

SAMPLING OPTIMIZATION FOR ROOT LESION NEMATODES IN THE IRRIGATED WHEAT FIELDS OF MARVDASHT REGION, FARS, IRAN

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Summary. The sample size or the number of small fields to be sampled for root lesion nematodes was estimated using CV as a measure of reliability and Taylor's power law model as a descriptor of nematode spatial distribution in the irrigated wheat fields of Marvdasht region, in autumn 2006. Fifty-six fields were sampled following eight different sampling patterns including: 1-2, sampling the first and the second diagonal transects (D1 and D2 transects, respectively), 3-4, sampling in a line along/across cultivation rows in the middle of field (D3/D4 transects), 5, taking a composite sample from D1 to D4 (Mixed transect), 6-8, the average of D1 to D4 transects (D1-4), D1 and D2 transects (D1-2) and D3 and D4 transects (D3-4), respectively. The number of samples for estimation of population densities of root lesion nematodes with 85% reliability (the most reasonable reliability level for management decisions) were greatest for D3 and D4 patterns (73 and 72 fields, respectively) and smallest for D1-4 pattern (15 fields). According to reliability and cost analysis, the optimum sampling pattern for root lesion nematodes was proved to be sampling on D1-2 transect (cores on two diagonal transects of fields). The data did not fit a negative binomial distribution, which is considered the best probability distribution for description of aggregated count data. The more robust Taylor power law was employed alternatively to verify the spatial pattern of the nematodes and estimate the optimum sample size using its estimated parameters ("a" and "b"). The approach used in the present study can be considered for sampling optimizations of root lesion or other nematodes in wheat or other row crops of regions with soils that are disturbed by heavy cultivation.

Key words: Cost, distribution, reliability, sample size, Taylor's power law.

Studies to determine optimum sampling patterns for plant parasitic nematodes have been carried out at the scale of field or small plots (Goodell and Ferris, 1980, 1981; Alby *et al.*, 1983; McSorley *et al.*, 1985; Francl, 1986; Ferris *et al.*, 1990; Wheeler *et al.*, 2000; Souza *et al.*, 2007; Monfort *et al.*, 2008) to large geographic areas (Duncan *et al.*, 1989; Prot and Ferris, 1992; Neher and Campbell, 1996). Studies have also been performed to determine an appropriate sample size for plant parasitic nematodes (Goodell and Ferris, 1981; McSorley, 1982; Duncan *et al.*, 1989; Prot and Ferris, 1992; Neher and Campbell, 1996; Wheeler *et al.*, 2000).

Abundance of pathogen propagules (e.g. plant parasitic nematodes) at sampling unit level can be regular, random or aggregated. Data that are used for monitoring the pattern of occurrence of a pathogen can be grouped into three types, including incidence data, severity data and density or counts (Madden *et al.*, 2007).

The fact that the presence of a nematode in soil makes the occurrence of other nematodes more likely indicates that nematode occurrences are not independent events and thus spatial dispersion of a nematode population is usually aggregated, which can be described by a negative binomial model (Proctor and Marks, 1974; McSorley, 1982; Boag and Topham, 1984; Campbell and Noe, 1985; Farias *et al.*, 2002; Been and

Schomakher, 2006). The negative binomial (NB) model describes populations where their variance is greater than their mean (Ferris *et al.*, 1990). However, in some instances, nematode population is uniformly or randomly distributed within fields (Koenning *et al.*, 2004; Been and Schomaker, 2006).

The degree of aggregation found depends on different factors, including nematode biology (Ferris *et al.*, 1990; Ciancio *et al.*, 1995), nematode population density (Prot and Ferris, 1992), cultural operations (Francl, 1986; Farias *et al.*, 2002; Monfort *et al.*, 2008), host plants (Ferris *et al.*, 1990; Viketoft, 2007; Aballay *et al.*, 2009), soil characteristics (Ferris *et al.*, 1990), field size (Alby *et al.*, 1983; Wheeler *et al.*, 2000) and sampling pattern (McSorley *et al.*, 1985).

