BIOGEOGRAPHY OF ENTOMOPATHOGENIC NEMATODES IN ETHIOPIA

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ABSTRACT

Mekete, T., R. Gaugler, K. B. Nguyen, W. Mandefro, and M. Tessera. 2005. Biogeography of entomopathogenic nematodes in Ethiopia. Nematropica 35:31-36.

The biogeography of entomopathogenic nematodes in Ethiopia was determined in a survey conducted from June 2002 to April 2003 by use of the insect bait method. A total of 288 soil samples were collected from locations throughout Central, Southern, and Southwestern Ethiopia. Twenty sites (6.9%) were positive for entomopathogenic nematodes. Eighteen sites (6.3%) were positive for *Steinernema yirgalemense* and two sites (0.7%) were positive for *Heterorhabditis bacteriophora*. Entomopathogenic nematodes were not collected from the cereal-growing areas of central Ethiopia, which is dominated by heavy clay soils.

Key words: biogeography, *Heterorhabditis bacteriophora*, *Steinernema yirgalemense*, survey.

RESUMEN

Mekete, T., R. Gaugler, K. B. Nguyen, W. Mandefro, and M. Tessera. 2005. Biogeografía de nematodos entomopatógenos en Etiopía. Nematropica 35:31-36.

En un estudio realizado de junio de 2002 a abril de 2003, se determinó la biogeografía de nematodos entomopatógenos en Etiopía, utilizando el método de insecto carnada. Se recolectaron 288 muestras de suelo de diferentes lugares del centro, sur y suroeste de Etiopía. Se encontraron nematodos entomopatógenos en 20 sitios (6.9%). En 18 sitios (6.3%) se encontró *Steinernema yirgalemense*, y en dos sitios (0.7%) se encontró *Heterorhabditis bacteriophora*. No se hallaron nematodos entomopatógenos en áreas cultivadas con cereales en el centro de Etiopía, en donde predominan los suelos arcillosos pesados.

Palabras clave: biogeografía, *Heterorhabditis bacteriophora*, *Steinernema yirgalemense*, censo.

INTRODUCTION

Ethiopia is the third largest country in Africa with an area of over one million km^2. The country is endowed with a variety of agro-ecological conditions ranging from desert to rainforest and from 200 m below sea level to highlands with altitudes of over 4500 meters above sea level. This diversity in climatic conditions has enabled Ethiopia to grow a large number of crops. Its complex topography and wide altitudinal variations also ensure a variety of temperature and rainfall patterns. As a result of this, it is common for most parts of the country to suffer from drought, while there is excess rainfall in other parts. The central, southern and southwest parts of Ethiopia have high and reliable rainfall and forest cover. The forest, by protecting the soil from erosion, has helped the region maintain a considerable agricultural potential for a wide range of crops (Wood, 1993).

Insect pests are considered to be the major constraints in agriculture. The most important biopesticide for soil insect pests is entomopathogenic nematodes (Gaugler,

2002). Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are biological alternatives to chemical insecticides. These nematodes occur naturally in soils throughout the world, where they play an important role as biological control agents against soil-dwelling insect pests in the U.S., Europe, and Asia (Kaya, 1990; Kaya and Gaugler, 1993; Georgis, 2002). These insect parasites possess high virulence to target insect pests yet pose no threat to crops, wildlife, or humans. The only free-living stage is the soil-inhabiting infective juvenile, which seeks, infects, and kills new insect hosts.

Entomopathogenic nematodes have a unique and highly specific association with symbiotic bacteria (*Xenorhabdus* spp. for *Steinernema* spp., and *Photorhabdus* spp. for *Heterorhabditis* spp.) held in the gut of the infective-stage juvenile. The juveniles enter the insect host via natural body openings or penetrate the cuticle at intersegmental areas. Once in the host haemocoel, they release their associated bacteria. These bacteria multiply rapidly to cause a lethal septicemia, usually within 24-48 hr. The nematodes feed on the bacteria and the decomposing insect cadaver, complete their development, and produce two to three generations. As the host becomes depleted, infective juveniles disperse into the soil in search of new hosts (Akhurst and Boemare, 1990; Poinar, 1979).

