Russian Journal of Nematology, 1997, 5 (1), 55 - 65

Natural distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in Belgian soils

John S. Miduturi, Lieven Waeyenberge and Maurice Moens

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

Accepted for publication 5 February 1997

Summary. In eight provinces of Belgium, 248 soil samples were collected in 72 ecologically diverse habitats to isolate indigenous entomopathogenic nematodes (epns). Using the *Galleria* larva bait technique, 21 soil samples were found positive for epns. The morphometric study of infective juveniles (IJs) classified 20 populations as *Steinernema* spp., and one population as *Heterorhabditis* sp. Further biochemical characterisation differentiated eight *Steinernema feltiae* A1 type, eight *S. affinis*, four *Steinernema* sp. B3 and one *Heterorhabditis megidis* (northwest European type). The entomopathogenic nematodes were recovered from 38.1%, 28.5%, 23.8% and 9.6% of the samples taken in woodlands, roadside verges, cultivated land and grassland respectively. *Steinernema feltiae* A1 type was isolated in all the habitats except in roadside verges, whereas, *S. affinis* was not found in cultivated land. *Steinernema* sp. B3 was isolated from cultivated fields and roadside verges; *H. megidis* from grassland. *S. feltiae* was prevalent in sand to loamy sand soils with a wide range of organic matter content. *S. affinis* was recovered in sand and sandy clay loam soils. *Heterorhabditis* sp. and *Steinernema* species B3 were isolated in sandy loam and loam soils with organic matter content of 5.6% and 7.5%, respectively. All the isolates were found in soils with a pH range of 3.6-7.8. However, most of the *S. feltiae* populations were isolated in acidic soils.
Key words: entomopathogenic nematodes, morphometrics, habitat, soil type, RFLPs.

Nematode species of the genus *Steinernema* and *Heterorhabditis* are obligate parasites of insects and are mutualistically associated with bacteria, *Xenorhabdus* and *Photorhabdus* respectively. The search for new species and strains of entomopathogenic nematodes (epns) was intensified due to a possible use of these nematodes for the biological control of soil-borne insect pests (Ehlers & Peters, 1995). Epns have been isolated from all continents (Kaya, 1990).

In an initial survey of Belgium, epns were detected in 6.1% and 12.3% of the samples taken in the East-Flanders and West-Flanders provinces respectively (Miduturi *et al.*, 1996a & 1996b). In both surveys *Steinernema feltiae* Filipjev was predominant. An additional survey was conducted from October to November 1995 in the remaining eight provinces of the country. The aim of the study was to isolate new indigenous epn populations and relate their distribution to that of soil type and habitat.

MATERIALS AND METHODS

Collection of soil samples. A total of 248 soil samples were collected from 72 different locations of diverse habitat types (grassland, woodland, cultivated land and roadside verge) throughout the eight provinces of Belgium (Table 1). Five random subsamples of approximately 500 ml each were collected to a depth of 10 cm over an area of 50 m^2 at each sampling site. A representative sample of 250 ml was placed in a plastic box and baited with five last instar larvae of *Galleria mellonella* L. The boxes were stored at 20-25 °C. After five days dead *Galleria* larvae were collected and transferred onto a White trap (White, 1927) to extract the infective juveniles (IJs). All the samples were baited three times with *Galleria* to get the maximum number of positive soil samples. Nematodes isolated from the White traps were used to infect *Galleria* larvae to check their pathogenicity.

Preparation of infective juveniles for morphometric studies. Infective juveniles were killed and fixed in 4% hot formaldehyde. Fixed nematodes were transferred to anhydrous glycerine and permanent slides were prepared. All measurements were made using a drawing tube attached to the light microscope. Identification was made using morphological criteria described by Poinar (1990).

Soil parameters. Soil pH was measured using 20 g samples, suspended in 100 ml distilled water and shaken for 3 hours. The organic matter content of each soil sample was determined by the ignition process and calculated using the percentage by weight method (Andrews, 1973). Soil samples were processed with a Coulter LS 100 fluid module apparatus for particle size analysis. It gave the relative presence of the clay fraction (<4 μm), the silt fraction (4-63 μm) and the sand fraction (>63 μm).

Nematode characterization through RFLPs. DNA was extracted from single adults and used for PCR amplification (Joyce *et al.*, 1994b) of the rDNA ITS region. Amplification products were stored at -20 °C until used. Following PCR, each sample together with controls were digested with either *Alu*I or *Hin*fI (1 μl 10x enzyme buffer, 5 μl PCR product, 3.5 μl double distilled H_2O and 0.5 μl restriction enzyme). Digests were incubated at 37 °C for 4 hours and fragments separated by electrophoresis in 1.5% (w/v) agarose gel in 1 x TAE at 100 V for 3-4 hours. Gels were viewed on a UV-transilluminator. Nematodes were identified by comparing their RFLPs with those in a database of known isolates (Reid, 1994).

RESULTS

Twenty one isolates of entomopathogenic nematodes were recovered from different habitats. All the isolates were able to multiply on *G. mellonella* larvae and so confirmed their entomopathogenic character.

The morphometrics of the isolates are summarised in Tables 2 and 3. The morphological features distinguishing the infective juveniles are: mouth and anus closed, intestine collapsed, lateral lines visible on the cuticle, bacterial pouch at the base of the pharyngeal bulb, excretory pore anterior to nerve ring and a short tail with refractile spine in some individuals. The average length and average width of 20 out of 21 isolates were 727 μm (423-1047 μm) and 26 μm (18-33 μm), respectively. These features allowed these 20 isolates to be identified as *Steinernema*.