Taylor's power law can alternatively be used to provide a description of variability of count data. The relationship between observed variance and observed mean for count data such as a nematode population can also be described by Taylor's power law model (Boag and Topham, 1984; McSorley *et al.*, 1985; Ferris *et al.*, 1990). Taylor (1961) expressed observed variance of count data (S^2) as a power function of the observed mean (Y) of the data ($S^2_y = aY^b$). This model has two parameters: "a" and "b", which are descriptive of the power relationship between variance and mean, such that "b" is considered an aggregation parameter and "a" is a scaling factor, dependent on both sample size and field size (Ferris *et al.*, 1990). This model does not require a mathematical fit to a specific statistical distribution (McSorley *et al.*, 1985).

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Formulae for calculation of sample size (or the number of small fields to be sampled) for use in simple sampling of count data are given by Madden *et al.* (2007) using Poisson distribution, negative binomial distribution and Taylor's power law as descriptors of variability of nematode counts. After making a decision on the level of reliability expressed as CV (coefficient of variation), a fixed proportion of the sample mean, or half the length of the required confidence interval of the sample mean, appropriate estimated parameters (μ as the single parameter of Poisson distribution, μ and κ parameters of negative binomial distribution and "a" and "b" parameters of Taylor power law), the minimum sample size is calculated (Madden *et al.*, 2007).

Marvdasht (29°52'27"N 52°48'09"E) is an agriculturally important region with temperate climate located in Fars province, in the south of Iran (Fig. 1). Fars province is considered the most important bread wheat production area of the country and Marvdasht's contribution to the wheat production of the province is substantial. Fars province, 419,488 ha in area (5.9% of the total of Iran) and with 1,329,276 metric tonnes of wheat production in 2009/10 (8.8% of the total wheat produced in Iran), mainly from irrigated wheat fields, is

ranked second in the country and Marvdasht, with more than 250,000 metric tonnes of wheat production, is one of the most important wheat growing areas of Fars province (Anonymous, 2011). Winter wheat is sown during October and November in Marvdasht and harvested around June next year. Corn or summer fallow are alternatives that are rotated with wheat. Non chemical management options, such as destruction of plant residues by burning or ploughing and weed control, are used for controlling root lesion nematodes in wheat fields of Marvdasht region.

Pratylenchus neglectus (Rensch) Filipjev *et* Schuurmans Stekhoven and *P. thornei* Sher *et* Allen are the two most common root lesion nematodes in Marvdasht irrigated wheat fields. Spread and population density of the first species are greater than for the second species; however, both species may cause yield losses to wheat in this region (Ghaderi *et al.*, 2009a, b). Although the damage level to wheat by root lesion nematodes has not yet been evaluated in Marvdasht region or other localities in Iran, these nematodes were reported to cause up to 5% wheat loss in the USA (Koenig *et al.*, 1999). Other investigations have shown damage levels up to 70-85% for *P. thornei* and 16-40% for *P. neglectus*



Fig. 1. Geographical location of Marvdasht region in Fars province, Iran (coordinates: 29°52'27"N 52°48'09"E). Sampled area is showed with a star.

(Nicol *et al.*, 1999). Reliable information on the nematode soil population density would help in selecting the most appropriate tactic to manage these nematodes.

The objective of this study was to compare some selected standard sampling patterns and to determine the best pattern for sampling and the number of fields to be sampled (sample size) for relatively reliable and cost-effective estimation of the mean density of root lesion nematodes. To fulfill the objective, the best descriptor of the spatial pattern of the nematodes was investigated through fitting the data collected across the irrigated wheat fields of Marvdasht region to different probability distributions. Afterwards, different sampling patterns were compared through reliability and cost analysis approaches.

MATERIALS AND METHODS

Sampling was carried out on the 56 irrigated winter wheat fields (49 fields with sandy loam texture, five of sandy clay loam texture, and two of loamy sand texture), randomly selected across the Marvdasht region, before planting winter wheat in autumn 2006. Fields with an approximate area of 2-3 ha were selected in a way to give a more or less even distribution across the region and random samples were taken following each of the eight sampling patterns as follows (Fig. 2): 1 and 2, sampling the first and the second diagonal transects (D1 and D2 transects) respectively; 3 and 4, sampling a transect along/across cultivation rows in the middle of

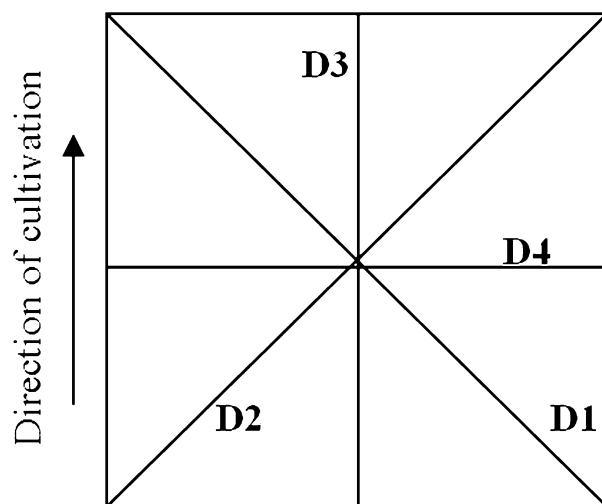


Fig. 2. Patterns used in sampling from irrigated wheat fields of Marvdasht region; first and second diagonal transects (D1 and D2, respectively), sampling along and across the central cultivation rows (D3 and D4, respectively); other patterns includes a composite sample from the above four patterns (Mixed), the average of the above four patterns (D1-4), the average of D1 and D2 patterns (D1-2), and the average of D3 and D4 patterns (D3-4).

field (D3/D4 transects); 5, taking a composite (bulk) sample from D1 to D4 transects (Mixed); 6 to 8, taking the average nematode counts of D1 to D4 transects (D1-4), D1 and D2 transects (D1-2), and D3 and D4 transects (D3-4), respectively.

Soils were sampled by taking 5 cm diameter cores to a depth of 20 cm, 20 m apart from each other (15 cores in each of D1, D2, D3 or D4 patterns). Soil from the 15 cores was pooled and each bulk sample was placed in a plastic bag and samples were transferred to the laboratory for further processing, which included extraction and counting. The counting of nematodes in soil extracts was repeated three times by sub-sampling each sample extract and therefore for each sampling pattern there was a total of 168 data points (i.e. 56 fields times 3 replicates per field per transect).

A 100 cm³ soil sub-sample was used for nematode extraction from each of the D1, D2, D3 or D4 pattern samples. Another sample was obtained by mixing the soil remaining from D1, D2, D3 and D4 (pattern 5), from which a sub-sample of 100 cm³ soil was processed. Nematodes were extracted by using the tray method (Whitehead and Hemming, 1965) at laboratory temperature, and collected after 48 hours. The nematode suspension was diluted to 10 ml, thoroughly mixed by blowing through a pipette, and the root lesion nematodes were counted in a volume of 2 ml of the suspension. Data were obtained after corrections for extraction efficiency and concentration. To assess extraction efficiency, three soil samples were randomly selected, from which two 100-cm³ sub-samples were taken. The six sub-samples were placed on the extraction trays and 2000 specimens of *Pratylenchus*, reared on carrot discs, were added to the soil of three trays but not the remaining three trays. After 48 h, the extracted nematodes were counted and extraction efficiency was determined and found to be 50%. Thus the whole survey data were multiplied by 2. However, efficiency was considered the same for different soil types (data not shown).

The negative binomial and Poisson models were fitted to the data (equations 1 and 2) using Fit-Distribution, an add-in written by the second author in the MS Excel environment (unpublished).

$$\Pr(Y) = \frac{\mu^Y e^{-\mu}}{Y!} \quad \text{eq.(1)}$$

$$\Pr(Y) = \left(\frac{\Gamma(k+Y)}{\Gamma(k)\Gamma(Y+1)} \right) \left(\frac{\mu}{k} \right)^Y \left(1 + \frac{\mu}{k} \right)^{-(k+Y)} \quad \text{eq.(2)}$$

Where e is the base of natural logarithms ($e = 2.718$), μ and k are distribution parameters and refer to the mean of the data and an over-dispersion parameter, respectively, and Γ represents the gamma function.

Due to the complexity of the dispersion patterns of the nematodes, neither Poisson nor negative binomial models had a satisfactory fit to the data. Taylor's power law model was fitted to the data as an alternative to in-

fer the possible spatial pattern of the nematodes and also borrow its parameters for sample size estimation. The data points estimated from samples from all fields were randomly divided into ten series (see numbers of observations per panel in Fig. 3) and the mean and variance of each set for all sampling patterns were determined. Parameters “*a*” and “*b*” were estimated by invoking a non-linear procedure using the Gauss method for optimization.

Sample size or the number of fields to be sampled (*N*) was determined for each of the eight sampling patterns at different degrees of reliability (*CV* = 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30) using the following formula (Madden *et al.*, 2007):

$$N = a\bar{Y}^{b-2} / CV^2 \quad \text{eq.(3)}$$

where \bar{Y} is the mean of nematode population density, *CV* is an indicator of reliability, and “*a*” and “*b*” are the estimated parameters for Taylor’s power law model.

The cost of the time required for sampling was determined for each of the eight sampling patterns, based on the formula [eq.(4)] developed by Goodell and Ferris (1981):

$$y = A + 24S + 0.5C + 1.1\sqrt{C^2 + (16)(S^2)} \quad \text{eq.(4)}$$

where: “*y*” is the time required in minutes for a certain sampling pattern; “*A*” is the set-up time; “*S*” is the number of samples; and “*C*” is the number of cores.

Finally, the best sampling pattern was selected, with regard to reliability and cost.

RESULTS AND DISCUSSION

Accurate estimation of nematode density in a field or larger area is important in making decisions for plant parasitic nematode management programmes. Reliable methods for sampling and extraction of nematodes are essential for accurate assessment of nematode densities in a field or region (Souza *et al.*, 2007). Additionally, to maximize benefits from sampling, estimates of mean and variance of nematode density (and hence aggregation) are important (Wheeler *et al.*, 2000).

The results of the current study indicate that the variances of the field data in each sampling pattern were much larger than their means. Thus, the dispersal of nematode populations was expected to be aggregated. Statistics for data frequency distributions are shown in Table I. Among many parameters that measure the degree of aggregation, coefficients of skewness (*y*) and kurtosis (*k*) are used to describe the degree of clumping of the population, with low numbers indicating high aggregation and vice versa (Been and Schomaker, 2006).

The results of the goodness of fit tests for Poisson and negative binomial models showed that neither model fitted the data. Poor fit of the Poisson model to data

indicates obvious deviation of spatial dispersion of nematodes from randomness. Deviation of the data from the negative binomial model in all sampling patterns, as a result of rejection of the goodness of fit hypothesis with both Chi square and log-likelihood methods, was an indication of a powerful factor that has affected the dispersion in the past. Taylor’s power law model was found robust enough to have a good fit to the data (Table II). Values of parameter estimates of Taylor’s power law model and the corresponding curve for each sampling pattern are presented in Table III and Fig 3. The number of samples required for estimation of mean density (i.e. minimum sample size) of root lesion nematodes in Marvdasht irrigated wheat fields, in different sampling patterns, with specific degrees of reliability, were determined (Table IV). It appears that estimated sample sizes for 80, 85 and 90% reliabilities (*CV* = 0.2, 0.15 and 0.10, respectively) were more practical in management decisions. However, many more samples were required to achieve the higher reliability of 95% (*CV* = 0.05), but conversely, a relatively lower reliability (*CV* = 0.25 or 0.3) may be less acceptable from the statistical point of view. There is no universally acceptable level for reliability and the reliability level acceptable as a basis for nematode management decisions may vary greatly with regard to location, crop, nematode species, and other factors. Goodell and Ferris (1981) stated that while precision levels of 50% might be acceptable for *Merlinius*, the variation of *Meloidogyne* estimates must be kept within 20% of the true mean. On the other hand, Ferris *et al.* (1990) indicated that a precision level of 15% might be reasonable and attainable for this aim generally.

At various pre-determined reliability levels ranging from 0.05 to 0.30, the required sample size was the smallest for the D1-4 pattern at all levels, but this pattern was also very costly and required a lot of time for sampling (372 minutes). Although the D1-2 and D3-4 patterns require longer sampling times than D1, D2, D3 or D4 patterns (195 minutes *vs* 108 minutes), they necessitated smaller sample sizes. The Mixed pattern (sampling pattern 5) gave a larger sample size and higher costs than D1-2 and D3-4. Thus, it appears that D1-2 and D3-4 are better options for sampling and, among the D1-2 and D3-4 patterns, the former was identified as the best sampling pattern and is recommended for sampling of the root lesion nematodes in Marvdasht irrigated wheat fields (Table V). The D1-2 pattern requires a smaller sample size at a comparable cost (Tables V and VI), possibly because estimated variance in population density of D1-2 was smaller than that of the D3-4 pattern. When one moves along the cultivation rows the population variability is expected to be lower, while across cultivation rows the variance is likely to be higher, perhaps due to a lower mixing and homogenising effect of tillage as farmers often plough their lands in the same direction every year. Consequently, count variance is expected to be lower in the direction of

Table I. Statistics of frequency distributions of actual population densities of root lesion nematodes in 56 Marvdasht wheat fields.

Coefficient of kurtosis (k)	Coefficient of skewness (y)	95% confidence interval for mean	Variance	Mean	Sampling pattern
2.0	1.1	47.5-88.5	5869.9	68.0	D1
3.0	1.8	41.3-84.6	6525.4	63.0	D2
2.5	1.7	38.3-78.5	5639.1	58.4	D3
3.4	1.9	41.7-87.8	7407.4	64.7	D4
3.3	1.8	45.2-89.3	6779.3	67.2	Mixed
1.4	1.4	45.5-85.5	5585.7	65.5	D1-2
1.8	1.6	40.9-82.2	5947.8	61.6	D3-4
1.3	1.4	43.7-83.3	5477.8	63.5	D1-4

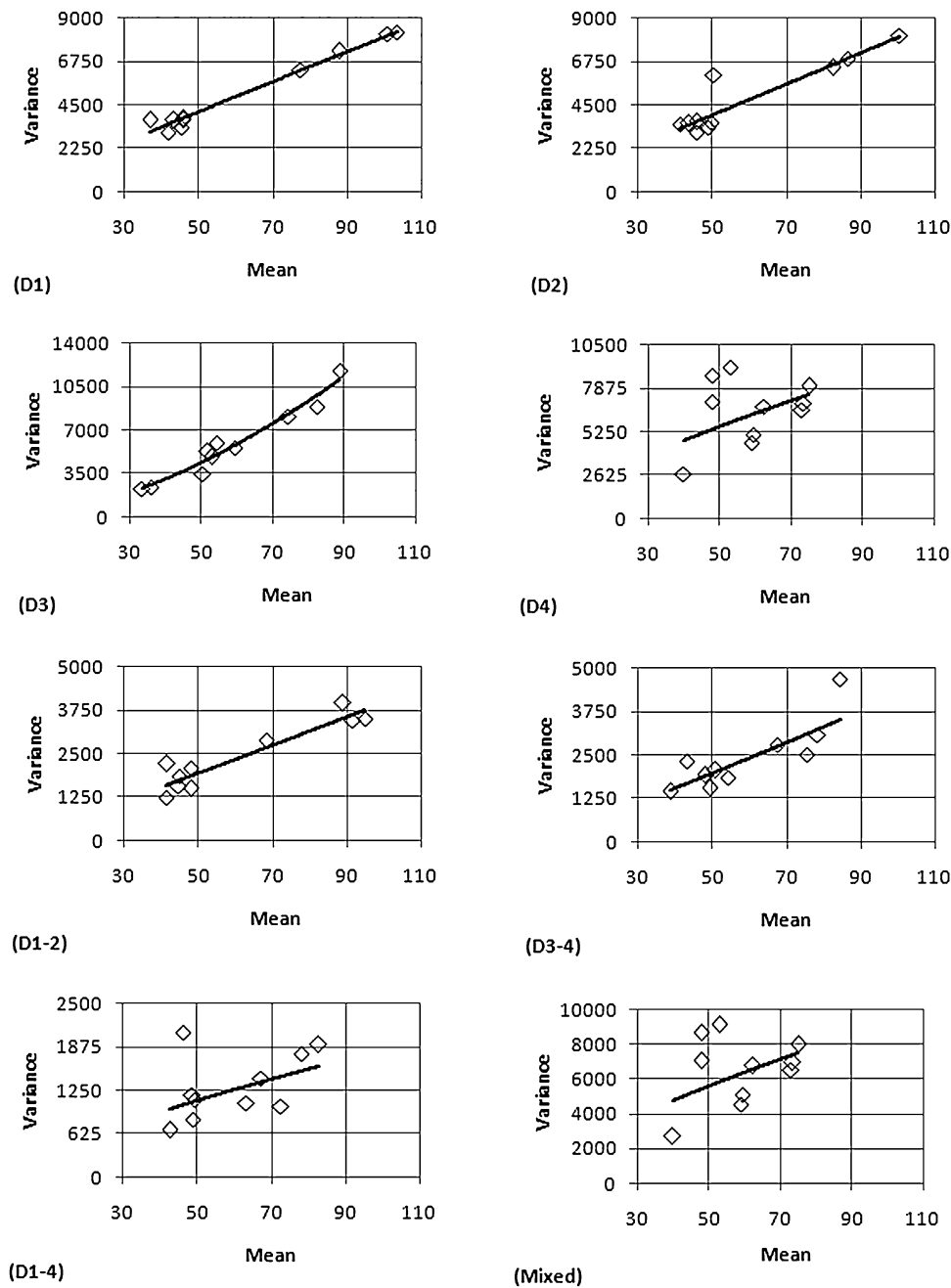


Fig. 3. Variance-mean curves of different sampling patterns (D1 to D4, D1-2, D3-4, D1-D4 and Mixed) for population estimation of root lesion nematodes of irrigated wheat fields in Marvdasht region.

Table II. Fitness parameters of Taylor's power law model for different sampling patterns in the irrigated wheat fields of Marvdasht region.

Sampling pattern	Adj-R ²	RMSE	Pr > F
D1	0.997	314.1	< 0.0001
D2	0.981	789.9	< 0.0001
D3	0.991	690.1	< 0.0001
D4	0.931	1995.8	< 0.0001
Mixed	0.991	654.5	< 0.0001
D1-2	0.984	361.2	< 0.0001
D3-4	0.966	532.9	< 0.0001
D1-4	0.914	453.8	< 0.0001

Adj-R², RMSE, Pr>F are model diagnostics and show how well the TPL model approximates the real data points of means and variances for each sampling design.

Adj-R² (= Adjusted-R²) is a measure of the proportion of variability in a data set that is accounted for by the TPL model. An Adj-R² of 1.0 indicates that the regression line perfectly fits the data.

RMSE (= Root Mean Squared Error) is a measure of accuracy or the differences between values predicted by TPL model and the values actually observed. An RMSE = 0 shows perfect agreement between TPL predicted and actual values but generally the smaller RMSE is the better it is.

Pr>F is another goodness of fit criteria and simply an indication of how well the TPL model fits the data and tests the null hypothesis or H₀; lack of association between variance and mean density. The null hypothesis is rejected if Pr < 0.01 and a statistical association between observed means and variances is concluded.

Table III. Parameter estimates by Taylor's power law model for different sampling patterns.

Sampling pattern	Parameter	Parameter estimate	Standard error	95% lower	95% upper
D1	a	90.213	20.334	43.321	137.1
	b	0.9759	0.0516	0.8569	1.095
D2	a	87.746	55.585	-40.433	215.9
	b	0.9799	0.1486	0.6373	1.323
D3	a	7.8138	4.7497	-3.1390	18.77
	b	1.6164	0.1420	1.2889	1.944
D4	a	1293.6	2620.2	-4748.7	7336
	b	0.3989	0.4945	-0.7414	1.539
Mixed	a	15.571	9.2483	-5.7560	36.89
	b	1.4370	0.1389	1.1167	1.757
D1-2	a	39.043	23.496	-15.138	93.22
	b	1.0032	0.1401	0.6802	1.326
D3-4	a	12.676	15.243	-22.475	47.83
	b	1.2842	0.2855	0.6260	1.943
D1-4	a	85.253	169.10	-304.80	475.3
	b	0.6684	0.4786	-0.4352	1.772

Table IV. Minimum sample size (number of fields to be sampled) for population estimation of root lesion nematodes in different sampling patterns with specific reliability (CV) for irrigated wheat fields of Marvdasht region.

CV \ Sampling pattern	D1	D2	D3	D4	Mixed	D1-2	D3-4	D1-4
0.05	480	513	657	652	583	242	268	136
0.10	120	128	164	163	146	60	67	34
0.15	53	57	73	72	65	27	30	15
0.20	30	32	41	41	36	15	17	8
0.25	19	21	26	26	23	10	11	5
0.30	13	14	18	18	16	7	7	4

Table V. Estimation of cost (required time, based on minutes) for different sampling patterns, based on formula developed by Goodell and Ferris (1981).

		D1	D2	D3	D4	Mixed	D1-2	D3-4	D1-4
Collect	Set up field	10	10	10	10	10	10	10	10
	Remove core from soil	15	15	15	15	60	30	30	60
	Tag, bag, bulk, sub-sample	2	2	2	2	8	4	4	10
Process	Set up extraction	2	2	2	2	8	4	4	8
	Extraction	10	10	10	10	10	10	10	10
Count	Count sample	20	20	20	20	20	40	40	80
Set up time (A)	Sum	59	59	59	59	116	98	98	178
No. of samples (S)		1	1	1	1	1	2	2	4
No. of cores (C)		15	15	15	15	60	30	30	60
Cost (min)		108	108	108	108	236	195	195	372

ploughing and higher in the across ploughing direction. By moving along a diagonal in the field, fluctuation in nematode populations is probably smoothed or buffered as the move is bi-directional. Also, a diagonal transect spans a considerably larger sampling area of the field, which gives a better snapshot of the spatial pattern of the nematode population than linear transects.

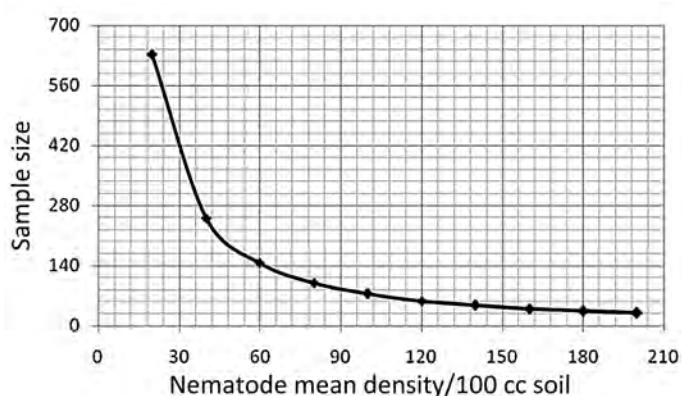
Sedentary endoparasitic nematodes deposit all their eggs in the same location, frequently in masses, resulting in a highly aggregated spatial pattern. Ectoparasitic nematodes move through the soil and deposit their eggs individually, thus showing a somewhat less aggregated pattern (Ferris *et al.*, 1990). It seems that migratory endoparasitic nematodes (including root lesion nematodes), which lay their eggs both in soil and in plant roots, occur in a less aggregated pattern than sedentary endoparasitic nematodes, but at the same time a more aggregated pattern than ectoparasitic nematodes.

Required sample size is negatively correlated with the degree of aggregation (Ciancio *et al.*, 1995). Thus, the number of samples required at the same reliability for estimating the mean count of ectoparasitic nematodes

(e.g. *Merlinius* spp., which are very common in Marvdasht wheat fields) is considerably smaller than that needed for root lesion or cyst nematodes. As it will often be appropriate to use distribution parameters for the most highly aggregated species as a general basis for determining sampling intensity (Ferris *et al.*, 1990), the results of the present study can be generalized, with some modifications, for sampling optimization of ectoparasitic nematodes or for root lesion nematodes in other row crops.

When parameter values for the Taylor's power law model are determined for a certain species of plant parasitic nematode, the values appear sufficiently robust to describe mean-variance relationships for the same species in various locations or times and to make inferences about its spatial heterogeneity (Ferris *et al.*, 1990). Furthermore, these parameter values provide a basis for evaluating the required sample size to measure the mean nematode population at various levels of reliability. Sample sizes required for estimation of different nematode mean densities were according to Fig. 4.

In the Taylor's power law model, " b " is considered an aggregation parameter. If " b " is greater than unity ($b > 1$), the nematode has an aggregated dispersal pattern and, if $b = 1$, a uniform dispersion for the nematode is conceivable. If $b < 1$, nematodes occur in a random pattern in the soil. In our research, " b " was less than unity in some sampling patterns even though root lesion nematodes would be expected to have an aggregated distribution pattern. The fact that these nematodes did not show aggregation in some fields of the irrigated wheat fields of Marvdasht region indicated the existence of a strong biasing factor. Presumably, tillage operations that are frequently done for seed-bed preparation, and also movement of bed preparation equipment through the field over many years, may have caused continual mixing of soil and frequent redistribution of root lesion nematodes, leading to a status intermediate between uniform, random and aggregated distributions. The degree

**Fig. 4.** Minimum sample size (number of fields to be sampled) required for estimation of root lesion nematode populations at different mean densities.

of the uniforming effect of soil tillage in the fields might depend on the history of crop rotation and land use. Further evidence for this phenomenon is the smaller variance of nematode counts along (D3) as compared to those across (D4) cultivation rows (Table I), indicating a homogenising effect of ploughing on the nematode population.

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