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Spatial pattern analysis of three nematode populations associated with chilli

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Summary – Spatial patterns of *Tylenchorhynchus curvus, Pratylenchus thornei* and *Aphelenchus avenae* were examined. Pattern was detected using Lloyd's and Morisita's indices of dispersion while the scale and intensity of pattern were analysed using Greig-Smith's mean square (variance)/block size analysis. Data were collected from a chilli field according to a systematic grid sampling scheme. Dispersion indices revealed contagious distribution of all three nematode populations. *Tylenchorhynchus curvus* and *P. thornei* exhibited highest mean squares at block size 16 (patch size of 16 m²) and *A. avenae* at block size 8 (patch size of 8 m²). *T. curvus* and *P. thornei* showed high covariance with *p*H and maximum water holding capacity at block size 16. The possible implications of spatial pattern of nematode populations in designing an efficient sampling design are discussed.

Résumé – ***Analyse de la distribution spatiale des populations de trois nématodes associés au poivron*** – La répartition spatiale de *Tylenchorhynchus curvus, Pratylenchus thornei* et *Aphelenchus avenae* a été étudiée. Les modèles de répartition ont été précisés en utilisant les indices de dispersion de Lloyd et Morisita tandis que l'échelle et la valeur de ces modèles ont été analysées par la méthode du carré des moyennes (variance)/ taille du bloc, suivant Greig-Smith. Les données ont été recueillies dans un champ de poivrons suivant un système codifié de prélèvements. Les indices de dispersion révèlent une répartition contagieuse des populations des trois nématodes *T. curvus* et *P. thornei* montrent la plus forte moyenne des carrés pour la taille de bloc 16 (parcelles de 16 m²) et *A. avenae* pour la taille de bloc 8 (parcelle de 8 m²). *T. curvus* et *P. thornei* montrent une covariance élevée avec le *p*H et la capacité maximum de rétention d'eau du sol pour la taille de bloc 16. Les implications possibles de la répartition spatiale des populations de nématodes pour la mise au point d'un système de prélèvements efficace sont discutées.

Key-words : distribution, *Tylenchorhynchus, Pratylenchus, Aphelenchus,* chilli.

Nematodes are seldom uniformly or randomly distributed in an area such as an agricultural field. Understanding of the spatial distribution patterns of plant-parasitic nematodes is essential in order to formulate efficient sampling strategies and also for the design of field experiments (Barker & Campbell, 1981; Ferris *et al.*, 1981; McSorley, 1982; Ferris, 1985). Since the spatial pattern of nematodes is usually contagious (Goodell & Ferris, 1980; Boag & Topham, 1984; McSorley *et al.*, 1985; Ferris *et al.*, 1990), the sampling variance is high.

Most of the previous studies on spatial pattern of nematodes were either concerned with fitting negative binomial distribution to observed nematode data (Goodell & Ferris, 1980; McSorley, 1982; McSorley & Parrado, 1982; Noe *et al.*, 1981) or describing the relationship between variance and mean by Taylor's power law (Mathias, 1969; McSorley *et al.*, 1985; Duncan *et al.*, 1989; Ferris *et al.*, 1990). However, Noe and Campbell (1985) and Francl (1986) used block-quadrat variance methods and Alby *et al.* (1983) used Morisita's index at various quadrat sizes to analyse the distribution pattern of plant parasitic nematodes.

The objectives of this investigation were : *i)* to test whether or not the distributions of three nematode populations were non-random, *ii)* to determine the scale and intensity of pattern in the nematode populations, and *iii)* to relate the observed pattern with two environmental variables.

Materials and methods

A chilli (*Capsicum annuum* L.) field plot located at the Crop Diseases Research Institute, Karachi University Campus, was sampled for population densities of three plant parasitic nematodes with a contagious grid of quadrats. The soil was sandy loam and was maintained at field capacity. The grid was 8 × 8 m divided into 64 1 m² grid units (quadrats). Each grid unit had two rows of three chilli plants. The distance between rows was 50 cm and the distance between plants was approximately 33 cm. A 66 × 50 cm area within the two rows in each quadrat was randomly sampled by drawing four replicate soil cores each 13-mm-diam. × 189-mm-deep (*ca* 25 cm³). The four replicate cores were pooled to obtain a composite sample of 100 cm³ soil for each quadrat. Two plant parasitic nematodes viz. *Tylenchorhynchus curvus* Williams, 1960 and *Pratylenchus thornei* Sher & Allen, 1953 and a fungus feeder *Aphelenchus avenae* Bastian, 1865 were separated from the composite soil sample by elutriation and centrifugation (Byrd *et al.*, 1976) and counted. For the purpose of soil analysis, a soil sample of 200 g was collected from a depth of 15 cm from the centre of each quadrat. Sieved (2 mm aper-

ture) soil samples were analysed for pH and maximum water holding capacity (Cox, 1976).

Pattern detection

An index of pattern detection that is relatively insensitive to changes in density is required to detect spatial pattern (Myers, 1978). Accordingly, Lloyd's (1967) index of patchiness *(c)* and Morisita's (1971) index (I_δ), that are unaffected by changes in density caused by random thinning, were used. First mean crowding $\overset{*}{m}$ (Llyod, 1967) is calculated as :

$$\overset{*}{m} = \frac{1}{N}\sum_{i=1}^{Q} X_i (X_i - 1)$$

where X_i is the number of individuals in the *i*th *quadrat*, Q is the number of quadrats and $N = \Sigma X_i$.

Index of patchiness C is then computed as $C = \overset{*}{m}/\lambda$ where λ equals the mean density per quadrat. Morisita's (1971) index which is almost equivalent to Lloyd's index of patchiness is :

$$I_\delta = \frac{Q\sum_{i=1}^{Q} X_i (X_i - 1)}{N (N - 1)}$$

Estimates of the pattern detection indices in literature are invariably reported without any indication of sampling variance. The jackknife method (Tukey, 1958) and the bootstrap method (Efron, 1979) not only allow estimation of variance and confidence interval but also permit bias reduction. Accordingly, jackknife and bootstrap estimates of the pattern detection indices and their variances were obtained. The variance/mean ratio for each nematode species was also computed.

The scale of heterogeneity was determined for the three nematode populations using the block-quadrat method developed by Greig-Smith (1961, 1983). This method involves a hierarchical analysis of variance of the data obtained from a grid of contiguous quadrats which are blocked in successive powers of two (Ludwig & Reynolds, 1988). The method is easily modified to compute covariance (or correlation) between a pair of variables at successive block sizes. The variances at the two smallest block sizes are calculated as :

$$\text{Variance (block size 1)} = \frac{2}{n}\left[\frac{1}{2}(X_1 - X_2)^2 + \frac{1}{2}(X_3 - X_4)^2 + \ldots + \frac{1}{2}(X_{n-1}) - X_n)^2\right]$$

$$\text{Variance (block size 2)} = \frac{4}{n}\left[\frac{1}{4}(X_1 + X_2 - X_3 - X_4)^2 \ldots + \frac{1}{4}(X_5 + X_6 - X_7 - X_8)^2 + \ldots + \frac{1}{4}(X_{n-3} + X_{n-2} - X_{n-1} - X_n)^2\right]$$

and the covariances are computed as :

$$\text{Covariance (block size 1)} = \frac{2}{n}\left[\frac{1}{2}(X_1 - X_2)(Y_1 - Y_2) \ldots + \frac{1}{2}(X_3 - X_4)(Y_3 - Y_4) + \ldots + \frac{1}{2}(X_{n-1} - X_n)(Y_{n-1} - X_n)\right]$$

$$\text{Covariance (block size 2)} = \frac{4}{n}\left[\frac{1}{4}(X_1 + X_2 - X_3 - X_4)(Y_1 + Y_2 - Y_3 - Y_4) \ldots + \frac{1}{4}(X_5 + X_6 - X_7 - X_8)(Y_5 + Y_6 - Y_7 - Y_8) \ldots + \frac{1}{4}(X_{n-3} + X_{n-2} - X_{n-1} - X_n)(Y_{n-3} + Y_{n-2} - Y_{n-1} - Y_n)\right]$$

where X_i is the number of individuals per quadrat of each species.

Computer programs were written in Applesoft BASIC to compute pattern detection indices using jackknife and bootstrap estimation procedures and to analyse variance and covariance using Greig-Smith's method.

Results and discussion

Detection of pattern

Figure 1 shows the maps of density distribution pattern of the three nematodes in the grid analysed. *Tylenchorhynchus curvus* shows a high density phase at the upper left and a large patch of high density in the lower right. *Pratylenchus thornei* exhibits high density phases in the middle and lower right portion of the grid. By contrast *Aphelenchus avenae* shows high density patches at three corners of the grid. Mean densities of *T. curvus, P. thornei* and *A. avenae* per 100 cm³ size were 83.75, 51.31 and 28.59 respectively while the variance/mean ratios were 95.87, 93.53 and 7.87 respectively. All the ratios are highly significantly different from 1 the expected ratio for random distribution ($p < 0.001$), thus exhibiting high degrees of aggregation.

Morisita's index and patchiness (Table 1) for all three species were high. In particular the indices were very high for *T. curvus* and *P. thornei* populations. Therefore, all the populations of nematodes show highly contagious distributions. Mean crowding was higher for *T. curvus* than for *P. thornei* but the reverse was true for Morisita's index and index of patchiness, because of the lower mean density of *P. thornei.*

Variances of bootstrap estimates of the pattern detection indices were invariably lower than those of jackknife estimates. This was presumably at the cost of slightly increased bias (Mueller & Altenberg, 1985).

The analysis of pattern

Pattern detection indices and dispersion indices indicate the degree of population aggregation. Analysis of

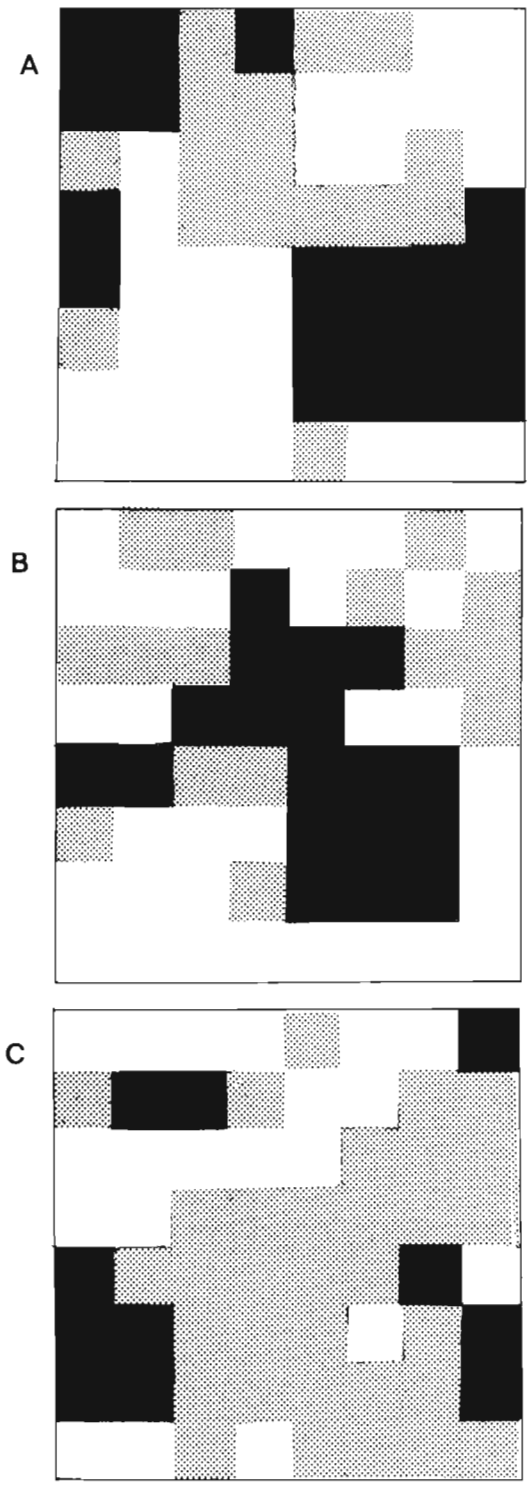

Table 1. Jackknife and bootstrap estimates of Lloyd's index of mean crowding index, Morisita's index and Lloyd's index of patchiness together with corresponding variances for three species of nematodes.

	Mean crowding index		Morisita's index		Patchiness index	
	Bias adjusted index	Variance	Bias adjusted index	Variance	Bias adjusted index	Variance
	JACKKNIFE ESTIMATES					
Tylenchorhynchus curvus	183.379	987.430	2.1597	0.0813	2.1598	0.0813
Pratylenchus thornei	145.557	878.90	2.7757	0.2338	2.7748	0.2339
Aphelenchus avenae	35.536	15.231	1.2444	0.0087	1.2445	0.0087
	BOOTSTRAP ESTIMATES					
Tylenchorhynchus curvus	184.451	937.40	2.1145	0.0650	2.1141	0.0650
Pratylenchus thornei	150.393	641.170	2.8580	0.1324	2.8570	0.1323
Aphelenchus avenae	35.109	11.6990	1.2197	0.00584	1.2190	0.00583

pattern discloses the scale and intensity of pattern. The mean square (variance)/block size graphs resulting from the pattern analysis of the three nematode populations (Fig. 2) indicate a primary peak at block size 16 and a secondary peak at block size 4 corresponding to patch sizes of 16 and 4 m² for *T. curvus*. *P. thornei* also showed a peak at block size 16 but it was less prominent than that of *T. curvus*. By contrast *A. avenae* showed a primary peak at block size 8 and a secondary peak at block size 2 corresponding to patches of size 8 and 2 m² (Fig. 2). The high mean square of *A. avenae* at block size 32 has no significance because of the lowest degrees of freedom at this block.

T. curvus and *P. thornei* showed a high positive covariance at block size 16 and a negative covariance at block size 4 (Fig. 3) indicating that the two species co-occur in patches of 16 m². On the other hand, *T. curvus* and *A. avenae* showed a high negative covariance at block size 16 and a smaller positive covariance at block size 8 suggesting that the high density patches of roughly 16 m² dimension of the two nematode species alternate with each other. Similarly, *P. thornei* and *A. avenae* also exhibited a high negative covariance at block size 16.

Fig. 1. The map of density distribution pattern of the three nematode species in the grid used for analysis. A : *Tylenchorhynchus curvus;* B : *Pratylenchus thornei;* C : *Aphelenchus avenae.*

(Black area represents > mean density + 0.5 standard deviation (SD), shaded area mean density – 0.5 SD to mean density + 0.5 SD, clear area < mean density – 0.5 SD.)

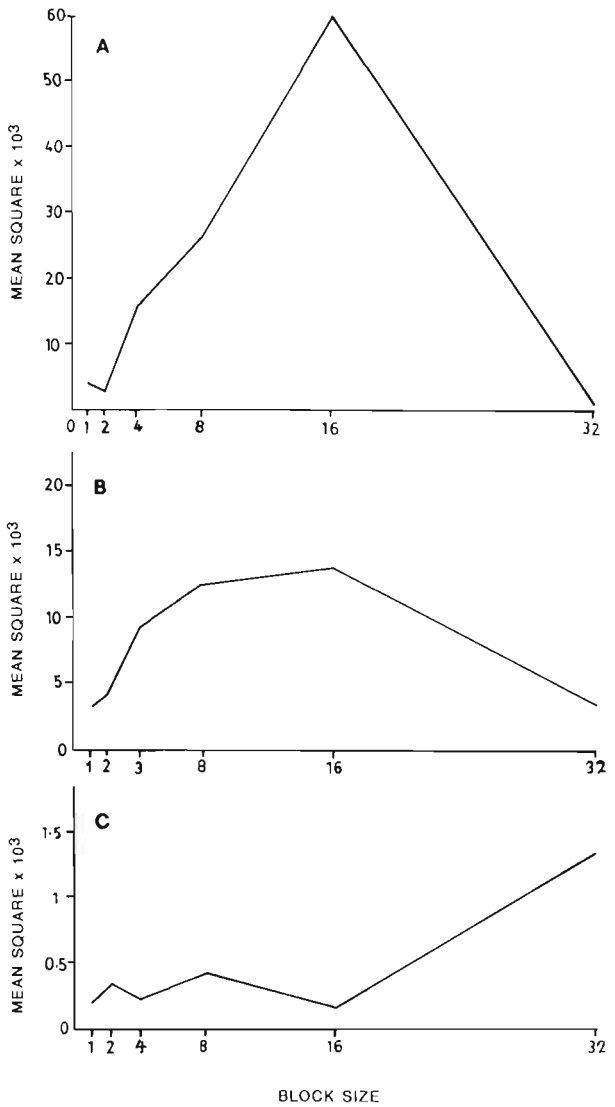

Fig. 2. Mean square/block-size graphs derived from block-quadrat variance analysis for the three nematode species. A : *Tylenchorhynchus curvus;* B : *Pratylenchus thornei;* C : *Aphelenchus avenae.*

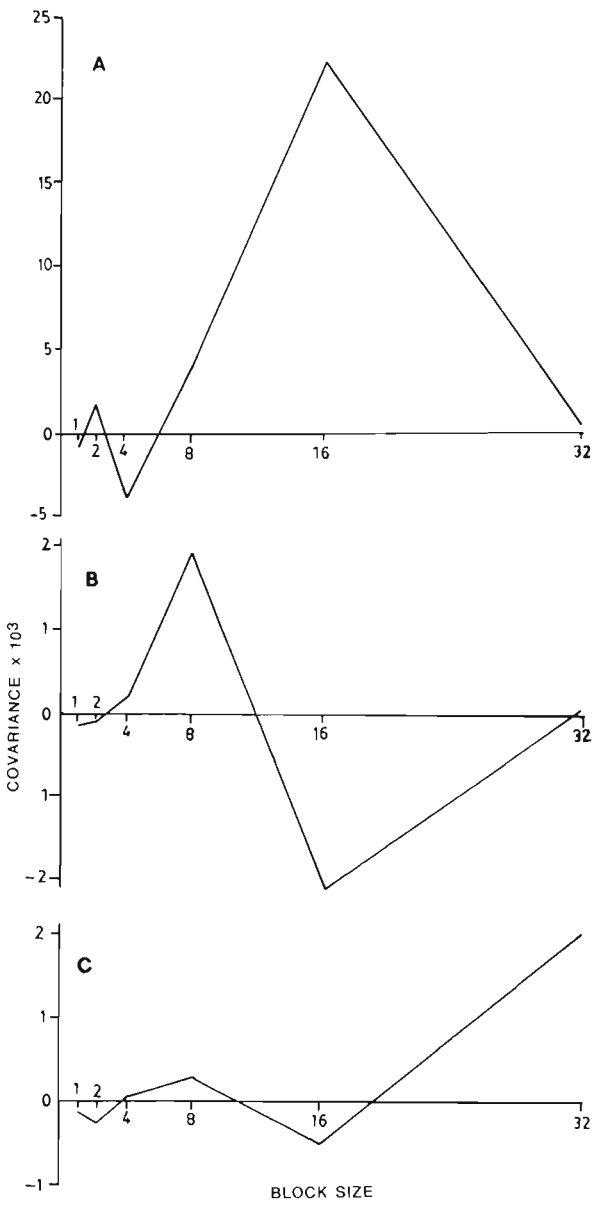

Fig. 3. Covariance/block-size graphs derived from block-quadrat covariance analysis between pairs of nematode species. A : *T. curvus/P. thornei;* B : *T. curvus/A. avenae;* C : *P. thornei/A. avenae.*

T. curvus and *P. thornei* populations exhibited high covariances with the maximum water holding capacity (MWHC) of soil at block size 16 (Fig. 4). These two species also had high variance at this block size. Therefore, soil moisture could be an important factor in controlling the distribution pattern of *T. curvus* and *P. thornei.* By contrast, *A. avenae* did not show significant covariance (correlation) with MWHC at any of the block sizes. *T. curvus* and *P. thornei* also appear to be correlated with soil pH at block size 16, showing increased covariance at this block size. Covariances of *A. avenae* with soil pH were of low order at all the block sizes examined. Thus, the distribution pattern of *A. avenae* does not seem to be correlated with pH. Besides the environmental factors considered for correlating the small-scale distribution pattern of the nematode populations, other soil factors such as soil organic matter content and spacing could be important (Francl, 1986). In addition, morphology of the crop plant root system may also be responsible for the observed pattern.

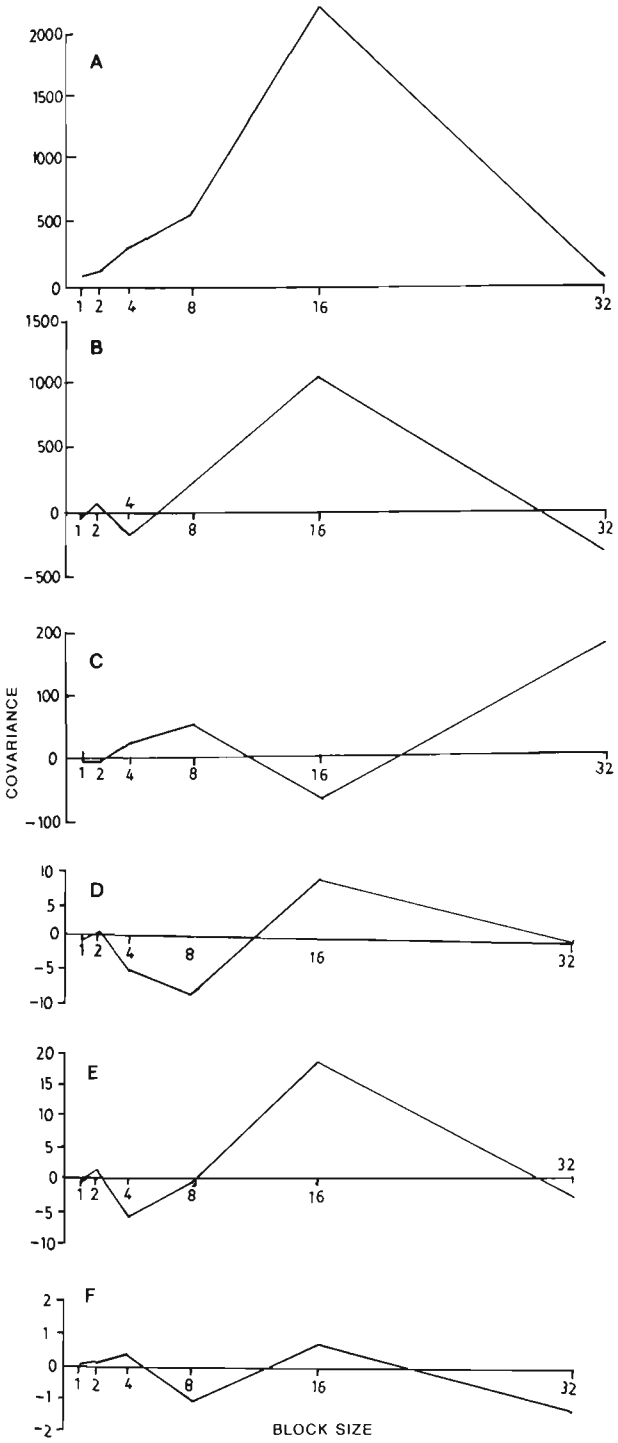

Fig. 4. Covariance/block-size graphs derived from block-quadrat covariance analysis between nematode species and edaphic factors. A : *T. curvus*/maximum water holding capacity (MWHC); B : *P. thornei*/MWHC; C : *A. avenae*/MWHC; D : *T. curvus*/pH; E : *P. thornei*/pH; F : *A. avenae*/pH.

Spatial heterogeneity in the nematode population densities as observed in the present study, may present serious problems in the analysis and interpretation of experimental data. In particular, lack of randomness should be anticipated. Undoubtedly, the information collected on the spatial distribution of a nematode species in a given field allows maximization of sampling accuracy and efficiency and precision in experimental design. Although the pattern analysis technique of Greig-Smith (1961) is restricted to blocks in powers of 2 of the grid unit (quadrat size) it gives a rough idea of patch size. Estimates of nematodes patch size provide a guide to the selection of optimum plot sizes. Patch sizes of single species are depicted by the variances (mean square) in the analysis of pattern. In experiments such as those evaluating the nematicidal efficacy or varietal trials the plot size in an experimental design should be chosen equivalent to that block size which shows low mean square in the analysis of pattern. Significant gain in efficiency may result at plot sizes corresponding to those yielding lower mean square. The information obtained in the spatial pattern analysis can also be useful in determining optimum shape and orientation of plots (Noe & Campbell, 1985). A greater number of experimental units should be taken to increase the reliability when the mean square at a specific block size in the experimental design is anticipated to be high or moderate. Furthermore analysis of covariance (ANCOVA) should be employed rather than the usual analysis of variance (ANOVA) taking into account that environmental variable which exhibits greater covariance at the specific block size used.

The design of experiments or sampling strategy involving two or more nematode species should also take into account the covariance structure in addition to variance of the species.

Most sampling designs for nematodes are directed at sampling for one particular species. Since often more than one nematode species are found to be associated with the crop, a multispecies sampling and a multivariate analysis is necessary as opposed to a univariate approach. The species covariance structure at different block sizes bears considerable significance in such a situation. The covariance (or correlation) matrices pertaining to different block sizes may be used to develop ordinations or dendrograms based on hierarchical agglomerative clustering which may be compared to look for consistency of pattern information and thereby arrive at an optimal plot size for multispecies sampling. Details of this procedure have been presented elsewhere (Shaukat, 1988).

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