The remaining isolate differed. Its mean body length and body width were 707 μm and 28 μm, respectively. The third stage infective juvenile was inside the second stage cuticle, which showed a number of longitudinal ridges. The head had a small projection on the dorsal side. The excretory pore was posterior to the nerve ring. Cells of symbiotic bacteria were found in the lumen of the intestine. These characters indicate that this isolate was a *Heterorhabditis* sp.

Morphometric identification to species level was carried out on all the twenty *Steinernema* isolates and these were compared with the revised descriptions of Poinar (1990). The colour of the spicules in males and the tail tip of the infective juveniles differentiated *S. feltiae* from *S. affinis* Bovien. Based on these differences twelve isolates were identified as "*S. feltiae* based on IJs" and eight as *S. affinis*. For all the twelve *S. feltiae* isolates, ratios C and D were consistent with the revised *S. feltiae* descriptions by Poinar (1990). However, mean values were overlaying the range of this description for the following characters and isolates: total length (MA25, LxM33), greatest width (MA41, LxM34), distance head to excretory pore (LxM33), distance head to nerve ring (MA25, LM16, LM19, NM25, LxM6 and LxM33), distance head to pha-rynx base (LxM33); tail length (MA25, MA41, LxM12, LxM33), ratio A (MA41, LxM12, LxM33), ratio B (MA38, LM16, NM25, LxM16 and LxM34), and ratio E (LxM12 and LxM33).

The mean values of the eight *S. affinis* isolates fit into the descriptions by Poinar (1990) for the following characteristics: greatest width, distance head to excretory pore, distance head to pharynx base, ratio C, D and ratio E (Table 2). Differences were found for: total length (HM13), distance head to nerve ring (VBM5, VBM19, HM13, NM6, NM30, LxM3 and LxM63), tail length (VBM5, VBM6, VBM19, HM13, NM6, NM30, LxM3), ratio A (VBM6, VBM19, LxM3 and LxM63) and ratio B (HM13).

The *Heterorhabditis* isolate (VBM30) had an average length of 707 μm (595-762 μm) and an average tail length of 117 μm (107-126 μm). The greatest width and all the ratios were consistent with the described measurements of *H. megidis* Poinar, Jackson & Klein (Table 3). Some of the remaining characters (distance from head to the excretory pore, to the nerve ring and to the pharynx base), however, were smaller than in the original descriptions.

RFLP examination of the isolates was used to confirm the morphometric results. All the isolates yielded an ca. 800-900 bp fragment upon PCR amplification with ITS primers. The following isolates were classified as *S. feltiae* A1 type: MA25, MA38, LM16, LM19, NM25, LxM6, LxM16, and LxM34; following as *S. affinis*: VBM5, VBM6, VBM19, HM13, NM6, NM30, LxM3 and LxM63. The remaining isolates: MA41, LxM12, LxM31 and LxM33 were classified as *Steinernema* sp. B3. The only *Heterorhabditis* population (VBM30) was classified as *H. megidis* NW European type (Fig. 1).

Epns were isolated from 21 (8.5%) of the 248 soil samples collected. The nematodes were recovered from 38.1%, 28.5%, 23.8% and 9.6% of the samples taken in respectively woodland, roadside verges, cultivated fields and grassland. *Steinernema feltiae* A1

Table 1. Habitat, soil characteristics and epns identity at different sampling sites.

Site	Habitat	Number of samples	pH	Organic matter content (%)	Soil type	Result (Sample code)
Antwerpen province						
Arendok	woodland	4	4.4	4.3	loamy sand	–
Baarle Hertog	woodland	2	3.8	6.2	sandy loam	–
Beerse	roadside verge	3	6.0	5.6	loam	–
Duffel	roadside verge	2	6.6	3.7	loam	–
Kalmthout	woodland	2	3.6	5.6	loamy sand	–
Kasterlee	woodland	5	4.0	9.1	loamy sand	–
Maria ter Heide	roadside verge	1	3.8	3.3	sand	+ (MA41)
Merksplas	cultivated field	1	4.6	8.5	loam	–
Oostmalle	woodland	1	3.9	7.1	sand	–
Poppel	woodland	1	4.7	3.9	loamy sand	–
Postel	woodland	10	4.5	12.3	sand	+ (MA25)
Putte	cultivated land	3	5.3	8.1	sandy loam	–
Ravels	woodland	1	3.8	6.4	loamy sand	–
St. Job-in-'t-Goor	woodland	6	4.7	12.2	sand	+ (MA38)
St. Lenaarts	cultivated land	1	5.2	9.4	clay loam	–
	woodland	2	3.5	6.2	sand	–
Vosselaar	woodland	2	3.6	6.1	loamy sand	–
	roadside verge	1	4.1	4.5	sandy loam	–
Wuustwezel	woodland	4	3.1	4.9	sand	–
Zwijndrecht	grassland	8	7.9	4.2	clay loam	–
Henegouwen province						
Beaumont	roadside verge	2	7.5	16.0	sandy loam	–
Doornik	roadside verge	2	6.6	5.8	sandy clay loam	–
Dottignies	cultivated land	1	6.6	3.1	sandy clay loam	–
	grassland	1	6.2	5.0	loamy sand	–
Givry	roadside verge	2	7.3	8.7	sandy clay loam	–
Loverval	woodland	5	6.0	14.0	sandy loam	–
Maisieres	woodland	2	6.0	4.6	loamy sand	+ (VBM30)
Rance	woodland	8	6.9	6.0	sandy loam	+ (HM13)
Stambruges	woodland	5	6.0	9.1	loamy sand	–
Limburg province						
Dilsen	woodland	3	4.0	4.5	sand	+ (LM19)
Grote-Brogel	woodland	2	3.6	12.9	sandy loam	–
Lommel	woodland	1	3.9	12.9	sandy loam	–
	grassland	1	5.5	5.6	sandy loam	–
Maaseik	cultivated land	1	4.8	6.0	loamy sand	+ (LM16)
	roadside verge	2	5.4	3.0	sandy loam	–
Tessenderlo	roadside verge	2	4.8	5.8	sandy loam	–
Tongeren	cultivated land	1	5.7	4.9	sandy clay loam	–
Vlasmer	woodland	2	3.9	4.0	sandy loam	–
Zolder	woodland	5	3.8	9.4	sandy loam	–
Luik province						
Brachon	roadside verge	1	5.7	12.2	sandy loam	–
Huy	roadside verge	1	7.8	15.2	sandy clay loam	–
Malmedy	woodland	4	5.5	10.9	loamy sand	–
Raeren	woodland	5	3.5	22.7	loamy sand	–
Spa	woodland	5	4.8	18.4	loamy sand	–
St. Vith	grassland	4	5.1	10.7	loamy sand	+ (NM25)
Verviers	roadside verge	2	6.0	12.8	sandy loam	–
Waremme	grassland	1	5.7	6.3	loamy sand	–

Table 1 (continued). Habitat, soil characteristics and epn identity at different sampling sites.

Site	Habitat	Number of samples	pH	Organic matter content (%)	Soil type	Result (Sample code)
Luxembourg province						
Arlon	woodland	5	4.9	11.0	sandy loam	–
Bastogne	woodland	5	4.2	18.4	sandy loam	–
Bouillon	woodland	5	3.6	14.5	sandy loam	–
Champlon	woodland	3	6.2	21.5	loamy sand	+ (LxM16)
Florenville	cultivated land	5	5.7	3.6	loamy sand	+ (LxM31, 33, 34)
Havelange	woodland	5	5.7	8.7	sandy loam	–
Libramont	roadside verge	2	6.8	15.7	sandy loam	–
Marche	grassland	1	8.1	10.6	loamy sand	–
	roadside verge	4	7.8	8.3	sandy loam	+ (LxM12)
Neufchateau	roadside verge	1	6.0	8.9	sandy loam	–
	grassland	4	6.6	8.3	loamy sand	–
Recogne	woodland	5	4.0	20.3	loamy sand	–
Vielsalm	woodland	2	7.7	8.3	loamy sand	+(NM30)
Virton	grassland	5	6.6	9.0	loamy sand	–
Namen province						
Anseremme	woodland	1	7.7	13.8	loamy sand	–
Chiny	roadside verge	5	5.0	7.5	sandy loam	+ (LxM3)
Couvin	woodland	5	5.9	8.8	sandy loam	–
Danssoulx	roadside verge	2	7.6	16.4	sandy clay loam	+ (LxM63)
Gembloux	roadside verge	2	7.6	8.3	sandy loam	+ (NM6)
Maillen	woodland	5	4.5	10.3	loamy sand	–
Namen	roadside verge	2	7.0	9.8	sandy loam	–
Philippeville	roadside verge	5	5.4	12.7	sandy loam	–
Villers/ lesse	woodland	5	5.7	35.0	sandy loam	+ (LxM6)
Vlaams-Brabant province						
Bost (Tienen)	grassland	1	7.0	9.3	sandy loam	–
	cultivated land	1	6.7	4.9	sandy clay loam	–
Brussel	roadside verge	5	7.8	3.6	sandy clay loam	–
Groot Bijgaarden	grassland	3	7.2	11.0	sandy loam	+ (VBM19)
Holsbeek	roadside verge	2	7.3	6.2	sandy clay	+ (VBM5)
	woodland	5	5.7	27.8	sand	+ (VBM6)
Keerbergen	woodland	3	3.8	7.5	sandy loam	–
Landen	grassland	1	7.7	6.3	sandy loam	–
	woodland	1	5.7	8.8	sandy loam	–
Leuven	roadside verge	2	7.4	8.0	sandy loam	–
Zonienwoud	woodland	5	4.3	6.7	sandy loam	–
Waals-Brabant province						
Nivelles	roadside verge	2	4.3	5.1	loam	–
Waterloo	grassland	1	5.9	4.3	sandy clay loam	–
	cultivated land	1	7.2	7.3	sandy clay loam	–

+ - entomopathogenic nematodes detected; – - entomopathogenic nematodes not detected.

type was recovered from all the habitats except roadside verges, whereas *S. affinis* was not found in cultivated fields. *Steinernema* sp. B3 was recovered from cultivated fields and roadside verges while *H. megidis* was detected in a grassland (Fig. 2A).

Sand to loamy sand soils with a wide range of organic matter content (3.1-35.0%) were associated with *S. feltiae,* whereas *S. affinis* was associated with sand and sandy clay loams with 6.0-27.8% organic matter content. *H. megidis* (NWE type) was isolated in sandy loam soil with an organic matter content of 5.6%, while species B3 was associated with loamy soils with an organic matter content of 7.5% (Fig. 2B). All the isolates were found in soils with a pH range of 3.6-7.8.

