240.
- Siddiqui, I.A., Shaukat, S.S., Hamid, M., 2002. Role of zinc in rhizobacteria-mediated suppression of root-infecting fungi and root-knot nematode. J. Phytopathol. 150, 569–575.
- Siddiqui, Z.A., Iqbal, A., Mahmood, I., 2001. Effects of *Pseudomonas fluorescens* and fertilizers on the reproduction of *Meloidogyne incognita* and growth of tomato. Appl. Soil Ecol. 16, 179–185.
- Siddiqui, Z.A., Mahmood, I., 1998. Effect of a plant growth promoting bacterium, an AM fungus and soil types on the morphometrics and reproduction of *Meloidogyne javanica* on tomato. Appl. Soil Ecol. 8, 77–84.
- Souza, R.M., Bressan-Smith, R., 2008. Coffee-associated *Meloidogyne* spp.—ecology and interaction with plants. In: Souza, R.M. (Ed.), Plant-parasitic Nematodes of Coffee. Springer, Berlin, pp. 123–147.
- Souza, R.M., Volpato, A.R., Viana, A.P., 2008. Epidemiology of *Meloidogyne exigua* in an upland coffee plantation in Brazil. Nematologia Mediterranea 36, 13–17.
- Taylor, A.L., Loegering, W.Q., 1953. Nematodes associated with root lesion in abaca. Turrialba 3, 8–13.
- Taylor, D.P., Netscher, C., 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica 20, 268–269.
- Umesh, K.C., Ferris, H., Bayer, D.E., 1994. Competition between the plant-parasitic nematodes *Pratylenchus neglectus* and *Meloidogyne chitwoodi*. J. Nematol. 26, 286–295.
- Upreti, G., Bittenbender, H., Ingamells, J.L., 1992. Rapid estimation of coffee yield. In: Proceedings of the Fourteenth International Scientific Colloquium on Coffee. San Francisco, 14–19 July 1991, ASIC, Paris pp. 585–593.
- Villain, L., 2008. Economic importance, epidemiology and management of *Praty-lenchus* sp. in coffee plantations. In: Souza, R.M. (Ed.), Plant-parasitic Nematodes of Coffee. Springer, Berlin, pp. 65–84.
- Villain, L., Hernández, A., Anzueto, F., 2008. Central America. In: Souza, R.M. (Ed.), Plant-parasitic Nematodes of Coffee. Springer, Berlin, pp. 261–275.Villain, L., Molina, A., Sierra, S., Decazy, B., Sarah, J.L., 2000. Effect of grafting and
- Villain, L., Molina, A., Sierra, S., Decazy, B., Sarah, J.L., 2000. Effect of grafting and nematicide treatments on damage by the root-lesion nematode *Pratylenchus* spp. on *Coffea arabica* L. in Guatemala. Nematropica 30, 87–100.
- Villenave, C., Cadet, P., 1999. Influence of soil organic matter and ion concentration on some Senegalese plant-parasitic nematodes. Eur. J. Soil Biol. 35, 189–197.
- Wardle, D.A., Yeates, G.W., Watson, R.N., Nicholson, K.S., 1995. The detritus foodweb and the diversity of soil fauna as indicators of disturbance regimes in agroecosystems. Plant Soil 170, 35–43.
- Widmer, T.L., Mitkowski, A., Abaw, G.S., 2002. Soil organic matter and management of plant-parasitic nematodes. J. Nematol. 34, 289–295.