Fundam. appl. Nematol., 1998, **21** (6), 667-672

Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Guadeloupe islands

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Accepted for publication 7 January 1998.

Summary - Soils from various habitats in Guadeloupe (Grande Terre, Basse Terre) and neighbouring islands (Marie-Galante, La Désirade, Petite Terre, Les Saintes, Saint-Barthélémy, Saint-Martin) were assessed for entomopathogenic nematodes using the *Galleria* baiting technique. Entomopathogenic nematodes were found in 35 of the 538 sites (6%). The genera observed were *Heterorhabditis* in 34 of these 35 sites (97%) and *Steinernema* in one site (3%). Two species of *Heterorhabditis* were found: *H. indica* (88%) and *H. bacteriophora* (12%). The one *Steinernema* was probably a new species, which is being studied. Nematodes occurred in coastal (91.4%), tropical low lands (5.7%), and tropical middle altitude (2.9%) areas. No entomopathogenic nematodes were found in mountain areas. © Orstom/Elsevier, Paris

Résumé - *Répartition des nématodes entomopathogénes (Rhabditida: Steinernematidae et Heterorhabditidae) dans les îles de la Guadeloupe* - Des prospections ont été réalisées sur 538 sites en Guadeloupe (Grande Terre, Basse Terre) et dans ses dépendances (Marie-Galante, La Désirade, Petite Terre, Les Saintes, Saint-Barthélémy, Saint-Martin) pour rechercher des nématodes entomopathogénes par la technique du piége à insecte (*Galleria mellonella*). Sur 35 sites positifs (6%), 34 hébergent des *Heterorhabditis* (97%) et l'un d'eux un *Steinernema* (3%). Deux espèces d'*Heterorhabditis* ont été identifiées : *H. indica* (88%) et *H. bacteriophora* (12%). Le *Steinernema* appartient sans doute à une nouvelle espèce en cours d'étude. Les nématodes ont été isolés en zone côtière (91,4%), en zone tropicale de basse (5,7%) et de moyenne altitude (2,9%). Aucun nématode n'a été trouvé en zone montagneuse. © Orstom/Elsevier, Paris

Keywords: entomopathogenic nematodes, geographical distribution, Guadeloupe, *Heterorhabditis bacteriophora*, *H. indica*, *Steinernema* sp.

Nematodes of the genera *Steinernema* and *Heterorhabditis* are pathogenic for insects. These organisms are used as biological control agents against insect pests. The nematode species and strains exhibit some variability in survival and infectivity potential, which impacts on their suitability for insect pest management programs (Gaugler & Kaya, 1990). The objective of this study was to identify indigenous entomopathogenic nematodes of the Caribbean area, particularly in Guadeloupe islands. Such nematodes should be adapted to the fluctuating conditions that are prevalent in Caribbean environments. Future studies will assess their entomopathogenic potentialities and genetic variability.

Materials and methods

Guadeloupe and the neighbouring islands were sampled every 2 km (Fig. 1). A total of 538 samples was collected between February and December of 1996. For each sample, three soil sub-samples were randomly selected in a 100 m^2 area . Each sub-sample consisted of a core 5.5 cm in diameter and 25 cm deep (about 0.6 dm^3 of soil). The three sub-samples were mixed together to make a 1.8 dm^3 sample, of which 0.6 dm^3 was used for recovering the nematodes and the rest for physical and chemical analyses.

The samples were placed in a sealed plastic bag, transported in coolers, and analysed individually in the laboratory. The nematodes were isolated by the *Galleria*-trap method (Bedding & Akhurst, 1975). Ten insects were exposed to each 0.6 dm^3 soil sample during 4 days and were changed four times (*i.e.*, four repeats for a total exposure duration of 16 days). Nematodes were identified using morphological criteria, a malate dehydrogenase (MDH) method (Mauléon & Laumond, unpubl.), and satellite DNA probes (Grenier *et al.*, 1996).

The spatial distribution of nematodes was related to elevation, rainfall, pH, and distance from the sea, and analysed by correspondence analysis after codification (software ANAMULT) (Febvay & Bonnot, 1991)

(Fig. 2). Parameters, site location, and soil type are given in Table 1.

The names of the nematode species cited in this study have been recently modified according to the International Code of Zoological Nomenclature (Hominick *et al.*, 1997); therefore, the following names will be used in this study: *Steinernema riobrave*, *S. cubanum*, *S. puertoricense*, and *Heterorhabditis indica*.

Results

Entomopathogenic nematodes were recovered from 35 of the 538 sites sampled (6%). Among the 35 positive sites, 34 yielded *Heterorhabditis* (97%) while only one produced *Steinernema* (3%) (Fig. 1).

Two distinct species of *Heterorhabditis* were found. They were identified as *Heterorhabditis indica* (88%) and *Heterorhabditis bacteriophora* (12%). The sole steinernematid was possibly a new species of *Steinernema* and is being studied. Nematodes were unequally present in the three geographical areas: coastal (91.4%), tropical low land (5.7%), and tropical middle altitude (2.9%) areas. No nematodes were found in mountains, which are mainly covered by rain-forests (Table 1). In the coastal area, nematodes were isolated from three littoral habitats: beaches and salt marsh (87.5%), limestone cliffs (6.25%), and cultivated fields (6.25%). The single sample from salt marsh was the only sample from which two species of entomopathogenic nematodes (*H. indica* and *Steinernema* sp.) were recovered. The beach and limestone cliff samples included only *H. indica*, except for the Saint-Martin sample. The two positive samples from cultivated fields included *H. bacteriophora*. Nematodes were more frequent between pH 8.0-9.3, which corresponds to calcareous sandy beach habitats.

In the positive samples at low elevation (vertisol; pH 6.5-7.2), two isolates of *H. indica* were found in pasture. Isolate HG 33 is included in the tropical low land group because of the an abnormally low pH value (6.21). The species in the single positive sample at middle elevation (woodland; pH 5.5) was identified as *H. bacteriophora*.

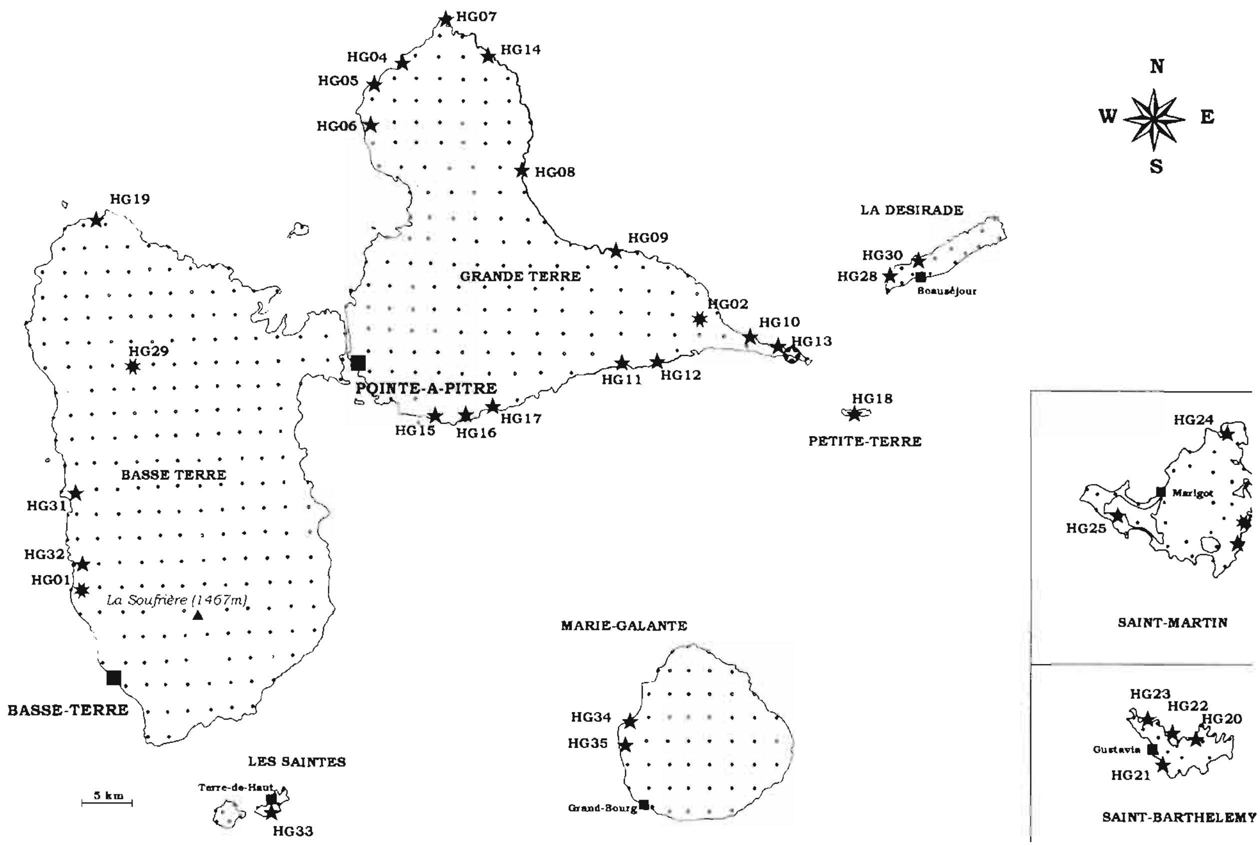
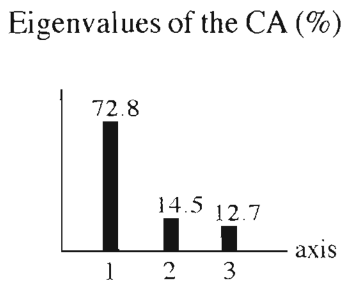


Fig. 1. Map of sampling sites for entomopathogenic nematodes in Guadeloupean islands (• = negative site; ✱ = Heterorhabditis bacteriophora*;* ∗ = Heterorhabditis indica*;* ✪ = Steinernema *sp.).*

Table 1. *Characteristics of the positive samples.*

Islands	Isolates	Genus/species	Soil	pH	Habitat	Rainfall (mm / year)	Elevation (m)	Distance from the sea (m)
Grande Terre	HG02	*H. bacteriophora*	Vertisol	7.20	cropland	1000/1250	25	1500
	HG04	*H. indica*	Sand	9.06	beach	1000/1250	0	25
	HG05	*H. indica*	Sand	9.23	beach	1000/1250	0	30
	HG06	*H. indica*	Sand	8.13	beach	1250/1500	0	20
	HG07	*H. indica*	Limestone	8.20	cliff	< 1000	75	30
	HG08	*H. indica*	Sand	8.69	beach	1000/1250	0	10
	HG09	*H. indica*	Sand	9.19	beach	1000/1250	0	5
	HG10	*H. indica*	Sand	8.82	beach	< 1000	0	30
	HG11	*H. indica*	Sand	9.80	beach	1000/1250	0	10
	HG12	*H. indica*	Sand	9.29	beach	1000/1250	0	5
	HG13	*H. indica*	Sand	8.67	salt marsh	< 1000	0	25
	HG14	*H. indica*	Sand	8.91	beach	< 1000	1	10
	HG15	*H. indica*	Sand	8.90	beach	1250/1500	0	5
	HG16	*H. indica*	Sand	9.22	beach	1250/1500	0	15
	HG17	*H. indica*	Limestone	8.19	cliff	1000/1250	70	25
	SG01	*Steinernema* sp.	Sand	8.67	salt marsh	< 1000	1	25
Basse Terre	HG01	*H. bacteriophora*	Vertisol	6.85	orchard	1000/1250	5	700
	HG19	*H. indica*	Sand	8.09	beach	1500/2000	0	50
	HG29	*H. bacteriophora*	Oxysol	5.50	woodland	2000/3000	350	8000
	HG31	*H. indica*	Vertisol	6.50	pasture	1500/2000	240	1500
	HG32	*H. indica*	Vertisol	6.85	pasture	1250/1500	170	1200
La Désirade	HG28	*H. indica*	Limestone	8.23	cliff	1000/1250	0	30
	HG30	*H. indica*	Limestone	8.03	cliff	1250/1500	0	30
Petite Terre	HG18	*H. indica*	Sand	7.86	beach	< 1000	0	300
Marie-Galante	HG34	*H. indica*	Sand	9.21	beach	1250/1500	1	20
	HG35	*H. indica*	Sand	9.17	beach	1250/1500	1	5
Les Saintes	HG33	*H. indica*	Sand	6.21	beach	1000/1250	1	30
Saint-Barthélémy	HG20	*H. indica*	Sand	9.21	beach	< 1000	1	20
	HG21	*H. indica*	Sand	8.78	beach	< 1000	1	10
	HG22	*H. indica*	Sand	8.35	beach	< 1000	1	15
	HG23	*H. indica*	Sand	8.98	beach	< 1000	1	15
Saint-Martin	HG24	*H. indica*	Sand	8.51	beach	< 1000	5	20
	HG25	*H. indica*	Limestone	8.05	golf course	< 1000	1	300
	HG26	*H. bacteriophora*	Sand	9.17	beach	< 1000	5	50
	HG27	*H. indica*	Sand	8.52	beach	< 1000	5	30

Fig. 2 illustrates these results. It should be noted, firstly, that the pH was a very important factor of nematode distribution as an indicator of soil composition (70% on the first factor of the analysis); *H. indica* was found mostly in soils, cliffs, and sandy beach of corral sediments basically composed of limestone, and the rare *H. bacteriophora* isolates were found in more acidic soils. Secondly, elevation (78% on the third factor of the analysis) and distance from the sea (61% on the second factor of the analysis) are two parameters that indicate that most nematodes were found near the sea, corresponding to the *H. indica* isolates, except for the few isolates corresponding mainly to the *H. bacteriophora* isolates. Rainfall does not seem to be an important factor (26% and 30% on the first and second factors of the analysis) because soils are kept



Weight and absolute contribution of column (%)

	Weight	CP1	CP2	CP3
pH	24.27	70.73819	0.24266	0.34413
Elevation	16.24	1.59596	3.47616	78.69091
Rainfall	37.12	26.57991	30.85352	5.44869
Dist. from the sea	22.37	1.08674	61.01952	15.51626

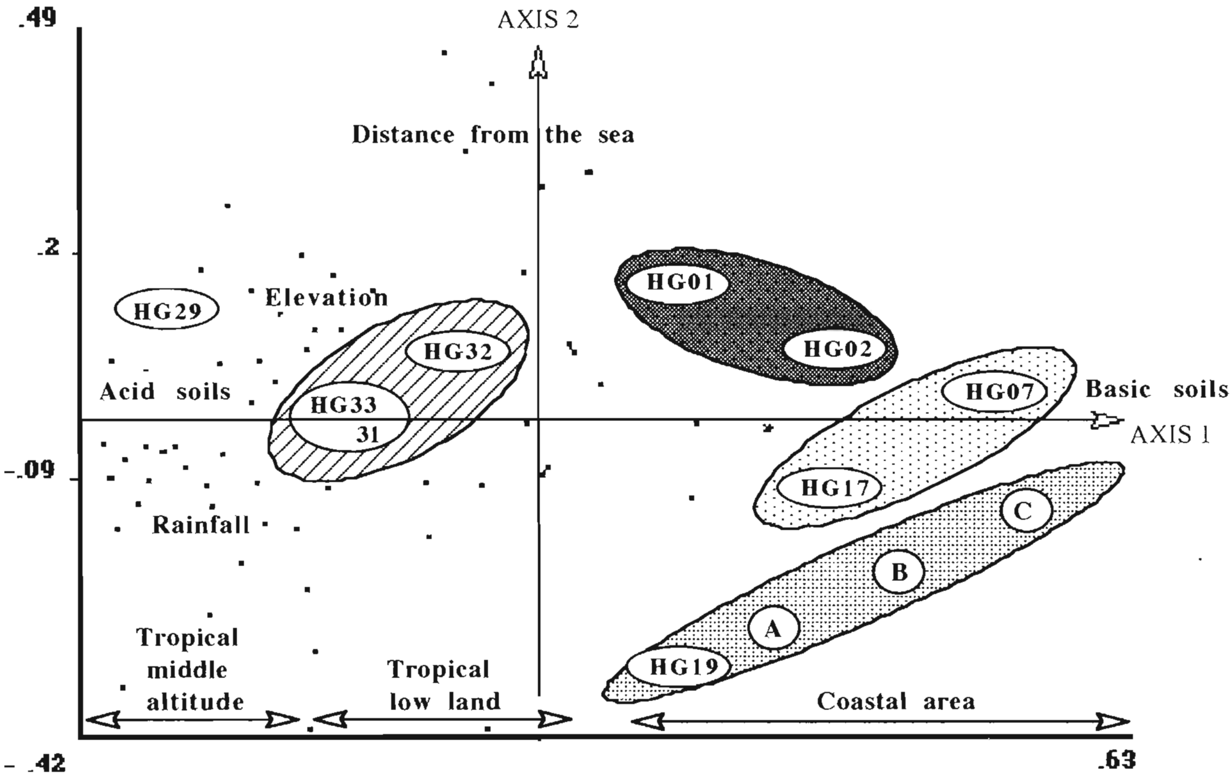


Fig. 2. Scattergraph of entomopathogenic nematodes samples analysed by correspondence analysis (538 sites and four environmental variables); ■ negative samples; ☐ woodland; ☐ pastures; ■ cultivated fields; ☐ limestone cliffs; ☐ beaches and salt marsh; Ⓐ HG06, 15, 16, 30, 34, 35; Ⓑ HG04, 08, 09, 11, 12, 28; © HG05, 10, 13, 14, 18, 20, 21, 22, 23, 24, 25, 26, 27, SG1.

quite wet under these latitudes. In fact, most of the negative samples were located in much wetter and more acid locations (Fig. 2) covered with dense tropical vegetation.

Discussion

Despite a very small sample grid (interval each 2 km), we collected entomopathogenic nematodes from only 6% of a total of 538 soil samples in Guadeloupe islands. This agrees with the findings of Deseö *et al.* (1988) from Italian soils and Hara *et al.* (1991) from Hawaiian soils, but is quite different from the results of northern European surveys reported by Burman *et al.* (1986) in Sweden (25%), Steiner (1994) in Swiss Alps (26.5%), Mráček (1980) in

Czechoslovakia (36.8%), and Hominick and Briscoe (1990) in Great Britain (48.6%).

In Guadeloupe, heterorhabditid isolates were more prevalent than steinernematids (97% and 3%, respectively). Similar observations were made in Puerto Rico (Roman & Beavers, 1983), Hawaiian islands (Hara *et al.*, 1991), and Egypt (Shamseldean & Abd-Elgawad, 1994).

Positive *H. indica* and *Steinernema* sp. sites occur almost exclusively in the beach and cliff habitats (elevation < 100 m; distance from the sea < 500 m), with average rainfall (< 1500 mm), and calcareous soils with moderately alcaline pH (8.0-9.3). These results are in agreement with a previous report concerning other species of *Heterorhabditis* (except *H. indica*) that were restricted to coastal areas of Ireland, Scotland, and Wales (Griffin *et al.*, 1994) and others countries (Poinar, 1993; Amarasinghe *et al.*, 1994). By contrast, *H. bacteriophora* isolates were found in neutral (vertisol) or acidic (oxysol) soils in croplands, orchards, and woodland habitats. Results of the soil analysis in progress should clarify the correlation between physical and chemical parameters and nematode isolates.

Generally, previous surveys show a prevalence of *Steinernema* under temperate and cold climates (Vänninen *et al.*, 1989; Griffin *et al.*, 1991; Hominick *et al.*, 1995). However, new species of *Steinernema* have been identified in tropical and warm countries, *e.g.*, *S. scapterisci* in Uruguay (Nguyen & Smart, 1990), *S. cubanum* in Cuba (Mráček *et al.*, 1994), *S. puertoricense* in Puerto Rico (Roman & Figueroa, 1994), and *S. riobrave* in Texas, USA (Cabanillas *et al.*, 1994). Moreover, several species of *Steinernema* originally found in cold countries have been reported in warm regions, *i.e.*, *S. glaseri* in Spain (Doucet & Gabarra, 1994) and *S. bicornutum* in Jamaica (Mauléon, unpubl.).

By contrast, *Heterorhabditis*, generally considered to be warm-climate nematodes, were also found in cold areas, *i.e.*, Ireland, Scotland and Wales (Griffin *et al.*, 1994), Finland (Vänninen & El Adawy, 1994) and New Jersey, USA (Stuart & Gaugler, 1994).

These observations suggest that adaptation of both steinernematids and heterorhabditids to climatic conditions is more a species than a genus characteristic. For example, in the case of heterorhabditids, *H. megidis* has been found only in cold regions (Poinar *et al.*, 1987; Mráček & Webster, 1993) but *H. indica* was found throughout the tropics (Hominick *et al.*, 1996). In the Caribbean region (Jamaica, Cuba, Puerto Rico, Dominican Republic, Guadeloupe and Martinique), *H. indica* is predominant over all the entomopathogenic nematodes (Mauléon, unpubl.). *H. indica* was reported in other warm regions, *e.g.*, in Egypt (Grenier *et al.*, 1996), India (Poinar *et al.*, 1992), Sri Lanka (Amarasinghe *et al.*, 1994) and northern Australia (Akhurst, 1987).

The clear habitat-linked occurrence for the two heterorhabditid species ought to facilitate the selection of the most suitable isolate for insect pest management. The advantage of using native isolates of entomopathogenic nematodes for biological control is basically founded on the concept that such isolates possess physiological traits that are adapted to local ecological conditions.

Acknowledgements

The authors thanks J. Pioche, S. Sassi, R. Le Saux and D. Fraipont for their technical assistance, E. Grenier (INRA Antibes) for identifying the nematode isolates using satellite DNA probes, and W. Hominick and N. Boemare for revising the manuscript .

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