DISCUSSION

Surveys document the occurrence of epns in various locations and habitats. They form the basis

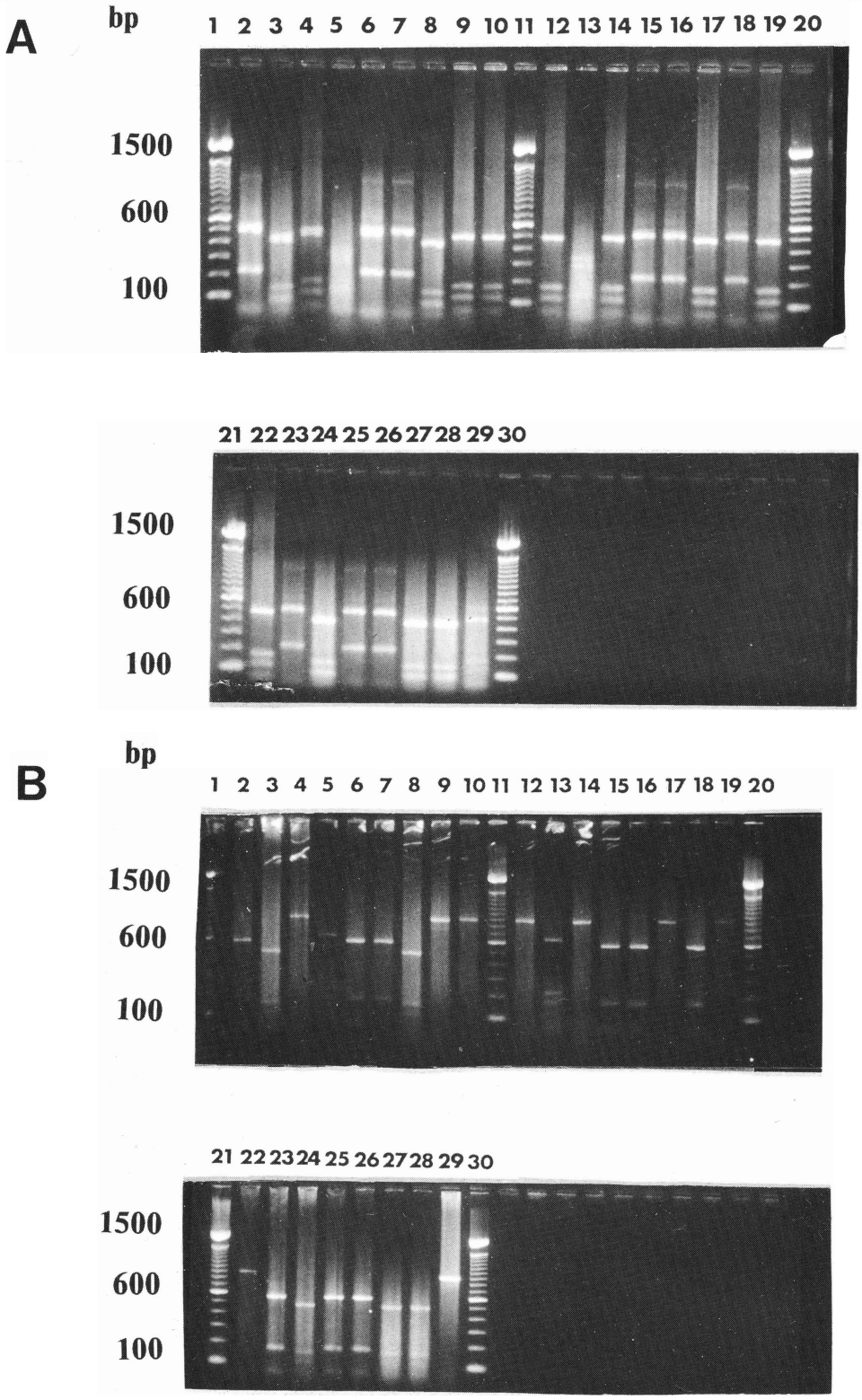

Fig. 1. Agarose gel electrophoresis profiles of ITS rDNA regions digested with restriction endonuclease enzymes. Panel A: RFLPs obtained by digestion with *Alu*I, Panel B: RFLPs obtained by digestion with *Hinf*I: Lane 1, 11, 20, 21 and 30 Marker lane 100 base pairs; 2, *Steinernema feltiae* (Belgium site RS71); 3, *Steinernema* sp. B3 (Belgium site RS50); 4 *S. affinis* (Belgium site Z16); 5 *Heterorhabditis megidis*, NW European type (Belgium site Sn5); Lane 6, 7, 14, 15, 16, 18, 23, 25 and 26 *S. feltiae* (MA25, MA38, LM16, LM19, NM25, LxM6, LxM16 and LxM31); Lane 8, 24, 27 and 28 *Steinernema* sp. B3 (MA41, LxM12, LxM33 and LxM34); Lane 9, 10, 12, 14, 17, 19, 22 and 29 *S. affinis* (VBM5, VBM6, VBM19, HM13, NM6, NM30, LxM3 and LxM63); and Lane 13 *H. megidis* NW European type (VBM30).

Table 2. Morphometrics of infective juveniles of isolates from Belgium comparable to *Steinernema feltiae*[a] and *S. affinis*[a]

Character	NM25	NM30	LxM3	LxM6	LxM12	LxM16	LxM31	LxM33	LxM34	LxM63	*S. affinis* (after Poinar, 1990)
Total length	807 (643-881)	616 (585-643)	673 (595-738)	818 (714-952)	738 (690-833)	889 (714-976)	805 (619-952)	528 (423-612)	859 (762-928)	701 (571-833)	693 (608-800)
Greatest width	27 (25-29)	26 (25-31)	24 (20-27)	25 (23-31)	26 (25-28)	29 (26-33)	25 (20-27)	25 (19-29)	30 (27-33)	25 (21-29)	30 (28-34)
Distance head to excretory pore	55 (48-69)	54 (44-66)	57 (49-64)	58 (48-63)	59 (51-65)	63 (57-69)	60 (49-68)	45 (37-52)	56 (46-63)	60 (52-67)	62 (51-69)
Distance head to nerve ring	83 (62-102)	80 (64-107)	87 (75-97)	84 (76-96)	90 (75-107)	98 (86-104)	92 (79-106)	64 (49-77)	89 (79-103)	85 (74-94)	95 (88-104)
Distance head to pharynx base	117 (97-132)	118 (93-130)	127 (110-137)	126 (116-140)	132 (108-149)	136 (128-142)	131 (120-140)	91 (74-110)	125 (109-139)	125 (108-142)	126 (115-134)
Tail length	78 (66-89)	54 (49-58)	61 (57-67)	76 (65-84)	68 (58-74)	82 (67-89)	79 (67-89)	45 (39-51)	79 (67-91)	71 (58-85)	66 (64-74)
Ratio A[b]	29 (22-33)	23 (20-26)	28 (28-32)	32 (26-40)	28 (25-32)	30 (27-33)	32 (27-36)	21 (17-25)	29 (25-34)	27 (21-32)	23 (21-28)
Ratio B[c]	6.9 (4.9-7.9)	5.3 (4.6-6.6)	5.3 (4.8-5.9)	6.5 (5.4-8.1)	5.6 (4.9-6.4)	6.5 (5.4-7.0)	6.1 (5.2-7.0)	5.8 (5.2-7.1)	6.9 (6.2-7.5)	5.6 (4.8-6.3)	5.5 (5.1-6.0)
Ratio C[d]	10.3 (8.4-12.0)	11.5 (10.3-12.7)	11.1 (10.2-12.1)	10.8 (9.4-12.4)	11 (9.3-12.0)	10.9 (10.1-11.9)	10.2 (9.0-12.3)	11.7 (10.3-14)	10.9 (9.9-11.5)	9.8 (8.6-11.8)	10.5 (9.5-11.5)
Ratio D[e]	0.47 (0.4-0.55)	0.46 (0.41-0.55)	0.45 (0.43-0.51)	0.46 (0.39-0.55)	0.45 (0.36-0.49)	0.47 (0.43-0.51)	0.46 (0.39-0.52)	0.49 (0.39-0.6)	0.45 (0.41-0.52)	0.48 (0.39-0.53)	0.49 (0.43-0.53)
Ratio E[f]	0.71 (0.61-0.9)	1.02 (0.86-1.32)	0.94 (0.87-1.02)	0.76 (0.68-0.88)	0.88 (0.76-1.0)	0.78 (0.72-0.87)	0.76 (0.66-0.97)	0.99 (0.85-1.13)	0.72 (0.62-0.88)	0.84 (0.71-0.94)	0.94 (0.74-1.08)
n	19	20	20	20	11	20	20	19	20	20	25
Species (Poinar, 1990)	*S. feltiae**	*S. affinis*	*S. affinis*	*S. feltiae**	*S. feltiae**	*S. feltiae**	*S. feltiae**	*S. feltiae**	*S. feltiae**	*S. affinis*	

Table 2 (continued). Morphometrics of infective juveniles of isolates from Belgium comparable to *Steinernema feltiae*[a] and *S. affinis*[a].

Character	MA25	MA38	MA41	VBM5	VBM6	VBM19	HM13	LM16	LM19	NM6	*S. feltiae* (after Poinar, 1990)
Total length	688 (534-833)	895 (643-1047)	812 (690-857)	627 (548-738)	682 (643-738)	665 (524-738)	590 (516-714)	785 (714-952)	739 (643-881)	630 (481-738)	849(736-950)
Greates width	23 (18-27)	24 (18-27)	31 (27-35)	25 (21-31)	24 (21-25)	24 (20-27)	25 (19-34)	27 (25-35)	25 (23-29)	25 (19-32)	26 (22-29)
Distance head to excretory pore	53 (48-63)	62 (52-70)	60 (48-71)	58 (49-74)	58 (56-61)	58 (46-66)	55 (44-66)	53 (48-67)	54 (43-68)	60 (48-89)	62 (53-67)
Distance head to nerve ring	81 (71-81)	91 (82-101)	91 (79-104)	85 (77-103)	88 (76-102)	85 (71-100)	81 (73-94)	81 (61-106)	82 (66-90)	85 (71-112)	99 (88-112)
Distance head to pharynx base	117 (93-132)	128 (112-140)	131 (96-139)	119 (105-130)	121 (99-132)	124 (104-134)	121 (95-133)	117 (98-146)	121 (101-136)	123 (108-131)	136 (115-150)
Tail length	68 (58-77)	75 (64-85)	66 (51-73)	57 (48-69)	60 (57-66)	56 (44-65)	54 (44-62)	70 (55-91)	70 (54-85)	58 (46-67)	81 (70-92)
Ratio A[b]	30 (22-37)	36 (29-40)	27 (20-31)	26 (23-31)	29 (26-31)	28 (26-32)	24 (21-27)	29 (22-39)	29 (27-33)	25 (19-30)	31 (29-33)
Ratio B[c]	5.9 (4.3-6.8)	7.0 (5.0-8.4)	6.2 (5.3-6.7)	5.4 (4.61-6.3)	5.7 (5.1-6.5)	5.4 (4.5-6.1)	4.9 (4.4-5.5)	6.8 (5.6-8.0)	6.1 (5.5-6.5)	5.1 (4.2-5.9)	6.0 (5.3-6.4)
Ratio C[d]	10.2 (8.0-12)	12.0 (9.6-14.0)	10.2 (9.3-13.0)	11.3 (9.0-12.8)	11.4 (10.9-12.1)	11.9 (10.7-13.0)	11.0 (9.4-12.7)	11.3 (9.8-13.5)	10.5 (9.6-12.0)	10.9 (9.3-12.5)	10.4 (9.2-12.6)
Ratio D[e]	0.45 (0.39-0.54)	0.49 (0.41-0.56)	0.46 (0.37-0.55)	0.49 (0.42-0.57)	0.48 (0.42-0.57)	0.47 (0.37-0.56)	0.44 (0.36-0.52)	0.45 (0.38-0.52)	0.45 (0.39-0.59)	0.49 (0.44-0.57)	0.45 (0.42-0.51)
Ratio E[f]	0.78 (0.69-0.90)	0.83 (0.71-0.93)	0.76 (0.64-1.0)	1.02 (0.8-1.25)	0.97 (0.95-1.04)	1.05 (0.81-1.24)	1.00 (0.88-1.24)	0.71 (0.52-1.0)	0.77 (0.61-1.0)	1.04 (0.94-1.22)	0.78 (0.69-0.86)
n	24	14	17	21	19	20	20	20	19	20	25
Species (Poinar, 1990)	*S. feltiae**	*S. feltiae**	*S. feltiae**	*S. affinis*	*S. affinis*	*S. affinis*	*S.affinis*	*S. feltiae**	*S. feltiae**	*S.affinis*	

* - *S. feltiae* identifiation based on IJs.
[a] - All measurements are in micrometers, range is given in brackets and follows the mean.
[b] - Length divided by width.
[c] - Length divided by distance from head to pharynx base.
[d] - Length divided by tail length.
[e] - Distance from head to excretory pore divided by distance from head to pharynx base.
[f] - Distance from head to excretory pore divided by tail length.

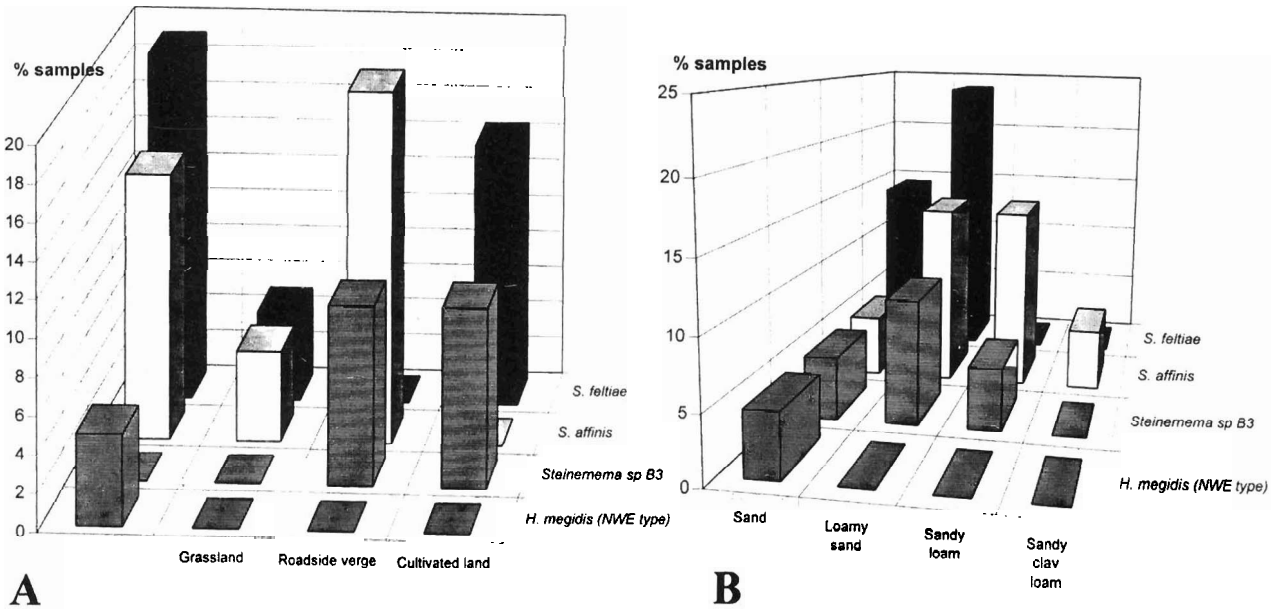

Fig. 2. A: Occurrence of entomopathogenic nematodes in different habitat types; B: Occurrence of entomopathogenic nematodes in different soil types.

for additional opportunities for commercial products and serve as a source of genetic diversity. The present survey of eight provinces in Belgium, yielded 20 steinernematid and 1 heterorhabditid populations. Previous surveys of the West-Flanders and East-Flanders provinces of Belgium, yielded a total 26 steinernematid and 1 heterorhabditid populations (Miduturi *et al.*, 1996a & 1996b). Moderate to slight variations in morphometric characters were observed within the isolates of *S. feltiae* and *S. affinis*. Gwynn (1993) observed similar variations in UK isolates of steinernematids. Nguyen & Smart (1995) attributed differences in morphometrics of steinernematids and heterorhabditid to the harvest time of IJs *in vivo* cultures. As in our morphometric studies for each of the populations, IJs of identical age were used, differences in morphometrics can not be explained by age differences but may be differences intrinsic to the populations.

