SPATIAL DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN SUGARCANE FIELDS

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ABSTRACT: Nematodes are important parasites of sugarcane in Brazil. Because of this, several studies have been conducted in recent years to evaluate the effectiveness of control methods. However, no studies have been reported on the spatial distribution of nematodes in sugarcane fields, and such studies are indispensable for the development of sampling plans, aimed at their application in integrated management programs. The spatial distribution of *Meloidogyne javanica, Pratylenchus zeae*, and mixed populations of *P. zeae* and *P. brachyurus* was studied in six commercial fields, with areas ranging from 0.26 to 0.50 ha; samples were obtained during the rainy season. The samples, represented by about 50 g roots, were collected within a rectangular grid measuring 7 × 12 m, 7 × 10 m or 5 × 13 m. Among the ten calculated semivariograms (four for *M. javanica* populations and six for *P. zeae* populations or the mixture between *P. zeae* + *P. brachyurus*), four could not be fitted to any model and presented a pure nugget effect; the spherical model showed the best fit to the semivariograms of data observed in the other six conditions. In those cases, values of range in semivariogram varied from 18 to 35 m, allowing the nematode aggregation area to be estimated at 2,110 m^2, on average, suggesting that at least five sampling points per hectare would be necessary, on average to obtain a reliable estimate for the population of these plant parasites in a given area.
Key words: *Saccharum*, *Meloidogyne*, *Pratylenchus*, spatial variability, population

DISTRIBUIÇÃO ESPACIAL DE NEMATÓIDES FITOPARASITOS EM ÁREAS CULTIVADAS COM CANA-DE-AÇÚCAR

RESUMO: Nematóides são importantes parasitos da cana-de-açúcar no Brasil e, em função disso, muitos estudos foram conduzidos, nos últimos anos, para avaliar a eficiência de medidas de controle. Entretanto, não há estudos sobre a distribuição espacial de nematóides em canaviais e esses estudos são essenciais para o desenvolvimento de planos de amostragem, visando aplicação em programas de manejo integrado. A distribuição espacial de *Meloidogyne javanica, Pratylenchus zeae* e de populações mistas de *P. zeae* e *P. brachyuurs* foi estudada em seis campos comerciais, com áreas de 0,26 a 0,50 ha, nas quais procedeu-se à amostragem na época chuvosa do ano. As amostras, representadas por cerca de 50 g de raízes, foram coletadas em grid retangular de 7 × 12 m, 7 × 10 m ou 5 × 13 m. Dos dez semivariogramas calculados (quatro para populações de *M. javanica* e seis para populações de *P. zeae* ou mistura de *P. zeae* + *P. brachyurus*), quatro não se ajustaram a qualquer modelo, apresentando efeito pepita puro; o modelo esférico foi o que melhor se ajustou aos semivariogramas dos dados observados nas outras seis situações. Nesses casos, o alcance variou de 18 a 35 m, permitindo estimar a área de agregação dos nematóides em 2110 m^2, em média, sugerindo que, seriam necessários, em média, pelo menos cinco pontos de amostragem por hectare para obter uma estimativa confiável da população desses fitoparasitos em determinada área.
Palavras-chaves: *Saccharum*, *Meloidogyne, Pratylenchus,* variabilidade espacial, populações

INTRODUCTION

Among the many species of nematodes found in association with sugarcane, there are three parasites for the Brazilian conditions: *Meloidogyne javanica* (Treub) Chitwood, *M. incognita* (Kofoid & White), and *Pratylenchus zeae* Graham (Lordello, 1981; Novaretti & Téran, 1983: Moura et al., 1990). On average, these species cause productivity losses between 20 and 40% in the first cut of susceptible varieties, also reducing the productivity - and consequently the longevity - of sugarcane ratoon stands (Dinardo-Miranda, 2006).

