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Spatial distribution of the phytonematode community in Egyptian citrus groves

Mahfouz M. Abd-Elgawad

Pests and Plant Protection Department, National Research Centre, El-Tahrir St., Dokki, Giza, P.O. Box 12311, Egypt.

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Summary — Taylor's power law was fit ($P \leqslant 0.01$) to population data of *Tylenchulus semipenetrans*, *Helicotylenchus pseudorobustus* and *Criconemella* spp. obtained from stratified random sampling (seven cores/sample) and of *T. semipenetrans* collected in systematic pattern (one core/sample) from citrus trees in the Northern and Southern Tahrir Province, Egypt. Differences between each of *a* and *b* values for the regression lines of these nematodes were statistically investigated. Sample size optimization needed to achieve a predetermined level of sampling error for the nematodes was calculated. Data transformation of $X^{0.03}$ for *T. semipenetrans*, 10 $(1 - X^{-0.03})$ for *H. pseudorobustus* and 10 $(1 - X^{-0.09})$ for *Criconemella* spp. were calculated from parameters of Taylor's power law fit to the survey data.

Résumé — ***Répartition spatiale de la communauté des nématodes phytoparasites dans des vergers de citrus égyptiens*** — La règle de puissance de Taylor ($P \leqslant 0.01$) s'applique aux données concernant les populations de *Tylenchulus semipenetrans*, *Helicotylenchus pseudorobustus* et *Criconemella* spp. obtenues lors des prélèvements randomisés et stratifiés (sept carottes par échantillon), ainsi qu'à *T. semipenetrans* collecté suivant un schéma systématique (une carotte par prélèvement) dans des vergers de citrus du nord et du sud de la Province de Tahrir, Égypte. Les différences entre chacune des valeurs de *a* et *b* de la droite de régression concernant ces nématodes ont été étudiées statistiquement. La taille optimale de l'échantillon nécessaire pour un niveau prédéterminé de l'erreur d'échantillonnage a été calculée. Les données transformées de $X^{0.03}$ pour *T. semipenetrans* $10(1 - X^{-0.03})$ pour *H. pseudorobustus* et $10(1 - X^{-0.09})$ pour *Criconemella* spp. ont été calculées à partir des paramètres de la règle de puissance de Taylor convenant aux données de l'enquête.

Key-words : Citrus, Egypt, nematodes.

Sample size is a key factor in estimating the level of plant-parasitic nematodes in citrus orchards (Duncan & Cohn, 1990). Knowledge of the nematode spatial distribution can be used to develop sample size optimization by using equations derived from negative binomial models (McSorley, 1982; McSorley & Parrado, 1982) or by simulation from the data base even if the model is not negative binomial (Goodell & Ferris, 1980). Both of these approaches require specific computer programs and therefore Taylor's power law provides a convenient alternative for developing sampling plans. The power law (Taylor, 1961) states that : the variance (S^2) of a population is proportional to a fractional power (b) of the arithmetic mean ($\bar{x}$) :

$$S^2 = a\bar{x}^b \qquad \text{or} \qquad \log S^2 = \log a + b \log \bar{x}$$

where *a* and *b* are population parameters; *a* depends chiefly upon the sample size and *b* is an index of dispersion (Elliott, 1971). Thus, the power law is useful in determining transformations (Taylor, 1970) and developing nematode sampling plans (Ferris, 1984). It covers a wider range of distributions and the transformations derived from *b* are often easier to apply than these of the negative binomial (Elliott, 1971).

The presence of two or more nematode species parasitizing a single citrus tree is a common occurrence in nature. The present study considers relationships among the levels of some nematode species under orchard conditions and examines sample optimization by two different sampling plans based on surveys of nematode spatial distribution. The plans cover sampling schemes for large and moderate as well as small citrus orchards. The effect of transforming nematode data according to Taylor's power law to equalize variance prior to statistical analysis was tested.

Materials and methods

Two hundred and fourty five citrus tree blocks in Northern and Southern Tahrir Province in Egypt, each equal to 2.1 ha and bordered with raised soil burms, were sampled between May and August 1990 for soil stages of the phytonematodes encountered. The tree blocks were divided into 2.1 ha areas in order to get leveled divisions to control surface irrigation and each was surrounded by wind break trees. The blocks were 24-year-old but differed with respect to scion-rootstock combinations, edaphic conditions and management practices. Seven subsamples, each from a random tree, were taken with a hand trowel (*ca* 6 cm diam. × 30 cm deep) beneath the tree canopy at 1.5 m from the trunk and composited into a single sample representing a tree

Table 1. Taylor's power law regression equations for data from two sampling methods [y].

Sampling methods and nematode species	n	r^2	Power law parameters	
			a	b
STRATIFIED RANDOM SAMPLING				
Tylenchulus semipenetrans	35	0.853 **	0.83	1.95
Helicotylenchus pseudorobustus	26	0.659 **	0.54	2.05
Criconemella spp.	11	0.962 **	0.36	2.18
Pratylenchus brachyurus	7	0.599 $^{n.s.}$	—	—
Tylenchorhynchus spp.	6	0.613 $^{n.s.}$	—	—
SYSTEMATIC SAMPLING				
T. semipenetrans (2.1-ha sites)	24	0.929 **	1.175	1.95
T. semipenetrans (4.2-ha sites)	12	0.952 **	1.690	1.98
T. semipenetrans (6.3-ha sites)	8	0.935 **	3.972	1.90
T. semipenetrans (8.4-ha sites)	6	0.974 **	4.932	1.89

[y] n = number of nonzero points. r^2 = coefficient of determination for fit to equation $\log s^2 = \log a + b \log \bar{x}$, where $\bar{x}$ = mean, s^2 = variance. Asterisks (**) indicate significance at P = 0.01; $^{n.s.}$ = no significant regression.

block (2.1 ha). In a second survey of *T. semipenetrans* conducted in March 1991, in the same province, one soil and root core (*ca* 6 cm diam. × 30 cm deep) for each sample was collected from beneath the canopy of a single tree. Samples from seven trees were obtained from each of 24 adjacent 2.1 ha sites. An every K th (K = 3 × 5 = 15 citrus trees) systematic sampling (Cochran, 1977) was followed where the sampled tree located at the centre of the fifteen trees.

In both surveys, a 250 g portion of each soil and root sample was analyzed. Soil nematodes were extracted with a modified sieving centrifugation technique (Jenkins, 1964). The extracted nematodes were counted at 80 × on a dissecting microscope and adult specimens were transferred to a compound microscope for species identification. Forty percent of each sample suspension was counted, corresponding to 100 g soil. All data analyses were performed on the actual, untransformed counts.

For each nematode, means ($\bar{x}$) and variances (S^2) for all stages extracted from soil were computed over seven samples from each 14.7-ha area (35 sites total). The systematic sampling pattern used in the second survey allowed the data base to be arranged as 24 2.1-ha sites of 7 samples each, 12 4.2-ha sites of 14 samples each, 8 6.3-ha sites of 21 samples each or 6 8.4-ha sites of 28 samples each. Means and variances of *T. semipenetrans* counts for each of these scenarios were calculated as well. The parameters *a* and *b* were determined for the equation $S^2 = a\bar{x}^b$ (Taylor, 1961) by regressing *Log 10* variances on *Log 10* means for the 35 points in the first survey and the 24, 12, 8 and 6 points in the second survey. A transformation procedure to normalize data in order to perform parametric statistics was determined according to the formulae $Y = x^{(1-0.5\,b)}$ for positive powers and $Y = 10(1 - x^{(1-0.5\,b)})$ for negative powers (Healy & Taylor, 1962; Taylor, 1970). Slope and intercept values from the regression lines were compared with t-tests (Kleinbaum & Kupper, 1978).

Sample data (unpubl.) obtained on eight dates from three field experiments were analysed to compare ratios of the largest to the smallest treatment variance estimates of untransformed data, log transformed data, and data transformed according to the above-mentioned formulae. Wilcox's signed rank test (Steel & Torrie, 1960) and the homogeneity of variance estimate test (Pearson & Hartly, 1966); were both used in the comparison. Samples were taken from individual trees of similar size and canopy density located in a completely randomized design. Three subsamples were taken with a hand trowel (*ca* 6 cm diam. × 30 cm deep) beneath each tree canopy and composited to form a sample representing a tree. In one experiment, each of four different doses of a growth regulator compound was sprayed on five trees to test its effect on *T. semipenetrans* reproduction. The other two experiments tested the effect of a nematicide on the populations of *Helicotylenchus pseudorobustus* and *Criconemella* spp. For each nematode genus, the nematicide was applied to five trees and other five trees were left to serve as a control in the same area. All three experiments were sampled simultaneously on each of three different dates whereas the first experiment was sampled alone on five additional dates. Each of the experimental areas was less than one-third of the survey block area (2.1 ha). Mean separation by Duncan's multiple-range test was performed using both log-trans-

Table 2. Number of samples needed to achieve a predetermined level of accuracy, as defined in terms of standard error to mean ratio[z] for nematode sampling.

Mean count per sample [y]	Minimum number of samples [x]			
	Stratified random sampling [w] Level of accuracy		Systematic sampling [w] Level of accuracy	
	20 %	25 %	20 %	25 %
Tylenchulus semipenetrans			(2.1-ha sites)	
100	17	11	23	15
300	16	10	22	14
1000	15	9	21	13
3000	14	9	20	13
Taylor's power law parameters : *a* = 0.83, *b* = 1.95			*a* = 1.175, *b* = 1.95	
Helicotylenchus pseudorobustus			(4.2-ha sites)	
100	17	11	39	25
300	18	12	38	24
1000	19	12	37	24
Taylor's power law parameters : *a* = 0.54, *b* = 2.05			*a* = 1.69, *b* = 1.98	
Criconemella spp.			(6.3-ha sites)	
100	21	13	63	40
300	25	16	56	36
1000	31	20	50	32
Taylor's power law parameters : *a* = 0.36, *b* = 2.18			*a* = 3.972, *b* = 1.90	
			(8.4-ha sites)	
100			74	48
300			66	42
1000			58	37
Taylor's power law parameters :			*a* = 4.932, *b* = 1.89	

[z] Assume $t = 2$ for 95 % confidence interval (Ferris, 1984).

[y] Based on a sample size of 100 g.

[x] All fractional values rounded up to nearest integer.

[w] The stratified random sampling was conducted in a 14.7-ha site for three nematode genera. Whereas the systematic sampling was applied to different areas for *T. semipenetrans* only.

formed data and data transformed according to Taylor's power Law.

Results

The nematodes found in the soil in the first survey and the average percentages of the total nematode community were : *Tylenchulus semipenetrans* Cobb (81.2 %), *Helicotylenchus pseudorobustus* (Steiner) Golden (11.7 %), *Criconemella* spp. (6.5 %), *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven (0.2 %) and *Tylenchorhynchus* spp. (0.4 %). Mean population levels of *T. semipenetrans* were 830 and 756/100 g soil in the first and second surveys, respectively.

In the first survey, Taylor's power law could be fit ($P \leqslant 0.01$) to data for *T. semipenetrans, H. pseudorobustus* and *Criconemella* spp. (Figs 1-3), but not those for *Pratylenchus brachyurus* and *Tylenchorhynchus* spp. (Table 1). Each sample in the stratified random sampling method had detectable nematode populations whereas eighteen samples were free of *T. semipenetrans* in the systematic survey. Using the parameters of Taylor's power law (Ferris, 1984), the number of samples needed to achieve a predetermined level of sampling error was calculated (Table 2). The nematode genera are listed (Table 2) in an increasing order of their degree of aggregation according to the parameter *b*. The number of samples required by a less aggregated nematode genus is fewer than that by a more aggregated one for a given specific level of nematodes and sampling error. For example, to sample *T. semipenetrans*, *H. pseudorobustus* or *Criconemella* spp. on citrus trees of 14.7-ha area with a 0.20 standard error to mean ratio and 1000 nematodes/100 g soil, one could collect 15, 19 or 31 samples, respectively (Table 2).

The slope values of the first geographic survey were used, resulting in the normalizing transformation $y = x^{0.03}$ for *T. semipenetrans*; $y = 10(1 - x^{-0.03})$ for *H. pseudorobustus* and $y = 10(1 - x^{-0.09})$ for *Criconemella* spp. The use of these equations as well as the base 10 logarithms in the transformation of data of three experiments reduced ($p \leqslant 0.01$) variance ratios compared to untransformed data. In this latter, heterogeneity of variances between treatments existed (Table 3) for both *T. semipenetrans* ($p \leqslant 0.05$; $p \leqslant 0.01$) and *Criconemella* spp. ($p \leqslant 0.05$). In contrast, the transformation served to equalized variances at these levels (Table 3). Use of data transformed according to Taylor's power law did not reduce the largest to smallest variance ratios compared to log transformation in 63 % of the cases considered (Table 3). Accordingly, the *F* values from analysis of variance and results of mean separation techniques were similar to the log-transformed data for the three nematode genera on the eight occasions; examples are given in Table 4.

Table 3. Comparison of the ratios of the highest treatment variance to the lowest treatment variance from nematode soil population counts and from transformed counts $^+$.

Sample	*Log x*	$x^{0.03}$	*Untransformed*
T. semipenetrans			
1	12.59	12.72	17.98
2	3.94	3.72	14.41
3	14.47	14.28	20.80 *
4	7.59	7.63	18.80
5	17.19	3.07	51.30 **
6	1.92	1.82	2.06
7	3.16	3.37	17.47
8	8.81	8.55	9.72
H. pseudorobustus		$10(1 - x^{-0.03})$	
9	1.19	1.13	6.46
10	1.73	1.81	2.23
11	1.11	1.12	1.09
Criconemella spp.		$10(1 - x^{-0.09})$	
12	2.55	2.41	12.78 *
13	1.15	1.15	1.31
14	1.95	1.81	4.74

Samples were obtained from citrus orchards on eight occasions and were from three field experiments, one experiment for each nematode genus.

+ Transformation calculated from $z = x^p$ for *T. semipenetrans* counts and $z = 10(1 - x^p)$ for counts of *H. pseudorobustus* and *Criconemella* spp. where $p = 1 - 0.5b$ (Healy & Taylor, 1962).

*, ** Significant at the 0.05 and 0.01 probability levels, respectively ($k = 4$, $v = 4$ for *T. semipenetrans* and $k = 2$, $v = 4$ foi *Criconemella* spp.); test of heterogeneity of variance (Pearson & Hartley, 1966 : Table 31).

Table 4. Results of mean separation of nematodes/100 g soil according to Duncan's multiple range test on transformed data $^+$.

Number of comparisons of ordered means	LSR$_{0.05}$		Treatment mean	
	(log x)	$(x^{0.03})^z$	*(log x)*	$(x^{0.03})$
Tylenchulus semipenetrans				
2	0.07	0.007	2.64 *a*	1.204 *a*
3	0.08	0.008	2.60 *a*	1.200 *a*
4	0.08	0.008	2.80 *b*	1.219 *b*
			2.76 *b*	1.216 *b*
$F_1 = 16.19$,	$F_2 = 15.16$	($v_1 = 3$,	$v_2 = 16$)	
Helicotylenchus pseudorobustus		$10(1 - x^{-0.03})$		$10(1 - x^{-0.03})$
2	0.65	0.39	1.87 *a*	1.21 *a*
			2.55 *b*	1.61 *b*
$F_1 = 5.75$,	$F_2 = 5.64$	($v_1 = 1$,	$v_2 = 8$)	
Criconemella spp.		$10(1 - x^{-0.09})$		
2	0.77	0.90	2.57	4.12
			2.82	4.39
$F_1 = 0.54$,	$F_2 = 0.46$	($v_1 = 1$,	$v_2 = 8$)	

$^+$ Data are from three experiments in Table 2, samples 8, 9 and 12 respectively. Treatments consisted of different doses of a growth regulator compound in the first experiment whereas a relatively low dose of systemic nematicide was used in the others.

^{z} Transformation calculated from $y = x^{(1 - 0.5b)}$ for *T. semipenetrans* and $y = 10(1 - x^{1 - 0.5b})$ for *H. pseudorobustus* and *Criconemella* spp. where *x* is the nematode count and *b* is the index of dispersion (Healy & Taylor, 1962).

F_1, F_2 values from analysis of variance for log transformed data and data transformed according to Taylor's power law, respectively (v_1, v_2 degrees of freedom for treatments and experimental error, respectively).

The regression intercept values increased as plot size increased in the systematic survey (Figs 4-7). The regression intercept through *T. semipenetrans* data from 14.7-ha sites was less than those from 6.3-ha sites with $0.05 < p < 0.10$ ($t = 1.93$; $df = 39$) and 8.4-ha sites with $p \leqslant 0.05$ ($t = 2.57$; $df = 37$). Also, the regression intercept for data from 2.1-ha sites was less than that from 8.4-ha sites with $0.05 < p < 0.10$ ($t = 1.8$; $df = 26$). The five slope values from the regression lines for *T. semipenetrans* soil population counts did not vary ($p > 0.05$) with field size or sampling method in the two surveys. Slope and intercept values for data from *H. pseudorobustus* were not different ($p \leqslant 0.05$) from those for *T. semipenetrans* and *Criconemella* spp. However, both *a* and *b* values for any of the regression lines for *T. semipenetrans* differed ($p \leqslant 0.05$) from those for *Criconemella* spp. (Table 1). The number of cores needed to fulfil a systematic sampling plan with a given level of nematodes and sampling error increased substantially as site size increased from 2.1 to 8.4 ha (Table 2).

Discussion

The values of the parameters *a* and *b* (Table 1) lie within the range reported by Taylor (1961) for other biological contexts. However, the higher slope value of *T. semipenetrans* (Table 1) than that reported previously (Duncan *et al.*, 1989) suggests more aggregated distribution of the pattern reported herein. In the first survey, each sampling area (14.7 ha) was divided into areas or strata of equal size (2.1 ha). These divisions are established by growers to separate different scion cultivars, control surface irrigation and ensure a well-managed harvest. In the government agricultural companies of such plots, there are usually pathways between the divisions to facilitate transportation of the citrus yield. Thus, the stratified random sampling of 14.7-ha areas should be useful to conduct a preliminary sampling plan (Table 2) for survey work in such large companies. On the other hand, the systematic sampling plan reported herein may be used by these companies and by citrus growers of smaller areas (2.1 to 8.4 ha) to estimate optimum sample size for *T. semipenetrans* in Tahrir Province (Table 2).

The parameter *b* proved stabile ($p \leqslant 0.05$) for *T. semipenetrans*, in spite of variations in sampling method, plot size and time of sampling. Its relative stability over time (Ferris, 1985) and with different plot size (McSorley *et al.*, 1985) was previously reported.

It is well known that the higher number of cores per sample does reduce the variation between samples (Proctor & Marks, 1974). Each sample in the first and second surveys consisted of seven and one core, respectively. Such a variation in the number of cores/sample could probably improve the stratified random sampling method because a low *a* value is associated with a low variance (Taylor, 1961). Nevertheless, because of the wide variety of crops, nematodes, and nematode distributions that may occur in any one geographical area, it is unlikely that any one sampling plan will suffice in all situations (McSorley & Parrado, 1982). Stratified random (McSorley & Parrado, 1982), simple random (Duncan *et al.*, 1989) and systematic (Duncan, 1988) sampling of citrus nematodes have been in use with a considerable variation in the optimum sample sizes among them (Duncan, 1988).

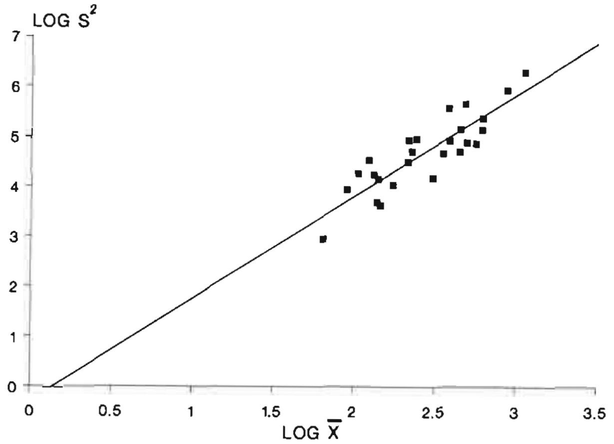

Fig 2. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *H. pseudorobustus* counts in a 14.7-ha area for composite samples from seven trees/2.1-ha. Regression equation of Taylor's power law : $log\ S^2 = 2.046\ log\ \bar{x} - 0.272$, $r^2 = 0.659$**.

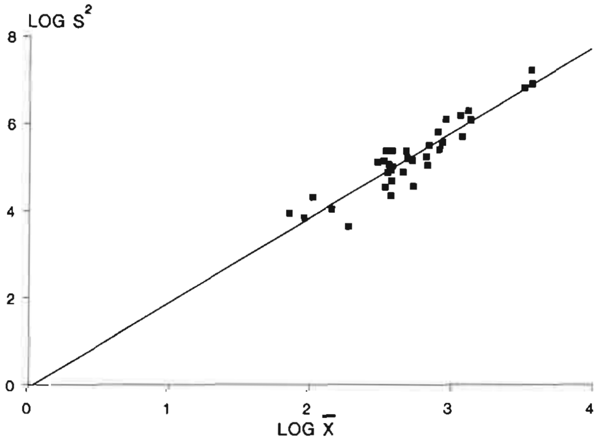

Fig. 1. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *T. semipenetrans* counts in a 14.7-ha area for composite samples from seven trees/2.1-ha. Regression equation of Taylor's power law : $log\ S^2 = 1.95\ log\ \bar{x} - 0.083$, $r^2 = 0.853$**.

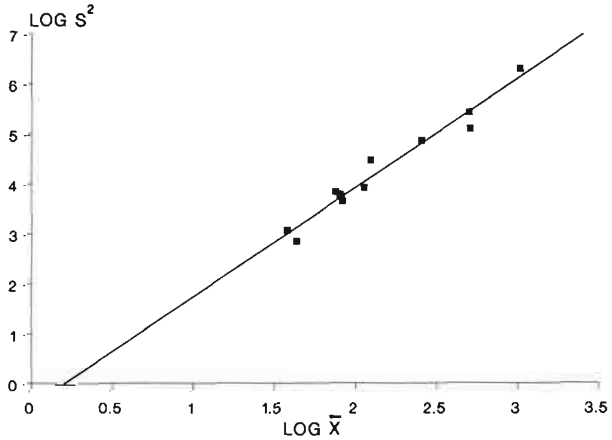

Fig. 3. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *Criconemella* spp. counts in a 14.7-ha area for composite samples from seven trees/2.1-ha. Regression equation of Taylor's power law : $log\ S^2 = 2.181\ log\ \bar{x} - 0.448$, $r^2 = 0.962$**.

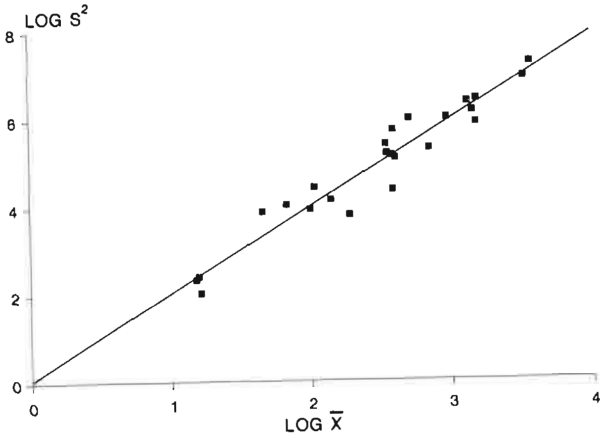

Fig. 4. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *T. semipenetrans* counts in a 2.1-ha area for single samples from seven trees/2.1-ha. Regression equation of Taylor's power law : $\log S^2 = 1.95 \log \bar{x} + 0.070, r^2 = 0.929$**.

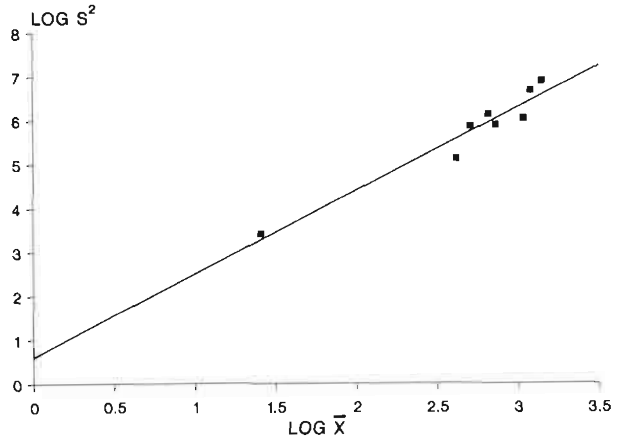

Fig. 6. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *T. semipenetrans* counts in a 6.3-ha area for single samples from 21 trees/6.3-ha. Regression equation of Taylor's power law : $\log S^2 = 1.9 \log \bar{x} + 0.599, r^2 = 0.935$**.

The size of the relative sampling error which can be tolerated should depend on the destructive potential of the nematode species involved (McSorley & Parrado, 1984). Thus, the criteria for sampling for *T. semipenetrans* are obviously more critical than those for *Criconemella* spp. and *H. pseudorobustus* since the former has been documented as a pathogen (Duncan & Cohn, 1990). It is reasonable to use the sampling plan for the most damaging nematode species in sampling several different species together on the same host. *Pratylenchus brachyurus* is generally not considered to be a major problem on mature trees (Duncan & Cohn, 1990) and pathogenicity to citrus trees by other nematodes (Figs 2 & 3) has not been investigated. However, if more than one pathogenic species were present in an orchard, it would be more conservative to use a sampling plan for the nematode species requiring the highest number of samples. Other nematode species would then be sampled with even greater levels of precision.

The number of samples required for a specific level of reliability showed general increases as *T. semipenetrans* population levels decreased. The reverse was true with regard to *H. pseudorobustus* and *Criconemella* spp. where $b > 2$. This case has also been reported ($b = 2.12$) for *Meloidogyne incognita* (McSorley *et al.*, 1985).

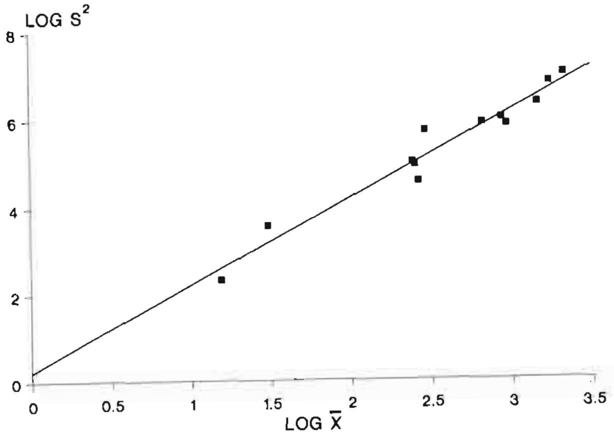

Fig. 5. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *T. semipenetrans* counts in a 4.2-ha area for single samples from fourteen trees/4.2-ha. Regression equation of Taylor's power law : $\log S^2 = 1.98 \log \bar{x} + 0.228, r^2 = 0.952$**.

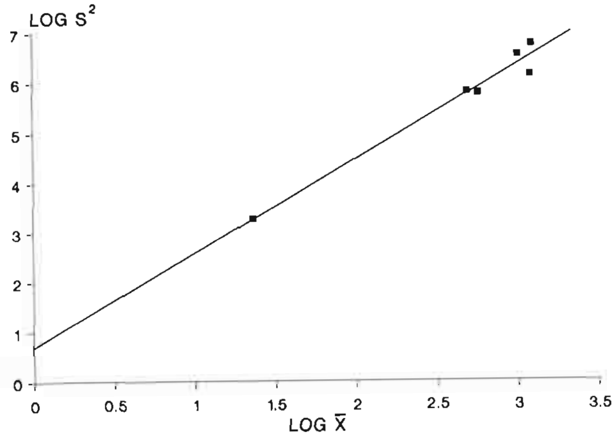

Fig. 7. Relationship between the logarithms of the mean ($\bar{x}$) and variance (S^2) of *T. semipenetrans* counts in a 8.4-ha area for single samples from 28 trees/8.4 ha. Regression equation of Taylor's power law : $\log S^2 = 1.89 \log \bar{x} + 0.693, r^2 = 0.974$**.

The use of log transformed data resulted in identical statistical interpretation of three experiments analyzed according to the parameter *b* of Taylor's power law (Table 4). Because the parameter $b \simeq 2.0$, log transformation is appropriate as well. Both transformations reduced the heterogeneity of variances ($p \leqslant 0.05$ and $p \leqslant 0.01$) approximating the assumption of equal variance underlying parametric statistical analysis. Nevertheless, the power law equations tended to reduce differences between treatment variance relative to log transformation (Table 3). Similar trends were recorded from orchards with small patches of *T. semipenetrans* (Duncan *et al.*, 1989).

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