A derivative of the intense interest in the use of entomopathogenic nematodes for biological control has been an explosion in exploration for new species. Hominick's (2002) exhaustive treatment of entomopathogenic nematode biodiversity notes that 23 of the 34 recognized species have been described since 1989. Although these nematodes seem nearly ubiquitous, Africa is virtually unexplored, receiving little treatment beyond the survey by Waturu (1998) (Hominick, 2002). The present effort is the first attempt to collect and determine the distribution of entomopathogenic nematodes in Ethiopia.

MATERIALS AND METHODS

Soil samples were collected from diverse habitats (agricultural fields, forest areas, and protected parks) of Central, Southern, and Southwestern (standard geographical divisions in Ethiopia) Ethiopia from June 2002 to April 2003. Three to five sites were selected in each sampling location, with a bias to accessibility by road. At each collection site, 1 kg of soil was taken with a hand shovel to a depth of 15 cm. Three random samples were taken over an area of 8-10 m^2. The three samples were placed into a plastic bag and mixed. Collection site, associated vegetation, and soil texture were recorded. The soil was transported to the laboratory in an insulated container for isolation of entomopathogenic nematodes.

The insect bait method devised by Bedding and Akhurst (1975) was used to isolate entomopathogenic nematodes from the soil samples. Wax moth larvae, *Galleria mellonella*, were cultured at 30°C in a wheat bran medium (200 g honey, 183 g glycerol, 47 g yeast extract, and 320 g wheat bran). A 500 g subsample of soil was transferred to a plastic pot (11-cm-diam), and five last-instar *G. mellonella* larvae were added to each pot. Pots were incubated for 10 days at 25°C. Pots were checked daily beginning on day four, and dead larvae were removed and rinsed with alcohol (95%) followed by a distilled water rinse. Dead larvae were individually incubated in modified White Traps (White, 1927) consisting of a Petri dish (12.5 cm) filled with distilled water and an inverted Petri dish placed within (7.5 cm). The dead larvae were incubated until nematode progeny emerged and migrated into the water reservoir. Koch's postulates were

completed by exposing field-collected chafer grubs (*Copegnatus curtipennis*) to harvested infective juveniles and noting host mortality and nematode reproduction. Identification to species level of nematode isolates recovered was accomplished via molecular identification using ITS regions of rDNA compared with entomopathogenic nematode sequences and deposited in GenBank (Nguyen *et al.*, 2001).

RESULTS AND DISCUSSION

A total of 288 soil samples were collected from sites throughout Central (59 samples), Southern (31) and Southwestern Ethiopia (98) (Fig. 1). Entomopathogenic nematodes were detected in twenty sites (6.9%): eighteen sites were positive for steinernematids (6.3%) and two sites were positive for heterorhabditids (0.7%) (Table 1). All of the isolated populations reproduced in *G. mellonella* and *C. curtipennis* larvae, confirming their entomopathogenic nature.

The present study is the first survey on the prevalence of entomopathogenic nematodes in Ethiopia. The steinernematid isolates detected were all of a recently described species, *Steinernema yirgalemense* (Nguyen *et al.*, 2004). The two heterorhabditid isolates were identified as *Heterorhabditis bacteriophora* and are new records for the country. *Steinernema yirgalemense* was nine times more prevalent than *H. bacteriophora.* Most positive samples were recovered from natural forest habitats, or coffee and fruit plantations. This is presumably because