The biochemical characterisation based on RFLPs of the ITS, proved to be a rapid and accurate method for species identification. In his paper on molecular taxonomy of *Steinernema* Reid (1994) published RFLPs of 15 species. Taking these RFLPs as a reference, our DNA study separated 8 *S. feltiae* A1, 8 *S. affinis*, and 4 *Steinernema* species B3 populations. This molecular observation confirmed our morphometric identifications. Joyce *et al.*, (1994a) identified six RFLP profiles for *Heterorhabditis* mtDNA and rDNA amplification products for species groups which included Irish, northwest European and tropical populations and the *H. bacteriophora* complex. The RFLP pattern of our *Heterorhabditis* isolate was identical to the northwest European type.

The recovery frequency of epns in the present survey (8.5%) was intermediate with the frequency of the previous surveys in West-Flanders (12.3%) and East-Flanders (6.1%) provinces and, as in previous surveys, steinernematids were predominant. The recovery rate of *Steinernema* spp. from the present survey was higher than in surveys undertaken in Scotland (2.2%) (Boag *et al.*, 1992) and Northern Ireland (3.8%) (Blackshaw, 1988) but was almost six times less than in a survey conducted in England, Wales and southern Scotland (48.6%) (Hominick & Briscoe, 1990b). The lower detection rate of epns in Belgium may reflect the occurrence of the nematodes or may be due to restricted access to some sampling sites. The distribution of sampling sites and habitats in each province was not the same and the number of samples collected from various provinces was different.

The A1 RFLP type of *S. feltiae* proved to be the most common type in all three surveys in the UK and the Netherlands with its prevalence ranging from 31.9% to 57.3% (Hominick *et al.*, 1995). In our survey S. feltiae A1 type and *S. affinis* were predominant (3.2% each), followed by *Steinernema* species B3 (1.6%). In Ireland *S. feltiae* and *S. affinis* were

Table 3. Infective juvenile body measurements and ratios of a Belgian isolate of *Heterorhabditis* sp[a].

Character	*H. megidis* (Poinar, Jackson & Klein, 1987)	Belgian isolate (VBM30)
Total length	768 (736-800)	707 (595-762)
Greatest length	29 (27-32)	28 (24-32)
Distance head to excretory pore	131 (123-142)	117 (107-126)
Distance head to nerve ring	109 (104-115)	95 (88-105)
Distance head to pharynx base	155 (147-160)	140 (130-148)
Tail length	119 (112-128)	108 (92-123)
Ratio A[b]	26 (23-28)	25 (21-28)
Ratio B[c]	5.0 (4.6-5.9)	5.0 (4.2-5.5)
Ratio C[d]	6.5 (6.1-6.9)	6.6 (5.9-8.0)
Ratio D[e]	0.85 (0.81-0.91)	0.84 (0.75-0.90)
Ratio E[f]	1.1 (1.03-1.2)	1.1 (0.97-1.35)
Ratio F[g]	0.25 (0.23-0.28)	0.26 (0.20-0.32)

[a] - All measurements are in micrometers, range is given in brackets and follows the mean.
[b] - Length divided by width.
[c] - Length divided by distance from head to pharynx base.
[d] - Length divided by tail length.
[e] - Distance from head to excretory pore divided by distance from head to pharynx base.
[f] - Distance from head to excretory pore divided by tail length.
[g] - Width divided by tail length.

found in 7.1% and 3.3% of the samples respectively (Griffin *et al.*, 1991) and *Heterorhabditids* were rare, being recovered from only one site in Britain and in Ireland (Hominick & Briscoe, 1990b). Subsequent reports showed that *Heterorhabditis* was more widespread and occurred more frequently from coastal sites (Griffin *et al.*, 1994; Hominick *et al.*, 1995; Liu & Berry, 1995). In the present survey only one population of *H. megidis* NWE type was recovered. The present sampling sites were inland, away from coastal areas, and, this may be the reason for the low detection of *H. megidis* in our survey.

Soil texture is one of the important factors determining the abundance of epns in soil with nematode survival being highest in soils with a high sand content and lowest in soils with a high clay content (Kung *et al.*, 1990b). In our survey 76% of the samples positive for epns were found in sand to loamy sand soils (Fig. 2B) which is similar to that reported by Burman *et al.* (1986), Blackshaw (1988), Hominick & Briscoe (1990a), Griffin *et al.* (1991), Hara *et al.* (1991), Glazer *et al.* (1991) and Liu & Berry (1995). These results can be explained by the effect that soil texture has on the movement of the IJs in the soil, with an increasing clay content in the soil having an adverse effect on nematode mobility and on parasitism of insects (Molyneux & Bedding, 1984).

Mraček & Webster (1993) found that epns occurred in ecosystems where human impact has been substantial (e.g. in cropland areas) rather than in natural habitats and suggested that it may be the result of outbreaks of insect pests associated with intensive crop monocultures. Crop monocultures may provide substantial food reserves enabling continuous breeding of insect populations, which are hosts for epns. In previous Belgium surveys, epns were isolated from 50%, 18.8% or 12.3% of the samples taken in sand dunes, grassland or woodlands, but not in samples from cultivated fields or roadside verges (Miduturi *et al.*, 1996a). In the present survey epns were recovered from roadside verges (28.5%), cultivated land (23.8%), woodland (38.1%) and grassland (9.6%) and *H. megidis* was detected in a sandy loam from a woodland (Fig. 2A). This differs from the results obtained during surveys in the Hawaiian Islands (Hara *et al.*, 1991), Ireland and Britain (Griffin *et al.*, 1994; Hominick *et al.*, 1995) where the detection areas were restricted to sandy, coastal soils and indicates that *H. megidis* is capable of surviving in a diverse group of higher soils.

The pH of normal soils does not affect the activity of epns (Kaya, 1990), however pH values of 10 and above adversely affect the survival and pathogenicity of epns (Kung *et al.*, 1990a). Soils at the sampling sites containing epns in our survey had a pH range 3.6-7.8 and most of the *S. feltiae* populations were found in acidic soils.

