Factors influencing the occurrence of entomopathogenic nematodes in the Central Rift Valley Region of Kenya

S. W. Mwaniki[1]*, J. H. Nderitu[2], F. Olubayo[2], J. W. Kimenju[2] and Khuong Nguyen[3]

[1]*Kenya Agricultural Research Institute, National Agricultural Research Laboratories, PO Box 14733-00800, Nairobi, Kenya,* [2]*Department of Crop Science and Crop protection, University of Nairobi, PO Box 30197, Nairobi, Kenya and* [3]*Department of Nematology and Entomology, University of Florida, Gainesville, FL 3211-0620, U.S.A.*

Abstract

A survey for entomopathogenic nematodes in the central Rift valley region of Kenya was conducted at altitudes between 1800 and 3000 m above sea level and from croplands and noncropland habitats. The sampling depth was 0–30 cm. GPS (global positioning system) was used to measure site positions. One hundred and twelve soil samples were collected and entomopathogenic nematodes trapped through *Galleria mellonella*. Entomopathogenic nematode presence was demonstrated by *G. mellonella* mortality and viable ones bulked through the same host. Nematode recoveries from two consecutive extractions were 30% per extraction and 52% for cumulative extractions. Recoveries from agro ecological zones ranged between 18% and 71%. Recovery frequency was higher from disturbed cropland habitats than the stable noncrop habitats. *Steinernema* species were more frequent than *Heterorhabditis (*9 : 1). Nematode occurrence clustered at 2–3% carbon and pH 5.3–6.3 with no specific pattern demonstrated from soil types. Nematode species of the two genera from high altitudes lost their culturing ability within 1 month of isolation. There was a tendency for recovering both nematode genera at the shores of water bodies. This is the first report of *Steinernema yirgalemense* and *S. weiseri* in Kenya and of *S. karii* in the central Rift valley region. The *Heterorhabditis* species has not been confirmed yet. This has widened the genetic base of entomopathogenic nematodes from Kenya. The entomopathogenic nematodes are available for developement as biological control agents of athropod pests.

Key words: agro-ecological zones, entomopathogenic nematodes, *Heterorhabditis, Steinernema*

*Correspondence: E-mail: shelmwaniki@yahoo.com

No conflict of interest has been declared by the authors

Introduction

Entomopathogenic nematodes occur in all soils except those in the Arctic and Antarctic circles (Hominick *et al.*, 1997). They are parasitic to arthropods and can be manipulated for use as biological control agents for crop pests. They have been successfully used for the control of the black vine weevil *Otiorhynchus sulcatus* F (Bedding & Miller, 1981), pests of turf *Popillia japonica* Newman (Georgis & Gaugler, 1991) and maize ear worm *Helicoverpa* (*Heliothis*) *zea* Boddie among others (Cabanillas & Raulston, 1995). The most notable study in Kenya was that carried out in the central highlands and coastal lowlands of Kenya where a new species *Steinernema karii,* and two described nematodes *Heterorhabditis indica* and *H. bactriophora* were isolated and effectiveness of these entomopathogenic nematodes on the stem borer (*Chilo partelus)* and cut worms *(Agrotis ipisilon)* tested (Waturu & Reid, 1997; Waturu, 1998). Several surveys have been carried out in other regions in the last two decades and several entomopathogenic nematode species isolated (Hominick, Reid & Briscoe, 1990; Gaugler *et al.*, 1992; Rosa *et al.*, 2000). The surveys have brought out some environmental factors that could affect entomopathogenic nematodes and therefore influence their survival.

The aim of this study was to broaden the genetic base of Kenyan entomopathogenic nematodes by expanding the part of Kenya surveyed. The study also aimed at finding the relationship between nematode occurrence and various environmental factors on a Kenyan perspective. The survey area was selected because of its diverse environment with most of the natural features in Kenya represented. Nematode species occurrence was expected to give a broad view of most of the entomopathogenic nematodes

in Kenya and predict environmental characteristics that are related to nematode recovery. The findings will assist in future one spot survey for fresh cultures for biological control of insect pests. The study area was to the immediate west of the previously surveyed area in Kenya (Waturu, 1998).

Materials and methods

Soil samples were collected from the central rift valley region of Kenya in October 2005. The survey covered all agro-ecological zones. The study area lies between 1°10′S–0°15′N and longitude 35°20′W–36°40′E and from 1600 to 3000 m above sea level. The mean temperatures in the area were 9.9–21°C and mean annual rainfall 600–1800 mm. The vegetation habitats covered included crops (annual crops; maize, beans, mixed cropping, vegetables, potatoes, arrowroots, pumpkins, peas, wheat, oats, barley, oil crops; rapeseed, perennial crops; Napier, Lucerne and undisturbed habitats (pasture lands, forests and under hedge canopies).

The sampling interval was 5 km. The number of sample plots per agro ecological zone was determined by the area of the agro ecological zone. Sample sites were plots of 1600 m^2. Ten soil samples were taken at random within the sample plots using garden hoes. The ten single point samples of 300 g were mixed to form a 3 kg composite field sample per site. The site location data in terms of altitude, latitude, longitude and proximity and direction to the nearest major town were recorded using GPS. The samples were placed in polythene bags and transported to the laboratory in sisal gunny bags.

Entomopathogenic nematodes were extracted from soils within the first week after the survey. The composite soils samples were shaken vigorously to ensure homogeneity. Sub samples of 400 ml were drawn and put in half-litre plastic containers for the extraction of entomopathogenic nematodes using the method described in Woodring & Kaya (1988). Four-third stage *Galleria mellonella* larvae were placed on the surface of each soil sample and contents covered with a perforated lid and incubated at room temperature (20–25°C) for 3 days. Both live and dead larvae were retrieved from soils on the third day. Four new *G. mellonella* larvae were used to replace those retrieved. The second batch was also retrieved after 3 days. Samples were considered positive for nematode occurrence, if *G. mellonella* larvae mortality occurred and if the cadaver took up the yellowish brown colour for steinernematids or brick red for heterorhabditids (Woodring & Kaya, 1988). Records of sites with nematode infected *G. mellonella* cadavers were taken in the two extractions. In each instance, cadavers were rinsed with distilled water to remove surface contaminants. The cadavers were then placed in sterilized petri dishes (9 by 3.5 cm) lined with Whatman filter paper. The dishes and contents were sealed with Parafilm and incubated for further 2 days at room temperature and then transferred to modified White traps for nematode emergence as described in Woodring & Kaya (1988). The infective nematode juveniles migrated into the larger bowl and were harvested for 4 days. *Galleria mellonella was* used to multiply the nematode isolates. The field sample sites were characterized based on altitude, agro-ecological zone (upper highlands, lower highlands, upper midlands and lower midlands and their finer subdivisions) as defined by Jaetzold & Schmidt (1983). Vegetation was classified as stable noncropland vegetation or unstable cropland vegetation. Per cent carbon was determined by the Walkley–Black wet oxidation method (Nelson & Sommers (1982). Soil pH was determined by 1 : 1 soil $CaCl_2$ suspensions and soil texture by the hydrometer method of Hinga, Muchena & Njihia (1980). Nematode culturing ability was determined by monthly re-culturing of the nematodes and proximity to water bodies from observations during sampling. The nematode cultures were stored at 15°C. Males, females and juvenile entomopathogenic nematodes that could culture were isolated for identification to species level by molecular techniques.

Results

One hundred and twelve composite samples (1120 single point samples) were collected from the area. The proportion of entomopathogenic nematode recovery from single extraction was 30% and 52% from cumulative double extraction. Recovery of entomopathogenic nematodes ranged 18–71% with most zones recording over 50% (Table 1). The samples from the upper highland zone three (UH3) were from the extended part of the zone in the aberdare ranges to the north east of L Elementaita. Although most nematodes were recovered from the altitude ranged 2400–2600 m above sea level (Table 2), those from higher altitudes had poor culturing ability, which declined rapidly, compared to those from lower altitudes. Three Heterorhabdtid isolates from lower altitudes

Table 1 Entomopathogenic nematode occurrence in different agro-ecological zones

AEZ	Total samples	*Steinernema* spp (%)	*Heterorhabditis* spp (%)	Total & % nematodes
UH0	7	4	0	4 (57)
UH1	12	1	1	2 (18)
UH2	26	14	2	16 (61)
UH3	13	8	1	9 (70)
LH2	8	3	0	3 (38)
LH4	15	9	0	9 (60)
LH5	13	6	1	7 (57)
UM5	7	4	1	5 (75)
UM6	11	4	0	4 (36)
	112	53 (47)	6 (5)	59 (52)

re-cultured after 8 months while the two isolated from above 2600 m could not culture beyond the first month.

The proportion of entomopathogenic nematodes positive sites from cropland vegetation was higher (56%) than that from the more stable noncropland vegetation habitats (35%). The samples per cent carbon ranged from <1 to 3–4 with most soils (46%) having a per cent carbon of 2–3%. Overall mean per cent carbon was 2.18, which was equal to the mean for samples positive with *Steinernema* species. Sites positive for *Heterhorhabditis* species had a mean of 2.35% carbon (Table 3). The differences of means were not significant.

Most soil samples had pH of 5.3–6.3, which was the same range with the maximum frequency of entomopathogenic nematodes (Table 4). The highest frequency of *Steinernema* species was within pH 5–6 but *Heterorhabditis* species although few were mainly in samples of pH >6. The soil texture classes were: clay loam (30%), clay (20%), loam (10%), sandy clay loam (6%), sandy loam (3%) and sandy silty loam (1%). Entomopathogenic nematode recovery was about 50% for most textural classes. Seventy-five per cent of samples from coastlines of large water bodies like Lake Elementaita, Nakuru and Naivasha and large man made dams were positive for nematodes while none of the samples from river banks and marshlands were positive for entomopathogenic nematodes (the number of samples from the two environments were equal). The two nematode genera were found occurring together near the water bodies.

Entomopathogenic nematode identification at molecular level has confirmed the occurrence of three described species in the region: *Steinernema yirgalemense*, *S. karii* and *S. weiseri*. These have been added to the entomopathogenic gene banks at three Kenya Agricultural Research Centers; National Agricultural Research Laboratories, National Horticultural Research Centre and National Fiber Research Centre Mwea.

The SAS Institute (1982) logistic was used for analysis of the data on soil texture, soil pH and per cent carbon while

Table 2 Entomopathogenic nematode positive samples at different altitudes in central Rift Valley

Altitude (m)	Total samples	Nematode sites overall (%)	*Steinernema* spp (%)	H*eterorhabditis* spp (%)
1800–2000	30	16 (53)	14 (46.6)	2 (6.6)
2001–2200	21	12 (57)	12 (57)	0
2201–2400	13	4 (31)	3 (23)	1 (8)
2401–2600	29	19 (66)	17 (59)	2 (7)
2601–2800	17	8 (48)	7 (42)	1 (6)
2801–3000	2	0	0	0
Total	112	59 (52.7)	53 (47)	6 (5)

Table 3 Entomopathogenic nematode occurrence in relation to per cent carbon

% carbon	Total samples (%)	*Steinernema* spp (%)	*Heterorhabditis* spp (%)	Overall occurrence (%)
<1	3 (3)	2 (66)	1	3 (100)
1–2	41 (38)	19 (46)	1	20 (49)
>2–3	50 (46)	23 (46)	1	24 (48)
>3–4	15 (14)	9 (60)	3 (20)	12 (87)
Total	109	53 (48)	6 (5)	59 (53)
Mean% carbon	–	2.18	2.35	–

pH	Total samples	*Steinernema* spp (%)	*Heterorhabditis* spp (%)	Overall nematode occurrence (%)
<4	1	1	0	1
4–5	20	10 (50)	1 (5)	11 (55)
5–6	53	28 (53)	1 (2)	29 (55)
6–7	30	11 (37)	2 (6)	13 (43)
>7	5	3 (60)	2 (40)	5 (100)
Total	109	53 (50)	6 (5)	59 (55)

Table 4 Entomopathogenic occurrence in relation to soil pH

chi-square was used for altitude and agro ecological zone data.

Discussion

The results from single extraction suggest that nematodes were well distributed in the survey area at the time of sampling. Additional positive sites in the second extraction were of *Steinernema* species only, evidence supporting the finding that in many surveys this nematode genus is more frequent. The results also suggest that more than one nematode assay would increase nematode frequency from surveys. The frequencies from assaying of soil once and twice are within the ranges reported by Ehlers in Gaugler et al. (1992). Some negative sites may have been positive all along but the nematodes remained noninfective. This could be a survival strategy adopted by some nematodes to avoid species extinction as entomopathogenic nematodes from some sites caused host mortality but were unable to reproduce in *G. mellonella.* Alternatively, *G. mellonella* may not have been a good host for some of these nematodes. Some nematodes have a narrow host range for instance *Steinernema scapterisci* (Nguyen & Smart, 1990). The environment in the culture laboratory may also not have been ideal for some of these nematodes. Per cent recovery of *Heterorhabditis* species was 5% compared to that of Steinernematids (47%). Both frequencies are within the ranges reported in continental Europe (Hominick *et al.*, 1990, 1996; Hominick, Reid & Briscoe, 1995; Steiner, 1996; Yoshida *et al.*, 1998 and Sturhan & Liscova, 1999) where steinernematids were more numerous. Low nematode recoveries have been reported from islands where *Heterorhabditis* species were more abundant (Rosa *et al.*, 2000).

More entomopathogenic nematodes occurred in crop lands (annual and perennial crops) than noncrop lands. Plots under crops are usually more aerated than undisturbed plots, which tend to be more compact. This could be one reason for the recovery trends. Most crop plots were small-scale farms, which were used for different crops in different seasons with some of the pests having resting stages in the soils. Different crops have different pests, which could have been used by nematodes for continued survival. In contrast, noncropland vegetation is usually one kind of vegetation over many seasons. The pests of one vegetation type are fewer than those of many crops and therefore having a reduced ability to sustain entomopathogenic nematodes. Entomopathogenic nematodes have been recovered in the natural attacking crop pests as is the case of *Steinernema riobravis,* which was discovered attacking the corn earworm *Helicoverpa zea* Boddie in Rio De Grande Valley (Cabanillas & Raulston, 1994).

Although the effect of altitude was not significant ($\chi^2 = 1.9$, $P = 0.16$) for nematode occurrence, the result of the study suggest that the culturing ability of entomopathogenic nematodes was influenced by the altitude of the area they were initially isolated from. Entomopathogenic nematodes from higher altitudes lost their viability more rapidly than those from medium and lower altitudes over an 8-month period. This could probably be related to the environmental conditions prevailing in the culturing centre (1850 m and 18–25°C). Lower altitude nematodes adapted better. Temperature has been reported as one of the major factors affecting nematodes with specific nematodes having an optimal and threshold temperature (Trdan *et al.*, 2005).

The study suggests that entomopathogenic nematodes had prevalence for soils of 2–3% carbon. Steinernematids were more suited to lower per cent carbon (mean 2.18%) than heterorhabditids (mean 2.35%). The mean was not significantly different but a similar trend was reported by Rosa *et al.* (2000).

The results of this study also suggest that pH 5.3–6.3 was the most suitable range for entomopathogenic nematode occurrence. Steinernematids were more suited for pH <6 and heterorhabditids for pH >6. This was in agreement with studies carried out by Rosa *et al.* (2000) who reported

pH 6 as borderline with Steinernematids preferring pH <6 and heterorhabdatid pH >6.

The basic consideration for nematode survival in soils is aeration and soil moisture, which are provided for in a soil environment by porosity (Kung, Gaugler & Kaya, 1990). Sandy soils have larger pore sizes making them ideal for nematode survival. No pattern was deduced for any soil type. The per cent nematode recovery was about 50% for all soil types. Preference for particular soils may be demonstrated when analysis is performed per nematode species as it has been shown that different nematodes behave differently in different soils (Kung *et al.*, 1990).

Nematode recovery from soils near water bodies whether fresh (Lake Naivasha and man made dam) or salty (Lake Elementaita) was higher than that from riverbanks and marshes. This may be related to the obvious better moisture levels in lake coastlines and the fact that heavy deposition occurs during heavy rains probably carrying nematodes from higher sites, while marshy soils are waterlogged and poorly aerated and therefore unsuitable to entomopathogenic nematodes, which are obligate aerobes.

The confirmation of the presence of three described entomopathogenic nematodes species in this region is understandable considering that *S. karii* was already isolated from the central highlands of Kenya (Waturu & Reid, 1997), S. *yirgalemense* from Ethiopia (Nguyen *et al.*, 2004) and *S. weiseri* from a roadside environment with apple trees in the Czech republic (Mracek et al., Sturhan & Reid, 2003). In this study, *S. weiseri* was also isolated from a roadside pasture in the Lower highlands in the eastern part of the study region at an altitude of 2050 m above sea level.

Acknowledgements

The study was supported by Kenya Agricultural Research Institute through the Kenya Agricultural Productivity Program (KAPP). We thank John Kamau and Simon Njinjo of KARI Kabete and Mwea for their assistance and Mr Mwangi and Wamai of KARI Biometric department for their assistance with data analysis.

References

Bedding, R.A. & Miller, L.A. (1981) Use of a nematode *Heterorhabditis heliothidis* to control the black vine weevil *Otiorhynchus sulcatus* in potted plants. *Ann. Appl. Biol.* **99**, 211–216.

Cabanillas, H.E. & Raulston, J.R. (1994) Pathogenicity of *Steinernema riobravis* against corn earworm, *Helicoverpa zea* (Boddie). *Fundam. Nematol.* **17**, 219–223.

Cabanillas, H.E. & Raulston, J.R. (1995) Effects of furrow irrigation and distribution and infectivity of *Steinernema riobr*avis against corn earworm. *Fundam. Nematol.* **19**, 273–281.

Gaugler, R.J., Campbell, F., Selvan, S. & Edwin, E.L. (1992) Large-scale inoculum release of entomopathogenic nematodes *Steinernema glaseri* assessment 50 years later. *Biol. Control* **2**, 181–187.

Georgis, R. & Gaugler, R. (1991) Predictability in biological control using Entomopathogenic nematodes. *J. Econ. Entomol.* **84**, 713–720.

Hinga, G., Muchena, F.N. & Njihia, C.M. (Eds) (1980) *Physical and Chemical Methods of Soil Analysis. A Handbook for Soil Analysis.* Agric Information Center, Nairobi, Kenya.

Hominick, W.M., Reid, A.P. & Briscoe, B.R. (1990) Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. *Parasitology* **100**, 295–302.

Hominick, W.M., Reid, A.P. & Briscoe, B.R. (1995) Prevalence and habitat specificity of steinernematids and heterorhabditids nematodes isolated during soil surveys of the UK and Netherlands. *J. Helminthol.* **69**, 27–32.

Hominick, W.M., Reid, A.P., Bohan, D.A. & Briscoe, B.R. (1996) Entomopathogenic nematodes: biodiversity, geographic distribution and the convention on biological diversity. *Biocontrol Sci. Technol.* **6**, 317–381.

Hominick, W.M., Reid, A.P., Briscoe, B.R., Pino De, F.G., Heng, J.I.A.N., Hunt, D.J., Kozody, E., Mracek, Z., Nguyen, K.B.R., Reid, A.P., Spiridnov, P., Sturhan, B., Waturu, C.N. & Yoshida, M. (1997) Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *J. Helminthol.* **71**, 271–298.

Jaetzold, R. & Schmidt, H. (1983) *Natural Conditions and Farm Management Information – Central Kenya (Rift Valley and Central Provinces). Farm Management Handbook of Kenya*, **Vol. 11B**. Ministry of Agriculture, Nairobi, Kenya.

Kung, P., Gaugler, R. & Kaya, H.K. (1990) Soil type and entomopathogenic nematode persistence. *J. Invertebr. Pathol.* **55**, 401–406.

Mracek, Z., Sturhan, D. & Reid, A. (2003) *Steinernema weiseri* n.sp. (Rhabditida, Steinernematidae), a new entomopathogenic nematode from Europe. *Syst. Parasitol.* **56**, 37–47.

Nelson, D.W. & Sommers, L.E. (1982) Total carbon, organic carbon and organic matter. In: *Methods of Soil Analysis. Part 2*, 2nd edn (Eds A. L. Page, R. H. Miller and D. R. Keeny). American Society of Agronomy, WI, USA.

Nguyen, K.B. & Smart, G.G. Jr (1990) *Steinernema scalpterisci* n.sp. Rhabditida Steinernematidae. *J. Nematol.* **22**, 187–199.

Nguyen, K.B., Tesfamariam, M., Gozel, U., Gaugler, R. & Adams, B.J. (2004) *Steinernema yirgalemense* n.sp. (Rhabditida: Steinernematidae) from Ethiopia. *Nematology* **6**, 839–856.

Rosa, J.S., Amaral, J., Lacey, L.A., Simoes, N. & Laumond, C. (2000) Natural occurrence of entomopathogenic nematodes

(Rhabditida: *Steinernema, Heterorhabditis)* in the Azores. *J. Nematol.* **32**, 215–222.

SAS Institute (1982) *SAS User's Guide Statistics*. SAS Institute, Cary, NC.

Steiner, W.A. (1996) Distribution of entomopathogenic nematodes in the Swiss Alps. *Rev. Suisse Zool.* **133**, 1–15.

Sturhan, D. & Liscova, M. (1999) Occurrence and distribution of entomopathogenic nematodes in Slovak Republic. *J. Nematol.* **1**, 275–277.

Trdan, S., Valid, N., Urek, G. & Milevoj, L.(2005) Concentration of suspension and temperature as factors of pathogenicity of entomopathogenic nematodes for the control of granary weevil, *Sitopilus granaries* (L.) Coleoptera: Curculionidae). *Acta Agric. Slov.* **85**, 117–124.

Waturu, C.N. (1998) *Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) from Kenya*. PhD, University of Reading, Reading, UK.

Waturu, C.N. & Reid, A.P. (1997) *Steinernema karii* sp.n. (Nematoda:Steinernematidae). A new species from Kenya. *J. Nematol.* **7**, 67–75.

Woodring, J.I. & Kaya, H.K. (1988) *Steinernematid and Heterorhabditid Nematodes: A Hand book of Biology and Techniques. South Cooperative Serries Bulletin 331*. Arkansas Agricultural Experimental Station, Fayetteville, AR.

Yoshida, M., Reid, B.R., Briscoe, B.R. & Hominick, W.M. (1998) Survey of entomopathogenic nematodes (Rhabditidae: Steinernematidiade and Heterorhabditidae) in Japan. *Fundam. Appl. Nematol.* **21**, 185–198.

(Manuscript accepted 3 December 2007)