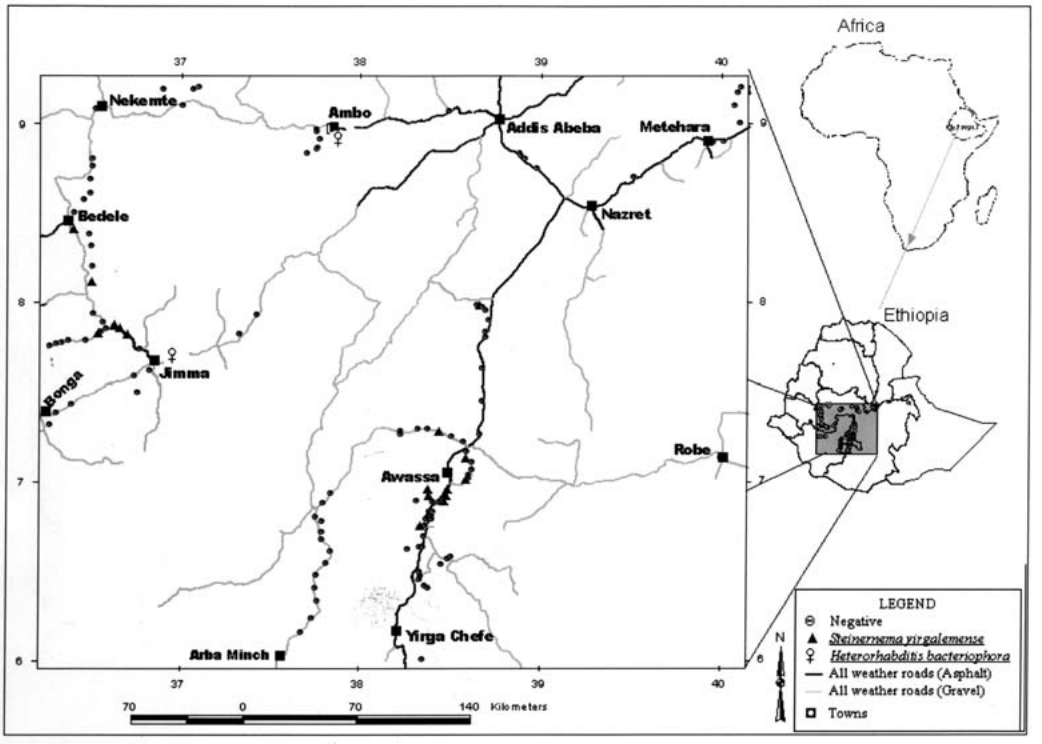

Fig. 1. The distribution of entomopathogenic nematodes in Ethiopia.

Table 1. Surveyed areas in Ethiopia positive for entomopathogenic nematodes.

Region (Total number of samples and % of positive samples)	GPS reading		Isolate designation	Soil texture	Elevation (masl)	Vegetation	Species identified
	Latitude	Longitude					
Central (59, 5.1%)	08°58.851'	37°51.166'	ANEPN 1	Sandy loam	2220	Citrus/garden flowers	*Heterorhabditis bacteriophora*
	08°57. 915'	37°51.364'	ANEPN 2	Vertisoil	2225	Garden flowers	*Steinernema yirgalemense*
	07°32.554'	38°41.031'	ANEPN 13	Sandy	1700	Orange	*Steinernema yirgalemense*
Southern (131, 7.6%)	07°06.246'	38°37.254'	ANEPN 3	Sandy loam	1900	Coffee/forest	*Steinernema yirgalemense*
	06°59.087'	38°31.156'	ANEPN 4	Sandy loam	1890	Enset	*Steinernema yirgalemense*
	06°59.087'	38°31 156'	ANEPN 5	Clay loam	1700	Coffee/Acacia	*Steinernema yirgalemense*
	06°41.280'	38°22.469'	ANEPN 6	Clay loam	1750	Coffee/Acacia	*Steinernema yirgalemense*
	06°40.072'	38°23.553'	ANEPN 7	Clay loam	1750	Coffee/Acacia	*Steinernema yirgalemense*
	06°59.087'	38°31.156'	ANEPN 8	Clay loam	1650	Avocado/Coffee	*Steinernema yirgalemense*
	06°52.128'	38°24.007'	ANEPN 9	Clay loam	1650	Avocado/Coffee	*Steinernema yirgalemense*
	06°46.221'	38°22.649'	ANEPN 10	Clay loam	1660	Sugarcane/Banana	*Steinernema yirgalemense*
	06°44.780'	38°23.272'	ANEPN 11	Clay loam	1650	Avocado/Coffee	*Steinernema yirgalemense*
	07°10.335'	37°57.457'	ANEPN 12	Sandy	1800	Fig	*Steinernema yirgalemense*
Southwestern (98, 7.14%)	07°32.113'	36°33.493'	ANEPN 14	Red clay	1734	Sugarcane	*Heterorhabditis bacteriophora*
	07°48.535'	36°41.499'	ANEPN 15	Red clay	2100	Coffee/Acacia	*Steinernema yirgalemense*
	07°51.218'	36°34.070'	ANEPN 16	Clay	1512	Shrubs	*Steinernema yirgalemense*
	08°22.408'	36°15.503'	ANEPN 17	Red clay	2100	Shrubs	*Steinernema yirgalemense*
	07°51.544'	36°34.497'	ANEPN 18	Red clay	2018	Coffee/Acacia	*Steinernema yirgalemense*
	07°34.947'	36°41.766'	ANEPN 19	Red clay	2100	Shrubs	*Steinernema yirgalemense*
	07°34.075'	36°38.096'	ANEPN 20	Red clay	1979	Coffee/Acacia	*Steinernema yirgalemense*

agricultural activities are reduced in perennial plantations, resulting in less disturbance to the soil ecosystem than annual row crops where tillage is required. This result supports the finding that entomopathogenic nematodes species are more prevalent in soils from indigenous forest habitats than in agricultural soils (Barker and Barker, 1998). However, Mracek and Webster (1993) found that heterorhabditid and steinernematid nematodes occurred in western Canadian sites where human impact had been substantial, and no nematode positive soil samples were recorded at sites where the impact of humans had been slight. These authors attributed their results to outbreaks of insect pests associated with crop monoculture.

Our study did not detect entomopathogenic nematodes in the cereal-growing areas of central Ethiopia, which is dominated by heavy clay soils (vertisols). Mobility and survival of entomopathogenic nematodes are favored in soils with high sand content, whereas soils with high clay content restrict nematode movement (Kung *et al.*, 1990).

Africa is represented in only 1 of 63 different surveys for entomopathogenic nematodes (Hominick, 2002). Waturu (1998, cited in Hominick [2002]) conducted a survey in Kenya, and detected entomopathogenic species in 154 of 641 (23.4%) samples taken in the central highlands, and 7 of 200 (3.5%) samples from the coastal lowlands. Three species were isolated in the Kenya survey: *H. bacteriophora*, *H. indica*, and a new species, *S. karii* (Waturu *et al.*, 1997). We also detected *H. bacteriophora*, which appears to have one of the broadest geographical distributions for entomopathogenic species, being found in five of the six inhabited continents. Although Ethiopia shares a common border with Kenya to the south, we did not collect *H. indica* or *S. karii*. The absence of *H. indica* from our samples was anticipated since Waturu (1998) found this species along the coast, whereas, our samples were collected more than 1000 km inland. The apparent lack of geographic overlap between *S. karii* and *S. yirgalemense* may be better understood as additional ecological information on these new species accumulates.

The occurrence of steinernematid and heterorhabditid nematodes from most surveyed areas indicates a potential role of these nematodes in regulating populations of soil insects in Ethiopia. Further studies are needed to determine whether these nematodes suppress pest populations and how to deploy them in pest management.

ACKNOWLEDGMENTS

This study was supported by a grant from the Lindbergh Foundation for the benefit of small-scale farmers in Ethiopia.

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Received: *11.II.2005*

Recibido:

Accepted for Publication: *29.VII.2005*

Aceptado para publicación: