Russian Journal of Nematology, 1997, 5 (1), 67 - 70

Distribution of entomopathogenic nematodes in a grassland

John S. Miduturi, Renaat Moermans and Maurice Moens

CLO-Research Station for Nematology and Entomology, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium.

Accepted for publication 7 February 1997

Summary. The distribution of *Steinernema feltiae* and *Heterorhabditis megidis* (NWE type) in a grassland habitat was studied during autumn 1994. Larvae of the wax moth, *Galleria mellonella*, were used as bait. Results indicated an aggregated distribution for both species. Sand fraction and organic matter content did not always influence the distribution of the nematodes. Largest numbers of *S. feltiae* were detected in samples with a sand fraction content higher than 90%. The area in which *H. megidis* was detected, was somewhat limited and had a sand fraction greater than 94%. Numbers of *S. feltiae* were negatively influenced by higher organic matter content.
Key-words: aggregation, granulometry, organic matter.

Nematodes of the families Steinernematidae and Heterorhabditidae are pathogenic to a diverse group of insects and have a unique symbiotic association with the bacteria *Xenorhabdus* and *Photorhabdus* respectively (Boemare *et al.*, 1993). The nematodes act as vectors for these bacteria which, inside the insect body, provide optimal conditions for reproduction and multiplication of the nematodes.

Entomopathogenic nematodes (epns) are increasingly used as biocontrol agents (Gaugler, 1988). To be effective, epns should be present in a way they can affect their hosts. This depends largely on the nematode distribution in the field but little information is available on the distribution of epns in natural habitats. Therefore, the natural dissemination of infective epns was studied and related to soil characteristics.

MATERIALS AND METHODS

Site and sampling. The study was done in a permanent pasture at Snellegem (2° 40' E, 51° 20' N) West-Flanders, Belgium at which *Steinernema feltiae* Filipjev (A1 type) and *Heterorhabditis megidis* Poinar, Jackson & Klein (NWE type) were present. The pasture was mainly composed of *Lolium perenne* L., *Festuca ovina* L., and *Agrostis canina* L. The overall soil texture of the site was sandy loam and the pasture was free of chemical fertilizers and pesticides.

Due to the irregular shape of the pasture, two sampling grids were laid out over the sampling area. The first grid had eleven rows (spaced 3 m) and six columns (spaced 4 m) and the second grid had eight rows and five columns spaced at half of the previous distances. Soil samples were collected in October 1994 at the intersections of the columns and rows. Samples were taken with a 10 cm diameter auger to a depth of 10 cm. The soil samples were thoroughly mixed in plastic bags and a representative subsample of 250 g was used for baiting.

Baiting and extraction of nematodes. Each soil sample was placed in a plastic box (13 x 15 x 3.5 cm) and five last instar larvae of *Galleria mellonella* L. were added. The lid of each box was sealed with Parafilm and the box kept inverted in darkness at 23-25 °C. After 3 days, the dead *Galleria* larvae were replaced with live specimens. This procedure was repeated twice. After the third baiting the samples were kept at 5 °C for 14 days and at the end of this period the samples were again baited with 5 *Galleria* larvae. After each baiting procedure the dead *Galleria* larvae were removed and digested with pepsin (Mauléon *et al.*, 1993). The preadults of both epns were counted. For the purpose of identifying the nematodes, one dead *Galleria* larva was incubated on a Petriplate for 7 days to obtain infective juveniles and adults. The identity of the isolates was determined based on the morphometrics of infective juveniles and the male tail shape and spicule colour.

Soil parameters. Soil samples were analysed for their organic matter content by the ignition method (Andrews, 1973). Granulometric analysis was performed using a Coulter LS 100 apparatus (fluid model). The particle size analysis gave the relative presence of the clay fraction (<4 μm), silt fraction

Table 1. Parameters of the negative binomial distribution for entomopathogenic nematodes in two sampling grids in a grassland.

Grid size (m x m)	Nematode species	Number of samples	Mean	Variance	K-value
3 x 4	*S. feltiae*	66	11.50	291.76	0.47
1.5 x 2	*S. feltiae*	40	15.22	864.92	0.27
	H. megidis	40	41.14	1930.69	0.89

(4-63 μm) and the sand fraction (>63 μm).

Statistical analysis. The mean and the variance for each species in both of the grids was calculated. The relationship between the epn numbers and the soil parameters *viz.*, particle size and organic matter content, was studied using stepwise multiple linear regression. Nematode numbers were represented by a bubble diagram as a function of the soil parameters (Chambers *et al.*, 1983).

RESULTS

Both sampling grids contained *S. feltiae* and 66 out of 106 samples contained the nematode. *Heterorhabditis megidis* was only present in the 2 m x 1.5 m grid with 7 out of 40 samples containing the nematode. None of the positive samples contained a mixed epn population. The parameters for the negative binomial distribution fitted to the nematode numbers are given in Table 1. In both sampling grids, the variances were greater than the mean resulting in k-values less than 1. This indicated that the distribution of the nematode numbers could be described by the above mentioned method.

The distribution of the nematodes in both grids is represented in Figure. 1A & B by means of a bubble diagram. The sizes of the symbols are proportional to the number of nematodes present at a given sample point. Figure 1A shows that in the first grid *S. feltiae* was evenly present over the sampling area and that some of the sample points contained much higher nematode numbers than others. In the second grid (Fig. 1B) the two nematode species were separated from each other. The area covered by *H. megidis* was much smaller than that occupied by *S. feltiae* and nematode numbers of both species peaked at some sample points.

In Fig. 1C, the bubbles represent the number of *S. feltiae* found in the first sampling grid as a function of the soil characteristics at the sample points. Increasing sand fraction corresponded with a higher nematode number; increasing organic matter content resulted in lower numbers. Nematodes were mainly found in soils with a sand fraction greater than 90% and an organic matter content less than 10%. The multiple linear regression relating nematode numbers to the soil characteristics, confirmed these observations. The F-values for the sand fraction and organic matter content have a probability of being exeeded of 0.014 and 0.016. The standardised coefficients, as absolute measures of the effects of both characteristics, are 0.300 and -0.294.

In Fig. 1D, *S. feltiae* and *H. megidis* numbers found in the second sampling grid are presented as bubbles as a function of the soil characteristics at the sample points. In general, *S. feltiae* was found in samples with a sand fraction of 91-96% and an organic matter content less than 8%. *H. megidis* was detected in samples with sand fractions higher than 92%; the organic matter content seemed to have no effect. The multiple linear regression relating nematode numbers to the soil characteristics, confirmed these observations. For *S. feltiae*, the F-values have a probability of being exceeded of 0.781 (sand fraction) and 0.396 (organic matter). These probabilities are too large to demonstrate any effect. For *H. megidis*, the F-values have a probability of being exceeded of 0.434 (sand fraction) and 0.071 (organic matter) with the small latter probability being due to an outlier.

DISCUSSION

Spatial distributions of nematodes can be studied by taking series of cores and computing their distribution parameters. A clumped or aggregated distribution is indicated by a variance greater than the mean and can be described by one of the contagious distributions, such as the negative binomial (Elliott, 1979). Plant parasitic nematodes are known to have an aggregated distribution which was described by a negative binomial model (Seinhorst, 1982; Davis, 1984). In general, natural populations of epns, like other soil dwelling nematodes, display a highly aggregated distribution (Fan, 1989; Harris, 1991; Cabanillas & Raulston, 1994) and the results of our study confirm *S. feltiae* and *H. megidis* to be highly aggregated. Both, the variance greater than the mean in both sampling grids and the estimated k-value of the negative binomial distribution less than 1 (Table 1) suggest a clustered distribution of both *S. feltiae* and *H. megidis.*

Soil texture influences nematode survival and mobility. Generally, higher clay content results in

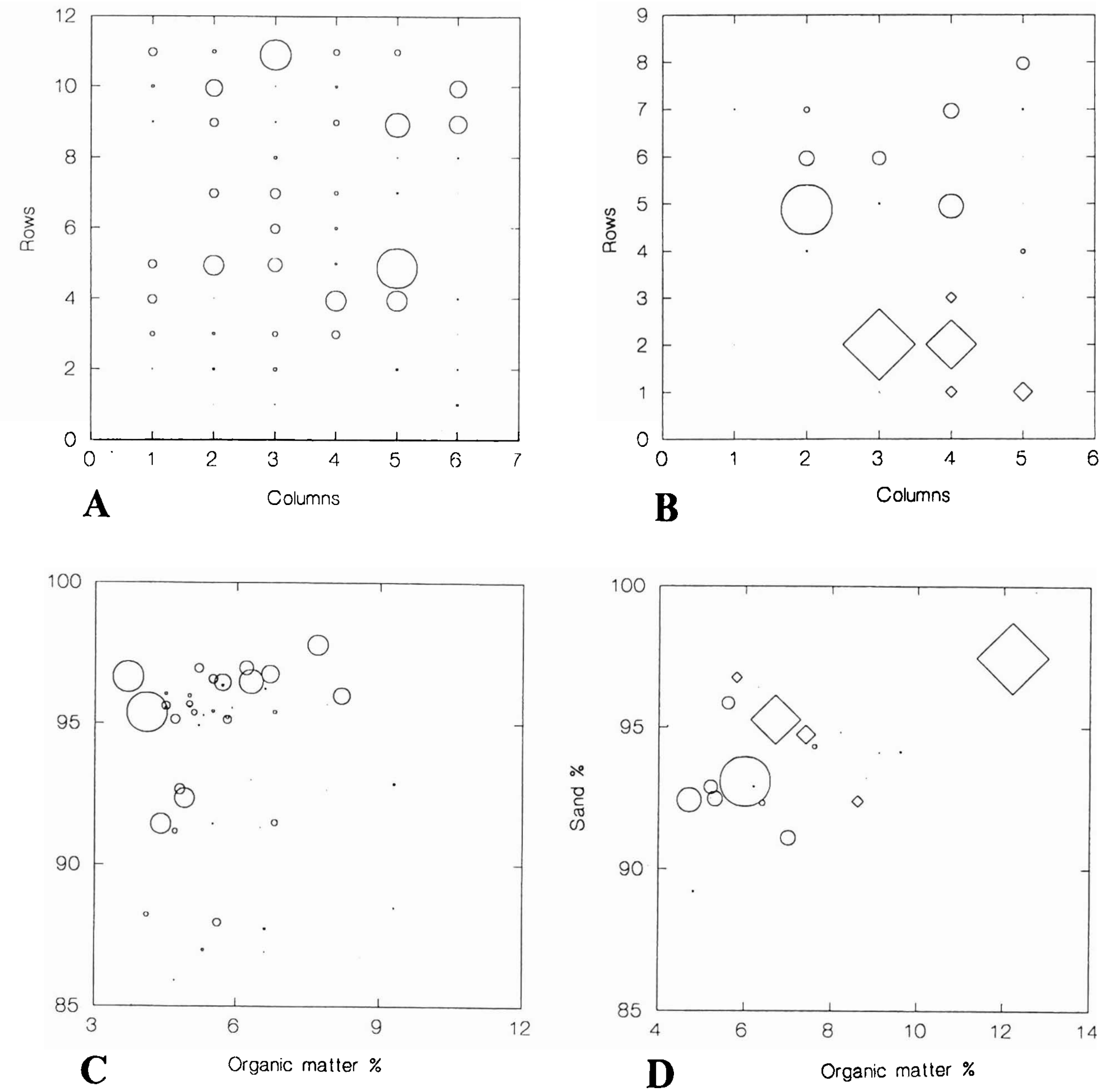

Fig. 1. A: Distribution of *Steinernema feltiae* in a sampling grid. The bubbles represent nematode numbers; the larger the bubble, the higher the nematode number; B: Distribution of *S. feltiae* (○) and *Heterorhabditis megidis* (□) in a second sampling grid; C: *S. feltiae* (○) numbers in relation to the sand fraction and the organic matter content of soil samples in a sampling grid; D: *S. feltiae* (○) and *H. megidis* (□) numbers in relation to the sand fraction and organic matter content of soil samples in a second sampling grid.

lower nematode survival. This is due to decreased pore size and reduced oxygen availability (Kung *et al.*, 1990a). Nematodes are generally more mobile in sandy soil and mobility decreases as the percentage clay and silt increases (Georgis & Poinar, 1983; Barbercheck & Kaya, 1991). These two factors *viz.* survival and mobility can greatly contribute to the distribution of nematodes in a particular habitat. Kung *et al.* (1990b) showed that the survival and infectivity of both *S. carpocapsae* and *S. glaseri* were positively affected by high sand fractions. Miduturi *et al.* (1996) reported that higher numbers of *S. feltiae* positive samples were obtained in sand to sandy loam soils than in clay loam soils. In the two grids we studied, *S. feltiae* was only occasionally detected when the sand fraction was less than 90%. As a results of the soil texture in both grids being almost identical, differences in numbers of *S. feltiae* were not detected. No significant effect of the sand fraction could be shown for *H. megidis*.

