SPATIAL PATTERN ANALYSIS OF ROOT ROT-ROOT KNOT DISEASE COMPLEX IN AN INFESTED EGG PLANT FIELD

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Summary. The spatial pattern of *Meloidogyne javanica* in soil, root gall intensity, and colonization frequency of two soilborne fungi (*Fusarium solani* and *Rhizoctonia solani*) on roots in an egg plant field, detected using various pattern detection indices, demonstrated non-randomness and patterns of aggregation. Morisita's index was computed for various quadrat (block) sizes to analyse the scale of pattern. Greatest heterogeneity was found at the smallest quadrat size and lowest at the larger sizes. Root gall intensity and nematode densities in soil were positively correlated at all block sizes. Colonizing frequency of the two fungi was positively correlated with galling intensity only at the smaller block sizes. The possible implications of spatial pattern of nematode populations with regard to sampling efficiency and interaction with plant pathogenic fungi are discussed.

Spatial analysis of diseases and pathogen populations are of central importance to the understanding and management of plant disease epidemics, because of the close relationship between pattern and disease increase and spread within plant populations (Nelson and Campbell, 1993). Infections caused by many soilborne plant pathogens generally occurs in clusters (Shew *et al.*, 1984; Yang *et al.*, 1991; Nelson and Campbell, 1993). The most frequently reported spatial distribution for nematodes and soilborne fungi is the negative binomial distribution (Godell and Ferris, 1980; McSorley and Parrado, 1982; Shew *et al.*, 1984; Campbell and Madden, 1990; Singh and Gaur, 1996; Rekah *et al.*, 1999). The parameter k of the negative binomial distribution has been interpreted as a measure of dispersion (Southwood and Henderson, 2000). Another measure of dispersion developed empirically which describes the relationship between variance and mean by Taylor's power law ($S^2 = am^b$, where S^2 equals the variance, m the mean and a and b the parameters of which b is considered a measure of aggregation while a is a scaling factor). Parameters for Taylor's power law have been estimated for several nematode species (Boag and Topham, 1984; McSorley *et al.*, 1985; Duncan *et al.*, 1989; Ferris *et al.*, 1990; Singh and Gaur, 1996). Knowledge of the spatial distribution are then used to develop sampling plans for specific situations using equations derived from negative binomial models (McSorley, 1982; McSorley and Parrado, 1982), by using Taylor's power law (Ferris, 1984) or by simulation from a database (Goodell and Ferris, 1981). However, analysis of frequency distributions indicates only whether nematodes have aggregated spatial patterns but provides no information on the precise nature and scale of aggregation (Noe and Campbell, 1985). Several techniques are available for analysing spatial patterns of plant-parasitic organisms and diseases for those pathosystems in which categorical or quantitative variates for disease severity or pathogen population estimates are available (Upton and Fingleton, 1985; Campbell and Madden 1990). Reports on nematode spatial analysis have generally dealt with areas larger than 0.5 ha (Goodell and Ferris, 1980; Davis, 1984). However, more accurate estimates are required in field experiments conducted in small plots. Disease intensity with regard to systemic diseases, such as those caused by plant-parasitic nematodes and fungi, are often expressed as binomial (presence/absence) data. However, better estimates of disease development may be obtained by examining the root system in greater detail such as root colonization frequency.

Egg plant is an important vegetable crop in Pakistan and is often badly infected, particularly in the Sindh province, by plant-parasitic nematodes and soilborne fungi (Ghaffar, 1995). The combination of nematode and fungus often results in a synergistic interaction wherein the crop loss is greater than expected from either pathogen alone. *Meloidogyne* species are known to interact with *Fusarium* and *Rhizoctonia* resulting in enormous losses in a variety of crops including egg plant (Smits and Noguera, 1982; Evans and Haydock, 1993).

The objectives of the present study were to test for non-randomness and analyse the spatial pattern of *Meloidogyne javanica* (the root-knot nematode) population in soil and root-knot infestation, as well as the incidence of two soilborne root-infecting fungi, *Fusarium solani* and *Rhizoctonia solani* and to examine the nematode-fungus relationship in the development and spatial pattern of root rot-root knot disease complex on egg plant.

MATERIALS AND METHODS

An egg plant (*Solanum melongena* L.) field plot located at Karachi University Campus was sampled for *M. javaniva* (Treub.) Chitw. and two root-infecting fungi, *F. solani* (Mart) Appel *et* Wollenw. emend. Snyd *et* Hans

and *R. solani* Kuehn in a grid of contiguous quadrats. The grid was 12 x 12 m divided into 1.2 x 1.2 m^2 grid units (quadrats). Each quadrat had two rows of three egg plants. The distance between rows was 60 cm and distance between plants was approx. 40 cm. Six replicate soil cores each 13 mm diam. x 189 mm deep (ca 25 cm^3) were drawn from soil near each plant in a quadrat (1 core/plant). The six replicate cores were pooled to obtain a composite sample of 150 cm^3 soil. The root-knot nematodes were extracted from the soil using Baermann funnels. To determine the root colonization by fungi, the root system from each plant was cut into five mm long pieces from which five were randomly chosen (30 pieces/quadrat). After surface sterilization with 1% $Ca(OCl)_2$, the root pieces were plated onto PDA plates supplemented with penicillin (100,000 units/l) and streptomycin sulphate (0.2 g/l). The plates were incubated at 28 ± 2 °C and after one week, root colonization by either *F. solani* or *R. solani* was recorded as follows:

Colonization % = (Number of root pieces colonized by a fungus/Total number of root pieces) x 100.

To analyse the spatial pattern of nematode population and fungal colonization, an index of pattern detection which is relatively insensitive to changes in density is required (Myers 1978). Accordingly Lloyd's (1967) index of patchiness (c) and Morisita index (I_d) that are unaffected by the changes in density caused by random thinning, were used. First mean crowding m* (Llyod 1967) was calculated as:

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

where X_i is the number of nematodes (or colonization frequency by the fungi) in the ith quadrat, Q is the number of quadrats and $N = \Sigma X_i$.

Index of patchiness c is then computed as $c = m/\lambda$ where λ equals the mean density per quadrat Morisita's (1964, 1971) index which is almost equivalent to Lloyd's index of patchiness is:

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

Estimation of the pattern detection indices in literature is almost invariably reported without any indication of sampling variance (e.g., Schuh *et al.*, 1986; Wheeler *et al.*, 1987; Johnson *et al.*, 1991). The jackknife method (Tukey, 1958) not only allows bias reduction but also provides estimation of variance and the confidence interval of the test statistics. Briefly, the value of any index θ is recalculated after removing one value from the pool of n observations. This creates n resampled values. Each of these resampled estimates is converted to a pseudovalue and the mean of pseudovalues gives the jackknife estimate. The variance of jackknife estimate can be estimated by s^2/n where s^2 is the sample variance of the pseudovalues (Manly, 1997). Accordingly, bias corrected estimates and the respective variances of the above statistics were computed. Since the values of patchiness were very close to those of Morisita's index, they are not reported. The value of Morisita's index is one for random distribution and >1 for aggregated pattern. The variance/mean ratio (Greig-Smith 1983) for each pathogen was also calculated to test non-randomness. Lloyd's and Morisita's indices were also calculated for various blocks of quadrats 2 x 2, 3 x 3 and 4 x 4.

Moran's *I* statistic of spatial autocorrelation was calculated for each pathogen. This statistic conserves the spatial information content of the field sampling by making use of information on the location of each sample point (Sokal and Oden, 1978). Thus spatial autocorrelation was used to assess autocorelation of the pathogens among the quadrats. The statistic was calculated from the following formula:

$$I = \frac{(1/W)\sum_{i=1}^{Q}\sum_{j=1}^{Q}(w_{ij}(x_i-x_m)(x_j-x_m))}{(1/Q)\sum_{i=1}^{Q}(x_i-x_m)^2}$$

where x_m is the mean, w_{ij} is the weight assigned to the join between quardats i and j, W is the sum of all weights. Moran's *I* statistic is approximately equal to zero when no trend is present in the spatial pattern. Values of *I* are positive (positive autocorrelation) when the variate values in joined quadrats vary in the same direction from the mean indicating that similar values tend to occur together (aggregated spatial pattern). Values of *I* are negative when variate values in joined quadrats tend to vary in opposite directions from the mean (regular spatial pattern). Autocorrelation coefficients were tested against the null hypothesis that sample *I* does not differ from the expected value $E(I) = -1/(Q-1)$. The significance of *I* was tested using the randomisation null hypothesis. Generally, several indices of pattern detection are required as they measure slightly different aspects of pattern (Greig-Smith, 1983). Computer programs for various methods of pattern detection and analysis have been developed in FORTRAN 77 and are available on request.

RESULTS AND DISCUSSION

Lloyd's index m*, Morisita's index I_δ, variance/mean ratio and autocorrelation for number of galls/root system, root-knot nematodes in the soil, and root colonization by *F. solani* and *R. solani* on egg plant at various block sizes are given in Table I. The advantage of using Lloyd's and Morisita's indices is that they are not influenced by any frequency distribution, *i.e.*, they are non-parametric. In general, mean crowding (the clumping

Table I. Jackknife estimates of Lloyd's index of mean crowding, Morisita's index together with their variances, and variance/mean (V/M) ratio and autocorrelation for root-knot infestation due to *Meloidogyne javanica*, nematode soil population and colonization by *Fusarium solani* and *Rhizoctonia solani*.

Variables	Blocks	Mean	V/M ratio	Lloyd's index		Morisita's index		Moran's index	
				Value	Variance	Value	Variance	*I*	P(*I*=E(*I*))
Galls									
	1 x 1	261.3	24.89	285.0	56.58	1.10	0.00013	0.043	<0.01
	2 x 2	–	41.3	1052.6	1530.8	1.03	0.00010	–	–
	3 x 3	–	62.0	2407.0	11015.4	1.02	0.00008	–	–
	4 x 4	–	72.3	4117.5	41940.8	1.01	0.00006	–	–
Nematode soil population	1 x 1	1865.6	646.75	2506.9	22604.9	1.34	0.0022	0.073	<0.01
	2 x 2	–	1544.0	18927.0	770828.1	1.20	0.0026	–	–
	3 x 3	–	519.2	24904.5	774211.3	1.02	0.000018	–	–
	4 x 4	–	2227.3	31694.2	114837.2	1.06	0.0014	–	–
F. solani									
	1 x 1	67.02	9.39	75.35	2.71	1.12	0.00032	0.035	<0.01
	2 x 2	–	17.07	283.87	98.16	1.05	0.00039	–	–
	3 x 3	–	13.82	620.89	498.86	1.01	0.00008	–	–
	4 x 4	–	39.50	1096.10	4227.6	1.03	0.00022	–	–
R. solani									
	1 x 1	30.51	15.47	44.80	5.05	1.47	0.0039	0.022	<0.05
	2 x 2	–	28.20	147.50	85.96	1.21	0.0029	–	–
	3 x 3	–	27.39	299.3	650.83	1.09	0.0011	–	–
	4 x 4	–	34.45	502.96	1704.5	1.06	0.0005	–	–

together of individuals within a quadrat) was high and increased with the increase in block size. Variances of Lloyd's index were mostly high compared to those of Morisita's index. The sample variance was much higher than the mean in every instance (high variance/mean ratios) implying aggregation of nematodes, soilborne fungi and number of galls (Greig-Smith, 1983). Moran's *I* statistic of spatial autocorrelation was found significantly greater than the expected value E(*I*) for the nematode population in soil, number of galls/root system and colonization frequency of the two fungi. Positive values for spatial autocorrelation implies that similar values occur near one another. The characteristic in which Morisita's index differs from Lloyd's index of mean crowding is that its value is independent of quadrat size. Thus any change in the values of Morisita's index with the change in quadrat size reflects the scale and intensity of pattern. Morisita's index was significantly larger than 1 (expected value for random distribution) at most of the block sizes (p at the most 0.05) though in general, Morisita's index declined with increasing block size indicating that increasingly larger blocks become larger than patch sizes. This resulted in a dilution effect wherein some sub-samples comprising a sample were taken outside the patch thus reducing heterogeneity among plots. By contrast, Alby *et al.* (1985) found greatest I_d values for intermediate plot size and lowest for small plot size. Morisita (1964) suggested that sampling was more reliable when I_d value could be minimized. Plot size selection and sampling intensity could have significant bearing on sampling efficiency (Shaukat and Khan, 1993). I_d was generally lowest at larger block sizes indicating that greater sampling precision can be achieved at these block sizes. Depending upon the pathogen, the most appropriate block size for increased sampling precision appears to be 3 x 3 or 4 x 4 m^2. The orientation of blocks could also greatly influence sampling efficiency (Noe and Campbell, 1985), although this was not tested in this study since all the blocks were square shaped. Besides sampling design, an applied use of the dispersion parameters estimated allows understanding of the risk associated with the management decisions for a given level of sampling intensity. The results suggest various plot sizes (blocks) that could be used for designing field experiments with the crop and the associated pathogens. The sampling intensity must be great enough to measure the populations so that the management decisions can be made at an acceptable level of risk (Ferris, 1984).

The galling frequency and nematode population densities in the soil were strongly positively correlated at all block sizes except 3 x 3 (Table II) indicating that disease severity increases with increasing nematode density. The colonization frequency of the two fungi showed positive correlation with galling intensity at the smaller block sizes indicating that they interact at a small spatial scale. Although interaction between root-knot nematode and root-infecting fungi (*Fusarium solani* and *Rhizoctonia solani*) is well known, the intensity of interaction at various scales in the field has largely been ignored. At individual plant level, the degree of correlation between nematode and *Fusarium* (or *Rhizoctonia*)

Table II. Correlation coefficients (r) and their significance (t) between root-knot nematode population in soil, root-knot infestation and colonization by *F. solani* and *R. solani*; n = number of observations.

Variables	Block size	r	n	t	p-level
Galls vs nematode soil population					
	1 x 1	0.7415	144	13.16	<0.001
	2 x 2	0.7557	36	6.72	<0.001
	3 x 3	0.3850	16	1.56	n.s.
	4 x 4	0.7754	9	3.24	<0.05
Galls vs *F. solani*					
	1 x 1	0.3077	144	3.85	<0.001
	2 x 2	0.3684	36	2.30	<0.05
	3 x 3	0.1960	16	0.74	n.s.
	4 x 4	0.4200	9	1.22	n.s.
Galls vs *R. solani*					
	1 x 1	0.5506	144	2.70	<0.01
	2 x 2	0.4640	36	2.71	<0.01
	3 x 3	-0.1390	16	0.52	n.s.
	4 x 4	0.3086	9	0.86	n.s.
Nematode soil population vs *F. solani*					
	1 x 1	0.3410	144	4.32	<0.001
	2 x 2	0.3940	36	2.50	<0.05
	3 x 3	0.2470	16	0.95	n.s.
	4 x 4	0.4060	9	1.17	n.s.
Nematode soil population vs *R. solani*					
	1 x 1	0.1863	144	2.24	<0.05
	2 x 2	0.2340	36	1.40	n.s.
	3 x 3	0.1637	16	0.62	n.s.
	4 x 4	0.2713	9	2.74	<0.05
F. solani vs *R. solani*					
	1 x 1	0.2053	144	2.49	<0.01
	2 x 2	0.3227	36	1.98	n.s.
	3 x 3	0.6074	16	2.85	<0.05
	4 x 4	0.7439	9	2.93	<0.05

may to some extent be determined by competition for infection loci or feeding sites and to a reduction in root growth (Griffin and Thyr, 1988). On the other hand, at the scale of quadrat or larger block sizes, these relationships can primarily be governed by factors like nematode dispersal, soil heterogeneity and the presence or absence of various antagonistic organisms of the root-knot nematode. Thus, for the work on the interactions of nematodes and root-infecting fungi it is suggested that small plot sizes (e.g., 2 x 2) should be employed together with an increased number of cores (sub-samples) to obtain greater sampling precision. It is apparent that the design of experiments or sampling strategy involving two or more pathogens should also take into account the covariance (correlation) structure in addition to dispersion or variances of the pathogen species. Further understanding of spatial pattern and the biological and environmental factors which influence the pattern of root-knot nematodes and the interacting fungi should ultimately lead to better management of root-knot nematodes and root-infecting fungi.

The study exposes the underlying spatial pattern of the root-knot and root-rot pathogens as well as the hitherto relatively unexplored interrelationships, in the spatial perspective, between soil fungi and the root-knot nematode.

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