Several studies have been conducted in order to evaluate the effectiveness of control methods such as chemical nematicides (Dinardo-Miranda & Garcia, 2002; Silva et al., 2006), resistant varieties (Dinardo-Miranda et al., 1996; 2003b; Garcia et al., 1997), organic matter (Novaretti & Nelli, 1985; Dinardo-Miranda et al., 2003a), and crop rotation (Rosa et al., 2003; Dinardo-Miranda & Gil, 2005). However, no studies report on the spatial distribution of nematodes in sugarcane fields. Such studies are indispensable for the development of sampling plans, aimed at their application in integrated management programs (Taylor, 1984; Southwood, 1978; Giles et al., 2000).

The spatial distribution of nematodes in the field is frequently described as aggregate (Ferris & Wilson, 1987), which means that there is spatial dependence between the populations in the sampled points. In view of this, geostatistics is the most adequate tool for the study of these populations, since it quantifies the spatial dependence between field-collected samples to be quantified and used to construct maps (Leibhold et al., 1993; Roberts et al., 1993; Ellsbeury et al., 1998).

The present study aimed to characterize the spatial distribution of *M. javanica*, *P. zeae* or mixed populations of *P. zeae* and *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven in sugarcane, using geostatistical analysis, so as to provide guidance for sampling procedures in the field.

MATERIAL AND METHODS

The spatial distribution of *M. javanica*, *P. zeae*, and mixed populations of *P. zeae* and *P. brachyurus* was studied in six commercial fields, whose characteristics are presented in Table 1. Areas ranging from 0.26 to 0.50 ha were selected in each field. Samplings were obtained in these areas during the rainy season, when populations usually peak due to favorable moisture and temperature conditions, as well as to abundant root growth. Samples were collected within rectangular grids measuring 7 × 12 m (areas 1 to 4), 7 × 10 m (area 5), or 5 × 13 m (area 6), (Table 1). Each sample was represented by about 50 g sugarcane roots, from which the nematodes were extracted by a combination of sifting and centrifugal flotation methods, in sucrose solution, according to Coolen & D´Herde (1972).

Data were initially analyzed by descriptive statistics and mean, standard deviation, coefficient of variation, maximum value, minimum value, skewness and kurtosis were obtained using the Statistical Analysis System (SAS Institute, 1995). In order to obtain lower coefficients of variation, data were transformed to log(x+1), and basic statistics were recalculated.

To verify the hypothesis of normality of the data, the Shapiro & Wilk (1965) test was carried out using the Statistical Analysis System (SAS Institute, 1995). After this, geostatistical analyses of the transformed data were run using semivariograms and kriging interpolation to construct maps, as described by Vieira et al. (1983). The semivariogram analyses were conducted using the GEOSTAT software (Vieira et al., 1983). Based on the models fitted to the semivariograms, the jackknifing test was used to verify whether the estimates of semivariogram parameters were adequate and to estimate the number of neighbors that should be used in kriging (Vieira, 2000). Once the parameters for the model were confirmed and the adequate numbers of neighbors were estimated, values were interpolated for the locations where they were not measured, by the kriging method, using the GEOSTAT software (Vieira et al., 1983). The kriging-estimated values were used in the Surfer software (Golden Software, 1999) to construct the maps.

Table 1 - Characteristics of the studied fields.

Characteristics	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6
	Itirapina 22°14'S; 47°48'W	S. Rita do Passa Quatro 21°42'S; 47°28'W			Guariba 21°21'S; 48°13'W	Piracicaba 22°43'S; 47°38'W
Variety	SP81-3250	RB72454	RB867515	SP81-3250	RB845210	RB855156
Cut (cicle)	2º	2º	2º	3º	2º	2º
Last harvest date	08/19/05	11/06/05	05/13/05	09/15/05	05/17/05	05/20/05
Sampling date	01/08/06	04/01/06	11/26/05	01/15/06	12/15/05	12/20/05
Sampled area (ha) (width × length)	0.50 ha (35 × 144 m)	0.50 ha (35 × 144 m)	0.50 ha (35 × 144 m)	0.33 ha (35 × 96 m)	0.28 ha (35 × 80 m)	0.26 ha (25 × 104 m)
Sampling grid	7 × 12 m	7 × 12 m	7 × 12 m	7 × 12 m	7 × 10 m	5 × 13 m
Nº of samples	78	78	78	54	54	54

RESULTS AND DISCUSSION

Among the important nematode species for sugarcane, *M. javanica* and the mixed population of *P. zeae* and *P. brachyurus* were found in area 1, *P. zeae* was found in area 2, *M. javanica* and the mixed population of *P. zeae* and *P. brachyurus* were found in area 3, *M. javanica* and *P. zeae* were found in area 4, *M. javanica* and *P. zeae* were found in area 5, and *P. zeae* was found in area 6. In all areas, great differences were observed between the maximum and minimum populations, both for *M. javanica* and *P. zeae* or for the *P. zeae* + *P. brachyurus* mixture, considering original data (Table 2) or transformed to log(x+1) data (Table 3).

Considering original data (Table 2) and the criteria proposed by Pimentel Gomes (2000), all populations presented very high coefficients of variation (CV > 30%). Analyzing transformed data, the variability was lower, and only *M. javanica* population in area 3, in area 4 and in area 5 still presented very high coefficients of variation (Table 3). For *M. javanica* population in area 1, the coefficient of variation was high and for *Pratylenchus* species the coefficients of variation were considered low or medium, with values between 5 and 12.3%.

For *M. javanica* the coefficients of variation were higher than the values obtained for *Pratylenchus* species, indicating that the variability of populations of that species was higher than the variability of *Pratylenchus* populations (Tables 2 and 3). The reasons for greater variability in *Meloidogyne* populations are related to its biology. Because *Meloidogyne* is a sedentary endoparasite, the females lay all their eggs at the same place, in masses, resulting in a highly-aggregated spatial pattern (Ferris et al., 1990). On the other hand, nematodes in the genus *Pratylenchus* are migratory and therefore move through the plant and the soil, laying their eggs individually, resulting in a more uniform distribution in the area.

Table 2 - Statistical parameters of the original data of plant-parasitic nematode populations in sugarcane.

Area	Nematode	Mean (nº/50 g of roots)	Minimum value	Maximum value	Coefficient of variation (%)	Variance	Skewness	Kurtosis	Shapiro-Wilk test
1	Mj	4371	0	24000	113.7	$2.4\ 10^7$	1.77	3.21	<0.0001
	Pz + Pb	13292	300	41000	68.7	$8.3\ 10^7$	1.15	1.09	<0.0001
2	Pz	15800	4000	35000	43.3	$4.6\ 10^7$	0.60	-0.09	0.0326
3	Mj	2410	0	17000	148.5	$1.2\ 10^7$	2.35	5.79	<0.0001
	Pz + Pb	2891	100	19800	120.6	$1.2\ 10^7$	3.16	11.14	<0.0001
4	Mj	753	0	6300	163.8	$1.5\ 10^6$	2.67	8.17	<0.0001
	Pz	18850	3400	47000	54.1	$1.0\ 10^8$	0.66	0.06	0.0455
5	Mj	698	0	16200	319.8	$4.9\ 10^7$	6.57	45.91	<0.0001
	Pz	7505	500	49000	110.3	$6.8\ 10^7$	3.13	12.28	<0.0001
6	Pz	4705	500	22700	84.7	$1.5\ 10^7$	2.25	7.16	<0.0001

Mj = *M. javanica;* Pz = *P. zeae;* Pb = *P. brachyurus.*

Table 3 - Statistical parameters of transformed data by log(x+1) of plant-parasitic nematodes populations in sugarcane.

Area	Nematode	Mean (nº/50 g of roots)	Median (nº/50 g of roots)	Minimum value	Maximum value	Coefficient of variation (%)	Variance	Skewness	Kurtosis	Shapiro-Wilk test
1	Mj	3.30	3.35	0	4.38	20.8	0.471	-1.58	5.63	<0.0001
	Pz + Pb	4.00	4.06	2.47	4.61	9.2	0.135	-1.27	3.25	0.0002
2	Pz	4.15	4.17	3.60	4.54	5.0	0.042	-0.54	0.32	0.0702
3	Mj	2.69	2.95	0	4.23	42.7	1.322	-1.26	1.00	<0.0001
	Pz + Pb	3.27	3.28	2.00	4.30	12.3	0.163	-0.18	1.82	0.0077
4	Mj	1.98	2.47	0	3.80	66.0	0.707	-0.67	-1.11	<0.0001
	Pz	4.20	4.21	3.53	4.67	6.5	0.075	-0.71	0.16	0.0262
5	Mj	1.60	2.00	0	4.20	87.1	1.943	-0.12	-1.68	<0.0001
	Pz	3.70	3.68	2.70	4.69	10.3	0.145	0.12	0.37	0.8840*
6	Pz	3.55	3.54	2.70	4.35	9.7	0.116	-0.05	-0.16	0.9974*

Mj = *M. javanica;* Pz = *P. zeae;* Pb = *P. brachyurus;* * = significant at 5%.

All variables original data did not follow normal distribution according to the Shapiro-Wilk test and to the skewness and kurtosis values (near zero) (Table 2). When transformed data were analyzed, two populations showed normal distribution (*P. zeae* in area 5 and area 6), with skewness and kurtosis values near zero (Table 3). However, for all *Pratylenchus* populations and for *M. javanica* populations in area 1, the mean values were very similar to the median values (Table 3), suggesting that the data presented a near to normal distribution in these cases.

Since using transformed data, the coefficients of variation, skewness and kurtosis were lower and most of then showed similar normal distributions, it was decided to use the transformed data to run the geostatistical analysis. However, according to Cressie (1991), it is not necessary that data present normal distribution to use geostatistical analysis; it is just convenient that the distribution curve does not show a long tail, endangering the results. So, considering the previous analysis of these data, it is possible to admit that the studied variables had appropriate distributions for geostatistical analysis.

Among the ten calculated semivariograms (four for *M. javanica* populations and six for *P. zeae* populations or the *P. zeae* + *P. brachyurus* mixture), those corresponding to *M. javanica* populations in areas 1, 3, and 5, and the semivariogram corresponding to the *P. zeae* population in area 4 exhibited a pure nugget effect (Table 4). This means that the spatial distribution of nematodes in the above-mentioned cases occurred simply at random, within the sampling distance used in the study, that is, the distance between sampling points was too large to allow the detection of a spatial dependence between them.

Regarding to studies involving insects, Liebhold et al. (1993) stated that the occurrence of a pure nugget effect is quite common and is mainly attributed to the fact that the spatial dependence occurs at a smaller spatial scale than the sampling scale that is often adopted. The same can be said of studies involving nematodes.

Mathematical models were fitted for the *M. javanica* populations in area 5, mixed *P. zeae* and *P. brachyurus* populations in areas 1 and 3, and *P. zeae* populations in areas 2, 5, and 6, and the spherical model was best fitted to the semivariograms of data of these six conditions (Figure 1, Table 4). Although the observed r^2 values were low, the parameters estimated for the spherical model (C_0, C_1, a) were endorsed by the jackknifing test, since the mean values for the reduced errors were near zero and the values for the variance of reduced errors were near 1 (Table 4).

The jackknifing test also revealed the ideal number of neighbors to be used in kriging. As a result, kriging was performed using 20 neighbors for the mixed population of *P. zeae* and *P. brachyurus* in area 1, 16 neighbors for the mixed population of *P. zeae* and *P. brachyurus* in area 3, 12 neighbors for *P. zeae* in area 2 and *M. javanica* in area 4, and 8 neighbors for *P. zeae* in areas 5 and 6. The constructed maps based on the data are presented in Figure 2.

The portion of variability attributed to spatial dependence, given by the $C_0/(C_0+ C_1)$ ratio, ranged from 0.29 to 0.68 (Table 4). These values indicate a moderate spatial dependence between samples (Cambardella et al., 1994). The range (a), representing the distance at which there is spatial dependence between samples, varied from 18 m for *P. zeae* in area

Table 4 - Parameters of fitted semivariograms and of jack kinifing, coefficients of determination (r^2), ratio $C_0/(C_0+C_1)$ of plant-parasitic nematodes populations in sugarcane.

Area	Nematode	Semivariogram parameters			Jack kinifing parameters (reduced errors)		r^2	$C_0/(C_0+C_1)$	Area (m^2)[1]
		C_0	C_1	a (m)	mean	variance			
1	Mj				pure nugget effect				
	Pz + Pb	0.07	0.063	20	0.010	1.070	0.32	0.53	1257
2	Pz	0.028	0.018	30	0.001	1.062	0.23	0.61	2827
3	Mj				pure nugget effect				
	Pz + Pb	0.1	0.063	20	-0.019	1.038	0.18	0.61	1256
4	Mj	1.2	0.63	35	-0.002	1.026	0.45	0.66	3848
	Pz				pure nugget effect				
5	Mj				pure nugget effect				
	Pz	0.04	0.1	18	-0.055	1.142	0.41	0.29	1018
6	Pz	0.085	0.04	28	-0.001	1.040	0.18	0.68	2463

Mj = *M. javanica;* Pz = *P. zeae;* Pb = *P. brachyurus.* [1] Area calculated by r^2, where p = 3.1416 and r = a.

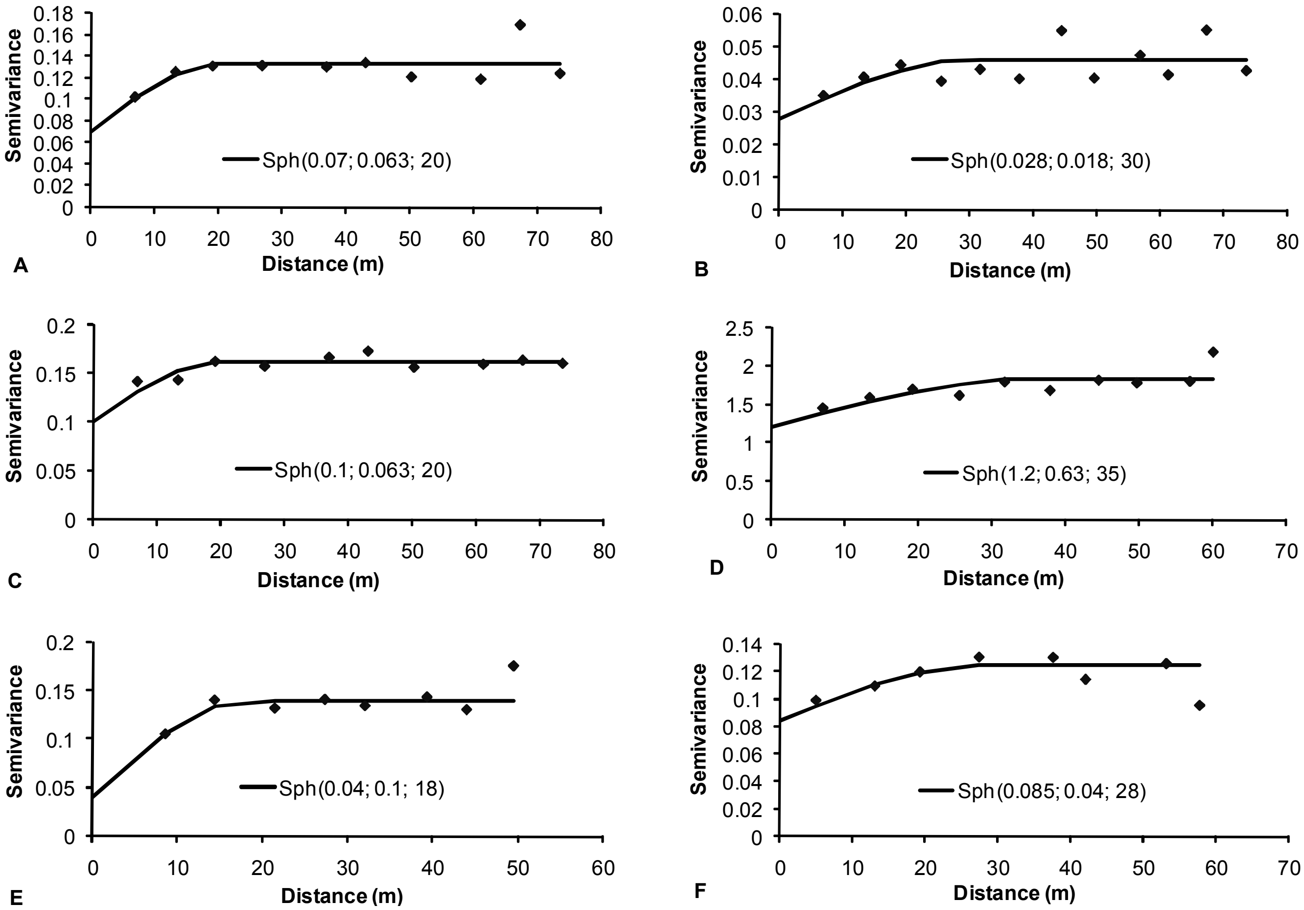

Figure 1 - Semivariograms for populations of (A) *P. zeae* + *P. brachyurus* in area 1, (B) *P. zeae* in area 2, (C) *P. zeae* + *P. brachyurus* in area 3, (D) *M. javanica* in area 4, (E) *P. zeae* in area 5 and (F) *P. zeae* in area 6. Numbers in parenthesis are nugget effect value (C_0), C_1 and range (a) of spherifal model (Sph).

5, to 35 m for *M. javanica* in area 4, therefore revealing that samples separated from one another by distances smaller than 18 or 35 m, for *P. zeae* or *M. javanica*, respectively, would not be statistically independent. These data allowed us to estimate that the nematode aggregation area ($A = \Pi^2$, in which r = a), in the various fields, varied from 1.018 m^2 to 3.848 m^2, with an average value of 2.106 m^2, suggesting that five sampling points per hectare would be necessary, on average, to obtain a reliable estimate for the nematode population in a given area. The samples would be collected at distances of 25 m. This value is higher than the value currently recommended for sampling in commercial areas (2 points/ha; Dinardo-Miranda, 2006), suggesting that changes should be made in the sampling plans in order to improve nematode population estimates.

Other authors have also revealed that the spherical model of the semivariogram best describes the spatial distribution of plant-parasite nematodes, with similar range values as those herein determined. Caswell & Chellemi (1986), studied the spatial distribution of *Rotylenchulus reniformis* for pineapple in Hawaii and found a 10 m range value, and by. Farias et al. (2002) worked with the same species in cotton in Brazil and determined a mean range value of 15 m. Webster & Boag (1992) worked with *Heterodera avenae* and *Globodera rostochiensis* and also verified that the spherical model best fitted to the obtained data, with an approximate range of 60m. In the same way, Wallace & Hawkins (1994) worked with a forage grass and fitted the spherical model to a semivariogram for *Tylenchorhynchus* spp. population data, with a range value around 40 m.

The data herein presented reveal that the use of geostatistics is viable to evaluate the spatial distribution of plant-parasite nematodes in sugarcane and to define sampling plans aimed to implement an integrated management program. However, further studies are required, especially those involving areas infested with *Meloidogyne*, since in the present work it was not possible to prepare maps for three of the four sampled areas, in which this species was present, because a larger sampling scale was adopted

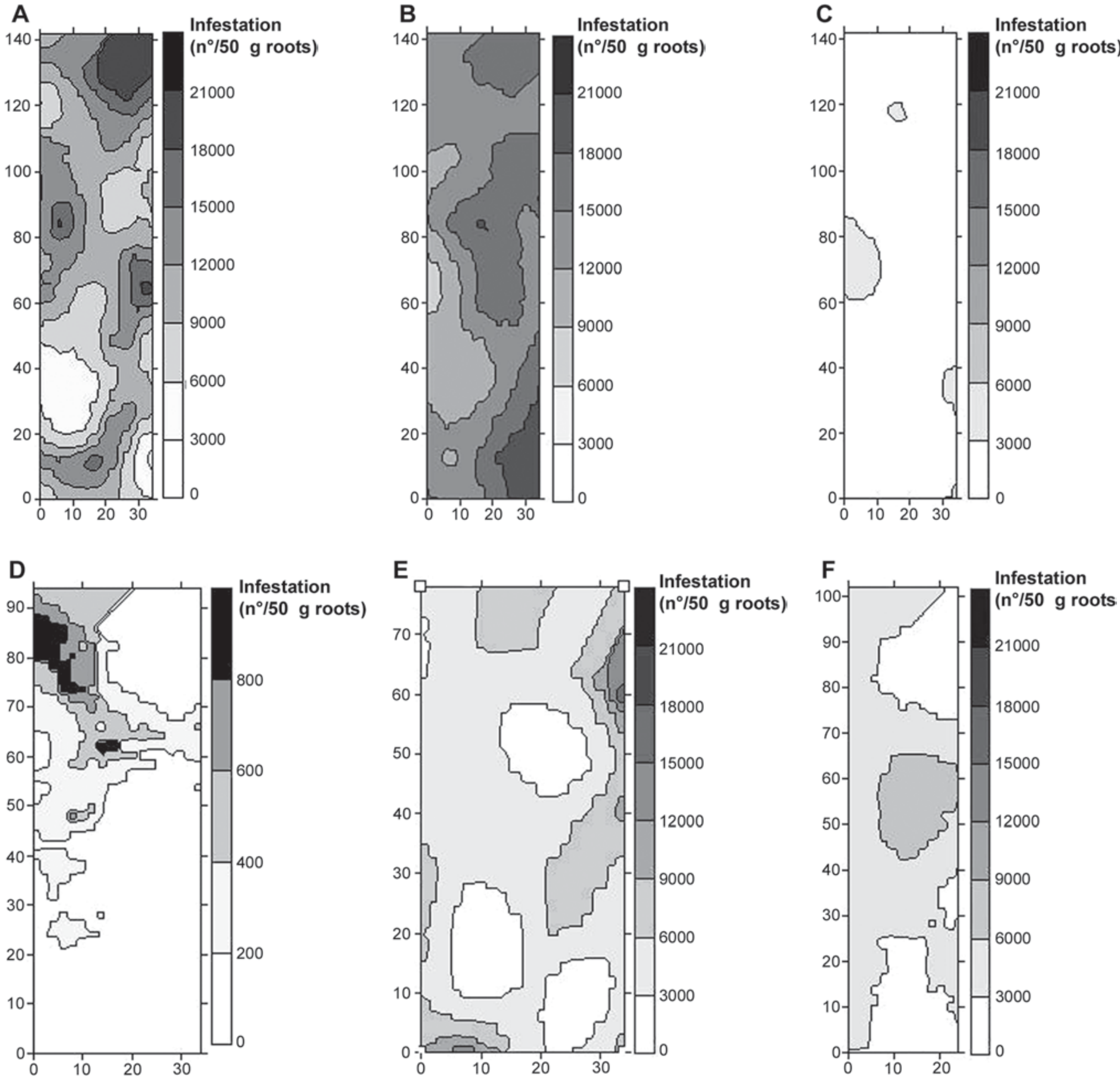

Figure 2 - Maps of the spatial distribution of (A) *P. zeae* + *P. brachyurus* in area 1, (B) *P. zeae* in area 2, (C) *P. zeae* + *P. brachyurus* in area 3, (D) *M. javanica* in area 4, (E) *P. zeae* in area 5 and (F) *P. zeae* in area 6.

than the scale at which spatial dependence occurs between the points. In addition, the commercial cultivation of sugarcane in Brazil involves a large number of varieties, grown at the most diverse production environments, which are factors that interfere with the spatial distribution of nematodes. Therefore, our data suggest that in order to obtain a reliable estimate of nematode populations in a given area, at least five samples per hectare must be collected, on average; however, more studies are required due to the great variations in sugarcane-cultivation conditions that exist in the country.

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