Both positive and negative correlations between nematodes and organic matter have been observed (Norton, 1978). Positive correlations between organic matter and members of Tylenchinae were found in soybean fields in a normal rainfall year and negative correlations were found between organic matter and Dorylaimidae and other stylet bearing nematodes (Norton *et al.*, 1971). The present study revealed that organic matter had a negative effect on the numbers of *S. feltiae* in the first sampling grid, as demonstrated by the negative value of the regression coefficient. No effect of organic matter content could be demonstrated for *H. megidis*.

The results of the present study suggest that both the sand fraction and the organic matter content of soil can affect the distribution of epns in grassland. Further laboratory observations are required to confirm the effect of sand and organic matter on the infectivity and persistence of epns.

ACKNOWLEDGEMENTS

We thank Ing. G. Hendrickx, Ms. N. de Sutter and Ms. T. Derycke for their help during the survey and the family Steyaert (Snellegem) for allowing us to take samples in their backyard. The senior author acknowledges the financial support from the Research Station for Nematology and Entomology (CLO), Merelbeke, Belgium.

REFERENCES

Andrews, W.A. 1973. *A Guide to Study of Soil Eology*. Scarborough, Ontario, Prentice-Hall of Canada Ltd. 198 pp.

Barbercheck, M.E. & Kaya, H.K. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditidae: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditidae: Steinernematidae). *Environmental Entomology* 20: 582-589.

Boemare, N.E., Akhurst, R.J. & Mourant, R.G. 1993. DNA relatedness between *Xenorhabdus* spp.(Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *International Journal of Systematic Bacteriology* 43: 249-255.

Cabanillas, H.E. & Raulston, J.R. 1994. Evaluation of the spatial pattern of *Steinernema riobravis* in corn plots. *Journal of Nematology* 26: 25-31.

Chambers, J.M., Cleveland, W.S., Kleiner, B. & Tukey, P.A. 1983. *Graphical Methods for Data Analysis.* North Scituate, Massachusetts, Duxbury press.

Davis, R.M. 1984. Distribution of *Tylenchulus semipenetrans* in a Texas grapefruit orchard. *Journal of Nematology* 16: 313-317.

Elliott, J.M. 1979. Statistical analysis samples of benthic invertebrates. *FBAS Publication* 25, 14-93: 126-137.

Fan, X. 1989. Bionomics of British strains of entomopathogenic nematodes (Steinernematidae). *PhD Thesis, University of London. U.K.*

Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agriculture, Ecosystems and Environment* 24: 351-360.

Georgis, R. & Poinar, G.O. Jr. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). *Journal of Nematology* 15: 329-332.

Harris, J.E. 1991. Dynamics of natural and inundatively released entomopathogenic nematodes. *B.Sc. Hons. Thesis*, Australian National University, Canberra.

Kung, S.P., Gaugler, R. & Kaya, H.K. 1990a. Influence of soil pH and oxygen on persistence of *Steinernema* spp. *Journal of Nematology* 22: 440-445.

Kung, S.P., Gaugler, R. & Kaya, H.K. 1990b. Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* 55: 401-406.

Mauléon, H., Briand, S., Laumond, C. & Bonifassi, E. 1993. Utilisation d'enzymes digestives pour l'ètude du parasitisme des *Steinernema* et des *Heterorhabditis* envers les larves d'insectes. *Fundamental and Applied Nematology* 16: 185-191.

Miduturi, J.S., Moens, M., Hominick, W.M., Briscoe, B.R. & Reid, A.P. 1996. Naturally occurring entomopathogenic nematodes in the province of West-Flanders, Belgium. *Journal of Helminthology* 70: 319-327.

Norton, D.C. 1978. *Ecology of Plant-Parasitic Nematodes.* New York, John Wiley & Sons. 269 pp.

Norton, D.C., Frederick, L.R., Ponchillia, P.E. & Nyhan, J.W. 1971. Correlations of nematodes and soil and properties in soybean fields. *Journal of Nematology* 3: 153-163.

Seinhorst, J.W. 1982. The distribution of cysts of *Globodera rostochiensis* in small plots and resulting standard error. *Nematologica* 28: 285-297.

Miduturi J. S., Moermans R., Moens M. Распределение энтомопатогенных нематод на участке луга.

Резюме. Исследовано распределение энтомопатогенных нематод *Steinernema feltiae* и *Heterorhabditis megidis* на участке луга. Для обоих видов было выявлено аггрегированное распределение. Механический состав и содержание органического вещества в почве не всегда определяли особенности распределения этих нематод. Максимальная численность *S. feltiae* была отмечена в почвах с содержанием песчаной фракции выше 90%. Нематоды *H. megidis* были довольно редки и встречались в почвах с содержанием песка выше 94%.