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Spatial distribution of the phytonematode community in Egyptian berseem clover (*Trifolium alexandrinum* L.) fields

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Summary – Taylor's power law was fitted ($P \leq 0.01$) to all phytonematode population data obtained from the rhizosphere soil of 41 Egyptian berseem clover fields between September and December 1993. Estimates of the parameter *b* of Taylor's power law, an index of nematode dispersion, ranged from 1.218 to 1.869 indicating different degrees of nematode aggregation. Differences in *b* values were not related ($P \leq 0.05$) to nematode feeding habits. Transformations based on Taylor's power law were more effective than log or square root transformations in stabilizing variances for nematode population data. Sample sizes to achieve a predetermined level of sampling error for the nematodes were determined. Differences between the intercept values of the power law, as indicators of sample size and habitat, for these nematodes were statistically investigated.

Résumé. ***Répartition spatiale d'une communauté de nématodes phytoparasites dans des champs de trèfle d'Égypte (Trifolium alexandrinum L.)*** – La loi de puissance de Taylor a été ajustée ($P \leq 0.01$) à l'ensemble de populations de nématodes phytoparasites prélevés dans la rhizosphère de 41 champs de trèfle d'Égypte entre septembre et décembre 1992. Les estimations du paramètre *b* de la loi de Taylor – un indice de dispersion des nématodes – s'étalent de 1,218 à 1,869, indiquant ainsi différents degrés d'agrégation des nématodes. Les différences dans la valeur de *b* ne sont pas liées ($P \leq 0,05$) au mode nutritionel du nématode. Les transformations opérées à partir de la loi de puissance de Taylor sont plus représentatives que les transformations fondées sur les log. ou racines carrées car elles stabilisent les variances des données relatives aux populations de nématodes. La taille des échantillons correspondant à un niveau prédéterminé d'erreur de prélèvements a été déterminée. Les différences entre les points d'alignement de la loi de puissance – en tant qu'indicateurs de la taille de l'échantillon et de l'habitat – ont été étudiées statistiquement.

Key-words : *Trifolium alexandrinum*, Egypt, nematodes.

Berseem clover or Egyptian clover (*Trifolium alexandrinum* L.) is an important forage legume because of its limited bloat problems as animal fodder, fast winter growth rate and relatively long growing season (Kretschmer, 1964). Greenhouse tests revealed that the three most common species of root-knot nematode are primary limiting factors to its production (Baltensperger *et al.*, 1985). Also, plants simultaneously infested with *Meloidogyne incognita, Heterodera trifolii* and *Rotylenchulus reniformis* showed much more growth reduction than those infected with either species alone (Massoud, 1980). In Egypt, many phytonematode species are encountered in field of berseem clover (Abd El-Massih *et al.*, 1982).

The aggregated distribution of nematodes in soil confounds parametric statistical analyses (Goodell & Ferris, 1980; Duncan *et al.*, 1989; Abd-Elgawad, 1992) necessary to study nematode effects on the plants. Approaches based on estimate of nematode dispersion indices have been used to solve these problems and in the development and evaluation of control measures (Barker & Campbell, 1981). Taylor's power law (Taylor, 1961) provides such an index. It states that : the variance (S^2) of a population is proportional to a fractional power *(b)* of the arithmetic mean *(X)* :

$$S^2 = a\overline{X}^b \text{ or } \log S^2 = \log a + b \log \overline{X} \qquad \text{(i)}$$

where *a* and *b* are population parameters; *a* depends chiefly upon the sample size and *b* is an index of nematode dispersion (McSorley *et al.*, 1985). The power law is useful in determining transformations (Taylor, 1970) and developing nematode sampling plans (Ferris, 1984). The objectives of this study were to determine spatial distribution patterns of phytonematodes in berseem clover fields. The study examines sample size optimization and the effect of transforming nematode population data according to the power law to equalize variance prior to statistical analysis.