The present study completes the survey of epns in Belgium and laboratory studies are being done to examine the effects of a selected group of ecological parameters on the survival and pathogenicity of the detected nematode isolates.

ACKNOWLEDGEMENTS

We thanks Ing. G. Hendrickx for technical assistance during the survey and also the CLO-Rijkssta-

tion voor Nematologie en Entomologie, Merelbeke for financial assistance.

REFERENCES

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

Bedding, R.A. & Akhurst, R.J. 1975. A simple technique for the determination of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110.

Blackshaw, R.P. 1988. A survey of the insect parasitic nematodes in Northern Ireland. *Annals of Applied Biology* 113: 561-565.

Boag, B., Neilson, R. & Gordon, S.C. 1992. Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. *Annals of Applied Biology* 121: 355-360.

Burman, M., Abrahamsson, K., Ascard, J., Sjoberg, A. & Eriksson, B. 1986. Distribution of insect parasitic nematodes in Sweden. In: *Fundamental and Applied Aspects of Invertebrate Pathology. Foundation of the 4th International Colloquium of Invertebrate Pathology.* (R.A. Samson, J.M. Vlak, & Peters, D. Eds.). pp. 312. Wageningen, The Netherlands.

Ehlers, R.U. & Peters, A. 1995. Entomopathogenic nematodes in biological control. Feasibility, perspectives and possible risks. In: *Biological Control - Benefits and Risks.* (H. Hokkanen & J. Lynch. Eds.). pp. 119-136. Cambridge, Cambridge University Press.

Glazer, I., Liran, N. & Steinberger, Y. 1991. A survey of entomopathogenic nematodes (Rhabditida) in the Negev desert. *Phytoparasitica* 19: 291-300.

Griffin, C.T., Moore, J. & Downes, M.J. 1991. Occurrence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematologica* 37: 92-100.

Griffin, C.T., Joyce, S.A., Dix, I., Burnell, A.M. & Downs, M.J. 1994. Characterisation of the entomopathogenic nematode *Heterorhabditis* (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and cross breeding techniques, and the occurrence of the genus in these islands. *Fundamental and Applied Nematology* 17: 245-253.

Gwynn, R.L. 1993. Development of cold active nematodes for insect control. *PhD Thesis, University of Reading,* 214 pp.

Hara, A.H., Gaugler, R., Kaya, H.K. & Lebeck, L.M. 1991. Natural populations of entomopathogenic nematodes (Rhabditida, Heterorhabditidae, Steinernematidae) from the Hawaiian Islands. *Environmental Entomology* 20: 211-216.

Hominick, W.M. & Briscoe, B.R. 1990a. Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). *Parasitology* 100: 289-294.

Hominick, W.M. & Briscoe, B.R. 1990b. Occurrence of entomopathogenic nematodes (Rhabditidae; Steinernematidae; Heterorhabditidae) in British soils. *Parasitology* 100: 295-302.

Hominick, W.M., Reid, A.P. & Briscoe, B.R. 1995. Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and the Netherlands. *Journal of Helminthology* 69: 27-32.

Joyce, S.A., Burnell, A.M. & Powers, T.O. 1994a. Characterization of *Heterorhabditis* isolates by PCR amplification of segments of mtDNA and rDNA genes. *Journal of Nematology* 26: 260-270.

Joyce, S.A., Reid, A.P., Driver, F. & Curran, J. 1994b. Application of polymerase chain reaction (PCR) methods to the identification of entomopathogenic nematodes. In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode - bacterium complexes.* Proceedings of a Symposium & Workshop. (A.M. Burnell, R.U. Ehlers & J.P.Masson. Eds.). pp. 178-187. St.Patrick's College, Maynooth, Co. Kildare, Ireland, E.C. DG XII, Luxembourg.

Kaya, H.K. 1990. Soil Ecology. In: *Entomopathogenic Nematodes in Biological Control.* (R. Gaugler & H.K. Kaya. Eds.). pp. 93-115. Boca Raton, Florida, CRC Press.

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.

Liu, J. & Berry, R.E. 1995. Natural distribution of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) in Oregon soils. *Biological Control* 24: 159-163.

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

Miduturi, J.S., Matata, G.J.M., Waeyenberge, L. & Moens, M. 1996b. Naturally occurring entomopathogenic nematodes in the province of East-Flanders, Belgium. *Nematologia mediterranea* 24: 287-293.

Mraček, Z. & Webster, J.M. 1994. Survey of Heterorhabditidae and Steinernematidae (Rhabditida, Nematoda) in Western Canada. *Journal of Nematology* 25: 710-717.

Molyneux, A.S. & Bedding, R.A. 1984. Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. D1 and *Steinernema glaseri* for larvae of the blowfly, *Lucilia cuprina. Nematologica* 30: 358-365.

Nguyen, K.B. & Smart, G.C.Jr. 1995. Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *Journal of Nematology* 27: 206-212.

Poinar, G.O.Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic Nematodes in Biological Control.* (R. Gaugler & H.K. Kaya. Eds.). pp. 23-61. Boca Raton, Florida, CRC Press.

Poinar, G.O.Jr., Jackson, T. & Klein, M. 1987. *Heterorhabditis megidis* sp. n. (Heterorhabditidae: Rhabditida) parasitic in the Japanese beetle, *Popillia japonica* (Scarabaeidae: Coleoptera), in Ohio. *Proceedings of the Helminthological Society of Washington* 54: 53-59.

Reid, A.P. 1994. