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DETERMINATION OF CLUSTER SIZE OF *PRATYLENCHUS PENETRANS* PHYTONEMATODE IN A ROSE FIELD

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ABSTRACT
A nursery field 21 m x 80 m was sampled sequentially for Pratylenchus penetrans by decreasing the plot sizes systematically. Plots sizes of 3.6 m x 8 m, 3.6 m x 3.6 m and 0.6 m x 0.6 m were sampled. Nematode counts were computed to obtain the respective sample mean and variance. The sample mean and variance were computed to obtain a and b values of the Taylor's power law. It is reported that the a and b-values of the Taylor's power law are influenced by plot size and number of cores per sample.

Keywords: core number, plot size, *Pratylenchus penetrans*, Cluster size.

INTRODUCTION
The damage potentials of nematodes depend on their population density at the time of planting host crop. There is therefore a relationship between nematode numbers and yield of crops (Seinhorst,1965). Thus the management of nematode populations in away, aims at manipulations that reduce numbers below, or prevent numbers from reaching economic thresholds (Brown and Kerry, 1987). These principles underscore the concept of integrated pest management (Weil, 1990), which evolved in response to environmental concerns arising from the use of inorganic nematicides (Duncan, 1991). Most plant parasitic nematodes are clustered in their distribution (Cotton, 1979; Goodell and Ferris, 1980; Seinhorst, 1982). The implications of a clustered distribution are, high variability in nematode counts with variance to mean ratios that are at disunity and confidence intervals around nematode population density estimates are often broad (McSorley, and Dickson 1991; Boag and Topham, 1984). Under this condition it has become very difficult to obtain accurate and precise nematode counts, which are fundamental to nematode management. To obtain accurate nematode count it is imperative to determine cluster size of nematode populations.

Taylor's power Law is one mathematical formula that has been extensively used to describe the horizontal clustered distribution of nematodes. (Taylor, 1961; 1970; 1984). This law relates the variance of a population to the mean by parameters that determine clustered distribution (Duncan *et al.,* 1989). The law is expressed by the equation ($S^2 = ax^b$). He observed that the variance (S^2) of a population is proportional to a fractional power (b) of the arithmetic mean (x). (a) depends on the sample size and b is an index of dispersion (Elliot, 1971; Mahfouz, 1992). This law has been credited with its usefulness in the development of sampling plans, transformation of field data and satisfactory quantitative measurement of nematode aggregation (Taylor, 1970; Caubel et al., 1972; Ripley, 1981; Perry, 1983). However, the weakness of Taylor's Power Law was outlined as its insensitivity in predicting variance of means close to zero and it lack the measure of distance which is inherently understood in the concept of aggregation (Boag and Topham, 1984; Boag *et al.,* 1992). Moreover, distinct differences were observed between different nematode species, this queries its general application among species (Mathias, 1969).

Pratylenchus penetrans, a root lesion nematode, is an endoparasite of nursery stocks of various ornamentals and tree crops. Due to its biology it is found both in the soil and plant roots. The aim of this investigation is to determine the relative cluster size of *P. penetrans* in a field using the coefficients of Taylor's power law. In this regard, the optimum plot size at which the distribution of the nematode tends to be random is investigated.

MATERIALS AND METHODS

Samples were collected from a parcel of land located in Wetteren, about 15 kilometers from the city of Ghent in Belgium. The plot of land is part of Gebroeders Dierick's farm and has been used for the cultivation of tree nursery of *Robinia pseudoacaeia* and *Aeer plaranoides,* (Rose Plant) in the past 5 years. Farm records indicate that no nematicide nor nematode control measure have been administered on the parcel since its inception. Tilling was done by ploughing and harrowing at a depth of about 30 cm with conventional machines.
The plot of land measuring 1680 m^2 (21 m x 80 m) was divided into grids of 3.65 m x 8 m and a total of 50 single core samples were collected at points of intersection of the grid lines. The samples were collected with an auger that is 20 cm in length and 2.5 cm in diameter. They were immediately put into plastic bags, labeled and taken to the laboratory for storage at 15 ^oC for subsequent extraction and counting.

Based on the nematode counts obtained from each sampling point in the first sampling stated above, three categories of sites were chosen to represent points of low, medium and high populations (0-100, 101-200 and >200 *P. penetrans*/ 100g soil and above respectively). Plots (3.60 m x 3.60 m) were obtained using the points of intersection as center. These plots were then subdivided by perpendicular grid lines, 0.6 m apart and 36 samples were collected at point of intersections of these grid lines. Furthermore, using these last points as centers, plots of 0.6 m x 0.6 m were mapped out and divided by perpendicular grid lines that are 0.1 m apart. Finally, at the point of intersection of these lines, 36 samples were collected. This procedure was repeated for each of the three categories of plots (3.6m x 8m; 3.6m x 3.6m and 0.6m x 0.6m) in figures 1, 2 and 3. Sections of figure 2, labeled A, B, C and figure 3, labeled D, E and F respectively represent areas of high, medium and low concentration within the same type of plot size. A total of 590 samples were taken for extraction and counting. All the samples taken were single core samples and were treated as such.

Nematodes were extracted from the soil samples using the centrifugal floatation methods introduced by Caveness and Jensen (1955) and modified by Coolen and D'Herde (1972). The principle behind this method is the flocculation of the nematodes by kaolin in the first centrifugation and the floating out of the nematodes into the denser liquid in the second centrifugation. They are finally collected on a 5 u*m* sieve. The nematodes were then washed off the sieve and suspended in 100 ml water. Two replicates of 10 ml each were counted in a De Grisse counting dish and the average count multiplied by 10 gave the nematode count per 100 gm soil. This procedure was repeated for all the 590 samples. The nematodes were then washed off the sieve and suspended in 100 ml water. The mean and variance (S^2) were calculated using the statistical package of Lotus 1-2-3. These parameters were calculated for each set and subset of samples and the parameter a and b of Taylor's Power Law ($S^2=ax^b$) were also calculated based on the equation $\log S^2 = \log a + b \log x$. A computer simulation was done to determine changes in a and b values with various numbers of cores at different sampling precision levels using a computer program written in FORTRAN. In addition the Wings statistical package, which runs under UNIXS, was used to obtain graphical presentation of contours indicating the horizontal distribution of the nematodes in the sampled field. Soil particle size was analyzed, using a COULTER^R LS analyzer. The particle sizes were determined by percentage volume of various particle size ranges occurring in a sub sample pulled from all the samples collected.

RESULTS

The results obtained for soil analysis, indicate that the soil is sandy with a particle size range of 50um to 350um. A graphical representation of the *P. penetrans* distribution in 3.6 m x 3.6 m plots for high (A); medium (B) and low (C) densities is shown in Figure 1. Similar, in Figure 2 the distribution of the nematode in 0.6 m x 0.6 m plots with high (D); medium (E) and low (F) densities are shown. In both figures maps on the same row are replicates. The parameters a and b of the Taylor's power law were obtained for 9 plots measuring 3.6 m x 3.6 m and 6 plots measuring 0.6 m x 0.6 m by regressing the sample variances (S^2) on the sample means (X) in table 1. The regression equation used was log S^2 =log a + b log x. From table 2, it is observed that the a-value for the smaller plots is higher than that of the bigger plots; the opposite is true for the b-values.

To obtain data on the influence of the number of cores composing samples on the a- and b-values of Taylor's power law, samples were generated through computer simulations. This was done for the 3.6 m x 3.6 m and 0.6 m x 0.6 m plots. Five samples, each composed of 1, 2, 3, 4 or 5 cores per low density area were randomly generated in the three replicates yielding 15 combinations of x and S^2 of samples composed of 6, 12, 18, 24 and 30 cores. This relationship was plotted for all the samples and sets of samples but only two are presented for clarity of explanation (fig.3). The a and b-values obtained as such were regressed on the number of cores. Because of their out laying characters a and b-values of the 24-cores and I8-cores, samples of the bigger and smaller plots respectively were omitted. The regression lines were linear; only the relationship between a-values and core numbers in smaller plots was of different nature (Table 3 and fig. 6).

DISCUSSION

The contour maps of figure1 suggest that nematodes are clustered in irregular patterns. It also indicates that cluster sizes are wider at higher densities than at lower densities. From figures 2 and 3, compared to the bigger plot in figure. 1, it is noticeable that nematodes are more evenly distributed in smaller plots especially when they occur at high densities.

The regression line fit the data well (high R^2). From the observation of the tables and the related figures it is obvious that for the bigger plots (3.6 m x 3.6 m) the higher the number of cores composing the samples the lower the a-value; for the smaller plots (0.6 m x 0.6 m) the opposite is true. The b-values have the opposite relationship with the number of cores. The a- and b-values of the Taylor's power law equation (S^2= a x^b) have been extensively used in the study of nematode horizontal distribution with less emphasis on cluster sizes. The b-values obtained from the present study as shown in Table 2 are in the range of those obtained by other workers. The *a* parameter which is related to sample size was reported to show a general decrease as plot size decreased from 1.2 to 0.2 ha (McSorley, 1987). **In** this study the *a* value increased as plot size decreased from 12.95 m^2 to 0.36 m^2. Thus it seems that the sensitivity of the a-parameter is variable in micro-plots. The b-parameter is less variable and decreases with decreasing plot size. Simulation experiments on the effects of increasing the number of cores per sample suggest that the b-value is only weakly influenced. The a-value decreases as the number of cores per sample increase. This relationship is only more pronounced in the smaller plots.

Generally, the overall results obtained in this study suggest that the possible range of sampling and experimental error accruable from quantitative nematological studies, particularly in horizontal distribution is wide and of diverse sources. This is particularly true when plot sizes used for quantitative studies are well in excess of relative cluster sizes. Consequent upon this view, most of the results reported in current literature on this subject may be influenced by plot size.

CONCLUSION

Based on the results obtained in this study it is concluded that populations of *Pratylenchus penetrans* occur in clusters. The size of these clusters in terms of horizontal distribution is irregular and dependents on the plot size. The parameters a and b of the Taylor's power law which are function of the sample size and index of dispersion respectively, are both influenced by plot size and the number of cores taken per sample. Consequently these parameters are more sensitive as experimental plot size approximates cluster size and may not be stable as it is reported in literature. Finally, it is suggested that the a and b parameters of the Taylor power law may be more useful in the estimation of nematode population. This will require further research on smaller plots.

Table 1:Table of Means and Standard deviation for *P. penetrans* counts obtained from field data

Plot size		Mean	S^2
3.6 m x 3.6 m	A	319.61	20391.96
		278.89	13104.32
	B	125.69	7557.15
		271.94	26226.77
		381.61	21861.96
	C	225.83	17934.30
		133.89	6323.77
		209.44	21044.00
0.6 m x 0.6 m	D	386.67	6811. 11
		336.39	9184.18
	E	411.11	22715.43
		277.50	17007.64
	F	136.94	9554.55
		76.94	9632.33

Table 2: Influence of plot size on the parameters of Taylor's power law

Plot Size (m x m)	**Parameters of Taylor's Power Law**	
	A	b
3.6 x 3.6	0.43	1.55
0.6 x 0.6	3.2	0.35

Table 3: Relationship between a-value (Y) and number of cores a sample (X) (Source: field Data)

Plot size	Regression Equation	R^2	P
3.6 x 3.6	$Y=0.8752 - 0.01544x$	0.958	0.0211
0.6 x 0.6	$Y=2.291x^{0.1008}$	0.988	0.0058

Table 4 : Relationship between b-value (Y) and number of cores a sample (X)

Plot size	Regression Equation	R^2	P
3.6 x 3.6	Y=1.3578+0.00742X	0.978	0.011
0.6 x 0.6	Y=0.5074-0.00533X	0.981	0.0094

(Source: field Data)

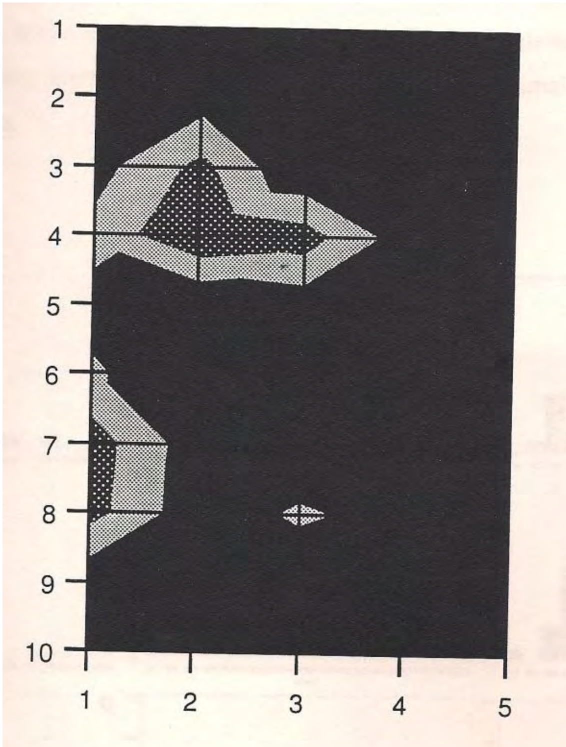

Fig 1: Contour map of P.penetrans distribution in 3.6 x 8m plot

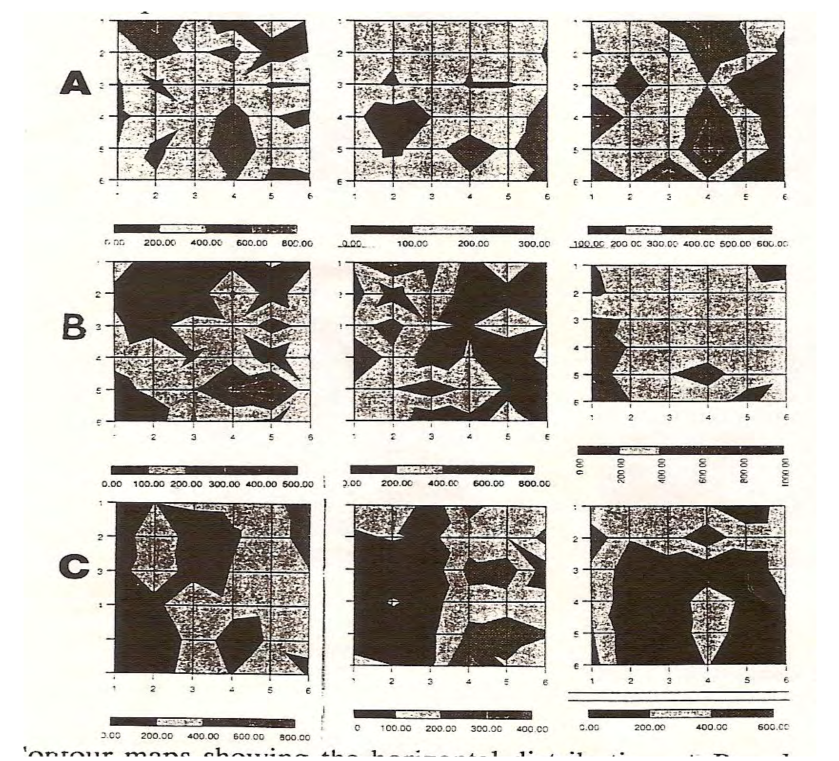

Fig 2 Cluster Pattern of *P. penetrans* in 3.6 x 3.6m plots, A, B, C indicate High, Medium and Low Densities respectively

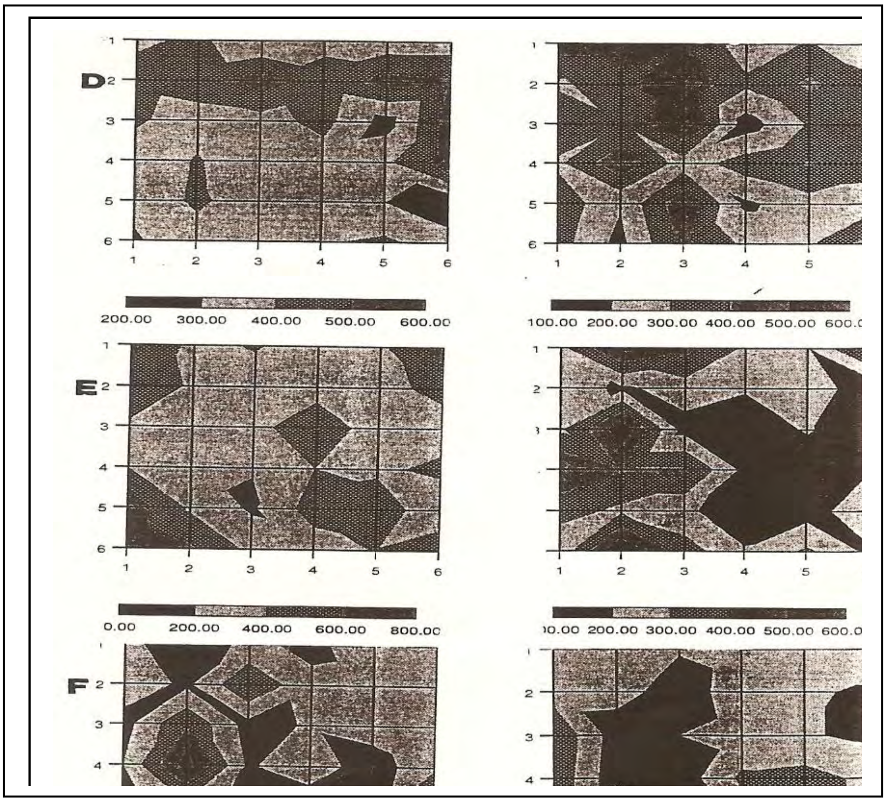

Fig. 3: Cluster Pattern of *P. penetrans* in 0.6 x 0.6m plots, E, F, D indicate High, Medium and Low Densities respectively

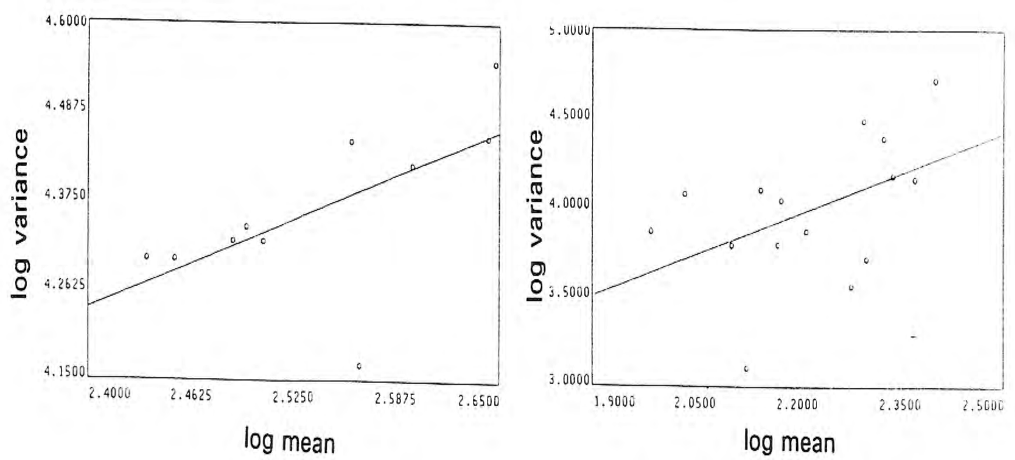

Fig.4 Two examples of regression lines showing the relationship between log variance and log mean for sets of *P. penetrans* count

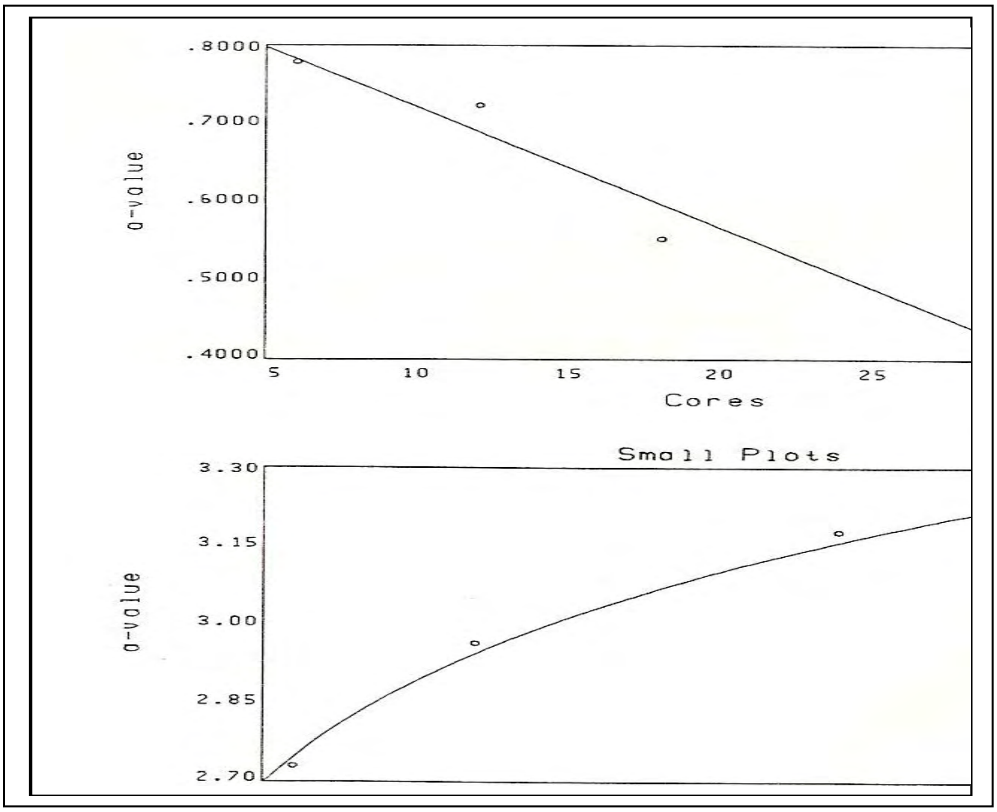

Fig 5: Regression lines of a-value of Taylor.s Power Law on number of cores per

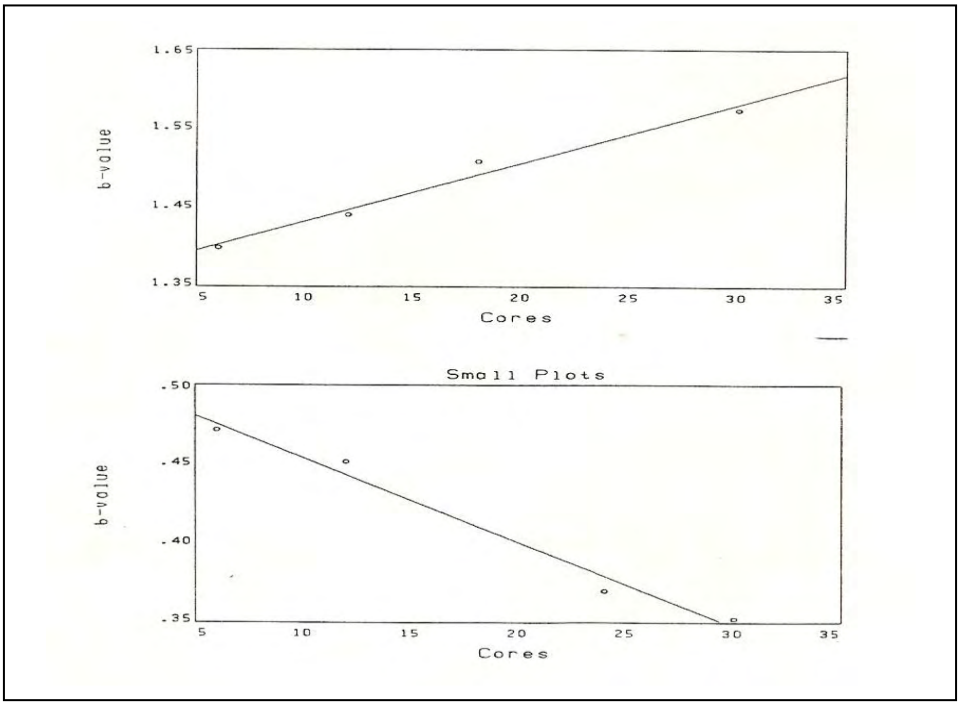

Fig 6: Regression lines of a-value of Taylor,s Power Law on number of cores per sample

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