Materials and methods

A geographic survey of nematodes encountered in berseem fields was conducted in five Egyptian governorates; (Behera, Gharbia, Giza, Kalioubia and Menoufia) between September and December, 1993. Forty-one fields with soils ranging from silt to clay were randomly selected. The fields differed with respect to soil texture, plant varieties, previous cultivated crop, planting time (September to November), and method of cultivation (Afir or Herati). Seeds of berseem varieties Miscawi, Khadrawi, Wafear or Saidi were broadcast 15-35 days before nematode sampling. A 4000 m² area was delimited in each field and divided into five equal sections.

Table 1. *Taylor's power law regression equations for nematode data from 4000 m² plots*[1].

Nematode	n	r^2	Power law coefficients		Effective range	
			a	b	$\bar{x}$	S^2
Aphelenchus avenae	6	0.984*	4.376	1.695	0.4-7.6	0.8-116.4
Criconemella spp.	6	0.912*	3.076	1.218	0.4-4.2	1.3-27.2
Ditylenchus spp.	8	0.933*	2.698	1.476	0.2-2.8	0.2-8.0
Helicotylenchus dihystera	11	0.992*	4.677	1.869	0.2-22.4	0.2-1629
Heterodera trifolii	26	0.852*	2.735	1.445	0.4-36	0.9-566
Hoplolaimus spp.	7	0.895*	3.388	1.59	0.4-2.4	0.8-16.2
Meloidogyne spp.**	22	0.92*	3.483	1.729	0.4-42	0.8-3767
Pratylenchus brachyurus	28	0.913*	3.767	1.398	1.0-44.4	3.8-2612
Rotylenchulus reniformis	5	0.98*	3.162	1.509	0.4-6.8	0.8-47.8
Trichodorus spp.	9	0.843*	5.445	1.327	0.2-21	0.2-745
Tylenchorhynchus clarus	39	0.77*	0.935	1.816	1.6-377	2.2-138357
Tylenchus spp.	32	0.887*	3.062	1.428	0.2-13.4	0.2-257

n = number of non-zero points, r^2 = coefficient of determination for fot to equation $\log s^2 = \log a + b \log \bar{x}$, where $\bar{x}$ = *mean*, s^2 = variance. Effective range = range of $\bar{x}$ and S^2 data. * = significance at P = 0.01.
** : Three species : *M. javanica, M. incognita* and *M. arenaria* are included.

Three subsamples (*ca* 6 cm diam × 20 cm deep), each from the rhizosphere of a random berseem plant, were taken with a hand trowel and composited into a single sample representing the section, thus achieving a stratified random sampling pattern (Cochran, 1977). Nematodes were immediately extracted with a modified sieving centrifugation technique (Jenkins, 1964) from a 250 g portion of each soil sample. Nematodes in 40 % of each sample suspension, corresponding to 100 g soil, were identified and counted using a compound microscope. All analyses were performed on the actual, untransformed counts.

For each nematode species, means $(\bar{X})$ and variances (S^2) for all stages extracted from soil were computed over the five samples from each field. The parameters *a* and *b* were determined for the equation $S^2 = aX^b$ (Taylor, 1961) by regressing Log_{10} variances on Log_{10} means. A transformation procedure to normalize data in order to perform parametric statistics was determined (Taylor, 1970) as follows :

$$Y = X^{(1-0.5b)} \qquad \text{(ii)}$$

Ratios of the largest to the smallest treatment variance estimates of untransformed data, log transformed data, square-root transformed data and data transformed according to Taylor's power law were compared using a homogeneity of variance test (Pearson & Hartley, 1966). Where possible, up to twelve fields (K = 12) were randomly selected from the original survey data for this test. Slope and intercept values from the regression lines were compared using t-tests (Kleinbaum & Kupper, 1978).

Results

The most common nematodes found in the soil and their average percentages in the total nematode community were : *Tylenchorhynchus clarus* (68.3 %), *Pratylenchus brachyurus* (11.3 %), *Heterodera trifolii* (6.6 %), *Tylenchus* spp. (4.1 %), *Meloidogyne* spp. (3.3 %), *Trichodorus* spp. (2.1 %), *Helicotylenchus dihystera* (1.4 %), *Rotylenchulus reniformis* (0.5 %), *Aphelenchus avenae* (0.4 %). The root-knot nematode populations were frequently composed of more than one of the three major species (*M. javanica, M. incognita* and *M. arenaria*). Because their severely galled roots indicated that berseem varieties were susceptible to the three species, confirming the report of Baltensperger *et al.* (1985), the root-knot nematodes were grouped together (Tables 1-4). The genera of *Mylonchulus, Xiphinema, Psilenchus* and *Dorylaimus* were rarely found and accounted for 0.8 % of the total nematode community. Their data were too few to be analysed.

Taylor's power law could be fitted ($P \leq 0.01$) to the common nematodes (Table 1). Based on coefficients of determination, the fungivorous nematodes, *Aphelenchus avenae* and *Tylenchus* spp., fit Taylor's power law almost as well as the nematodes that are likely parasites of ber-

seem clover. The intercept value (represented by *log a* of Taylor's power law and affected primarily by sample size and habitat) ranged from 0.431 for *Ditylenchus* spp. to 0.736 for *Trichodorus* spp. with the exception of *Tylenchorhynchus clarus* which showed a negative intercept value (log a = – 0.029). It is interesting that *T. clarus*, with the widest range of population levels and variances and the highest frequency of occurrence (Table 1) had intercept value different from many other species (Table 4). This does not necessitate the change of intercept values for more extensive data sets because no significant difference was noted between the intercepts of *T. clarus* and *T. reniformis* (Table 4) which had the lowest data sets (Table 1). The slope values (representing the index of nematode dispersion of the regression of *log* variance on *log* mean ranged from 1.218 for *Criconemella* spp. to 1.869 for *Helicotylenchus dihystera* indicating different degrees of nematode aggregation.

Using the parameters of Taylor's power law (Ferris, 1984), the number of samples needed to achieve a predetermined level of sampling error was estimated (Table 2). The number of samples required for a less aggregated nematode genus is generally fewer than that for a more aggregated genus for a given specific level of nematodes and sampling error. For example, to sample *H. trifolli*, *R. reniformis* or *Meloidogyne* spp. from one acre berseem clover area with a 0.25 standard error to mean ratio and 100 nematodes/100 g soil, one could collect three, five or sixteen samples, respectively (Table 2).

The slope values of the present survey were used in the normalizing transformation, producing equalized variances for all the nematode count data (Table 3). Use of data transformed according to Taylor's power law reduced the largest to smallest variance ratios compared to log or square root transformation for all the cases considered. In contrast, heterogeneity of variances ($P \leq 0.05$ or $P \leq 0.01$) between nematode data of berseem clover fields existed concerning untransformed, log-transformed or square root-transformed data of at least one or more nematode species (Table 3).

The slope values of the regressions of log variances on log means through data from the twelve nematode genera were significantly different in only nine cases (Table 4). Eleven significantly different cases were detected between the intercept values for these regression lines (Table 4). Such significant differences between *a* and *b* values could not be grouped according to a distinct basis (e.g. feeding habits).

Table 2. *Number of samples needed to achieve a 25 % level of reliability in 4000 m² berseem areas, as defined in terms of standard error to mean ratio (E) or confidence interval half-width to mean ratio* (D) (all fractional values rounded up to nearest integer).*

Mean count per sample**	Minimum number of samples	
	E	D
Aphelenchus avenae		
10	35	139
100	17	69
Criconemella spp.		
10	8	33
100	1	5
Ditylenchus spp.		
10	13	52
100	4	16
Helicotylenchus dihystera		
10	55	221
100	41	164
Heterodera trifolii		
10	12	49
100	3	14
Hoplolaimus spp.		
10	21	84
100	8	33
Meloidogyne spp.		
10	30	119
100	16	64
Pratylenchus brachyurus		
10	15	60
100	4	15
Rotylenchulus reniformis		
10	16	65
100	5	21
Trichodorus spp.		
10	19	74
100	4	16
Tylenchorhynchus clarus		
10	10	39
100	6	26
Tylenchus spp.		
10	13	53
100	4	14

* Assume t = 2 for 95 % confidence interval (Ferris, 1984).
** Based on sample size of 100 g soil.

Discussion

The nematode population assessment problems is one of magnitude and proportion (Ferris, 1985). The sampling area, number of cores/sample and number of samples vary widely for different purposes and between countries for the same purpose. For example, the area used as a sampling unit for the potato cyst nematode ranges from 0.33 ha in the Netherlands to 0. 125 – 1 ha in Denmark and Germany and from 1 to 6 ha in the United Kingdom (Southey, 1974, *in* Barker

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.*

The nematode	K+	*log x*	$X^{(1-0.5b)}$‡	$\sqrt{X}$	Untransformed
Aphelenchus avenae	6	21.3	2.2	12.1	149.2**
Criconemella spp.	6	14.8	4.0	5.8	34.9*
Ditylenchus spp.	8	9.3	1.8	3.2	10.3
Helicotylenchus dihystera	11	33.1	1.7	45.2	2 088.5**
Heterodera trifolii	12	56.2*	6.0	25.2	634.0**
Hoplolaimus spp.	7	12.5	1.9	4.5	20.8
Meloidogyne spp.	12	36.9	3.2	68.7*	4 833.3**
Pratylenchus brachyurus	12	5.9	5.1	15.0	224.3**
Rotylenchulus reniformis	5	17.3	2.8	7.7	61.3**
Trichodorus spp.	9	14.4	7.951	21.7	469.9**
Tylenchorhynchus clarus	12	4.6	2.4	104.0*	10 814.2**
Tylenchus spp.	12	8.3	1.3	8.6	73.3*

Samples were obtained from one-acre areas of Egyptian clover.
+ K = independent mean squares; $v = 4$ for test of heterogeneity of variance (Pearson & Hartley, 1966 : Table 31).
‡ b = index of the nematode dispersion (Taylor, 1961).
*, ** = Heterogeneity of variance is significant at the 0.05 and 0.01 probability levels, respectively.

Table 4. *The* t*-test values of comparing the slope* (b) *and intercept* (a) *estimates for the relationship between the mean* (m) *and variance* (S^2) *of nematode population densities in 4000 m² areas of berseem clover*+.

The nematode	*Aphelenchus avenae*	*Criconemella* spp.	*Ditylenchus* spp.	*Helicotylenchus dihystera*	*Heterodera trifolii*	*Hoplolaimus* spp.	*Meloidogyne* spp.	*Pratylenchus brachyurus*	*Rotylenchulus reniformis*	*Trichodorus* spp.	*Tylenchorhynchus clarus*	*Tylenchus* spp.
Aphelenchus avenae	–	2.24‡	1.04	1.438	0.731	0.43	0.125	0.917	1.141	0.961	0.241	0.957
Criconemella spp.	1.759	–	1.028	4.044**	0.625	1.208	1.756	0.523	1.26	0.263	1.133	0.71
Ditylenchus spp.	2.333*	0.558	–	2.655*	0.104	0.373	1.046	0.276	0.142	0.446	0.102	0.197
Helicotylenchus dihystera	0.03	2.6*	3.366**	–	2.197*	1.354	0.892	2.532*	2.707*	2.509*	0.188	2.791**
H. trifolii	1.252	0.315	0.041	1.849	–	0.29	1.632	0.272	0.173	0.515	1.734	0.113
Hoplolaimus spp.	1.442	0.452	1.076	2.222*	0.624	–	0.349	0.192	0.305	0.488	0.307	0.394
Meloidogyne spp.	0.812	0.443	0.991	1.391	1.479	0.106	–	1.936	0.75	1.905	0.377	2.304*
Pratylenchus brachyurus	0.03	0.515	0.912	0.68	0.897	0.288	0.254	–	0.319	0.32	1.926	0.20
R. reniformis	1.905	0.118	0.639	2.537*	0.435	0.074	0.288	0.386	–	0.43	0.57	0.27
Trichodorus spp.	0.473	1.21	1.658	0.44	1.661	1.108	1.293	0.83	1.00	–	1.641	0.53
Tylenchorhynchus clarus	2.141*	0.788	1.44	2.618*	1.828	1.889	2.369*	2.216*	1.52	2.42*	–	2.01*
Tylenchus spp.	1.26	0.016	0.05	1.979	0.467	0.393	0.651	0.709	0.09	1.786	2.299*	–

+ : $S^2 = a\ m^b$ or $log\ S^2 = log\ a + b\ log\ m$ (Taylor, 1961).
‡ : Upper values for the slope comparisons are separated by dashes from lower values for the intercept comparisons.
*, ** : Significant at the 0.05 and 0.01 probability levels, respectively.

& Campbell, 1981). In Egypt, however, raising cattle is mainly run by the peasants. Samples were stratified only in line with the cultural practices of the peasant who periodically cuts about one-fifth of the area planted with the multi-cut berseem clover varieties studied. Thus, sampling procedure for pest management should be easy and not damaging to the plants in recently cut section.

Little information exists on the effect of several kinds of the nematodes reported here on berseem clover plants. Hence, an appropriate experimental design using an efficient sampling plan (Table 2) should enable nematologists to develop functional advisory programmes through relating the initial numbers of each nematode species to the yield of berseem clover. The primary thrust of our present work, however, was to apply Taylor's power law to study the spatial distribution and efficient sampling of plant-parasitic nematodes in berseem clover fields. Such a thrust has motivated others (McSorley *et al.*, 1985) concerning the fauna of Perrine marl soils. Based on our investigation, further studies such as obtaining sufficient numbers of nematodes to identify to the species level and solving the problems arising from the polyspecific nematode community as well as the interactions of these nematodes may be conducted. The latter study, for example, may use the nematode indices of dispersion (Table 1) for appropriate transformation of nematode count data prior to ANOVA in order to compare between the nematode population levels.

The regressions of log variances on log means resulted in slope values between one and two (Table 1). The latter two values should indicate from equation (ii) that log (slope = 2.0) or square root (slope = 1.0) data transformation are of common use to stabilize variance of nematode population data (Taylor, 1970). Compared to these two transformations, the intermediate slope values are advantageous as variance-stabilizing transformations for all nematode count data reported here (Table 3). Most differences between the *b* values (indices of nematode dispersion), however, were insignificant (Table 4), possibly because many count data represented more than one species within a nematode genus. Also, seeds of berseem clover were (randomly) broadcast which is reflected in the plant root distribution, a major spatial distributional component of phytonematodes (Ferris, 1985; Ferris *et al.,* 1990). Differences between *b* values (Table 4) were not related to feeding habits (e.g., *Aphelenchus avenae* as fungal feeders and *Meloidogyne* spp. as parasites of higher plants) corroborating a previous report (McSorley *et al.*, 1985). Nevertheless, the relative stability of *b* over time (Ferris, 1985) and with different plot size (McSorley *et al.,* 1985) and sampling methodology was previously reported (Abd-Elgawad, 1992). Such a stability does not necessitate that *b* values in the present study represent global indices for nematode species which are sufficiently robust to be useful in any situation since many other spatial distributional components of phytonematodes may interfere.

The number of samples needed to achieve a given level of reliability is positively related to *a* and *b;* the parameters of Taylor's power law (Ferris, 1985; Duncan *et al.*, 1989). For example, these parameters were significantly higher for *Helicotylenchus dihystera* than for *Criconemella* spp. (Table 4) which resulted in nearly sevenfold to fortyfold increase in the sample size estimate relative to sampling error and nematode population level (Table 2). Such numbers of samples are costly. A lack of high sampling precision may alleviate this problem if further studies prove that the majority of population estimates are well below their damage threshold levels. On the other hand, it is possible that another sampling plan may reduce the optimum size of sampling for *Helicotylenchus dihystera.* With information on subsample variability, it would be possible to optimize numbers of cores/sample and numbers of samples/area of various sizes to produce a more efficient plan for a specific kind of nematode. The latter scheme may be facilitated by using systematic sampling pattern (McSorley *et al.*, 1985; Abd-Elgawad, 1992). Nevertheless, it is well known that any one sampling plan will unlikely suffice in all situations because of the wide variety of crops, nematodes and nematode distributions that may occur in any one geographical area. However, increasing the number of cores/sample would probably reduce the variation between samples (Abd-Elgawad, 1992) and consequently improve the present sampling method especially for those kinds of nematodes requiring a high number of samples (Table 2).

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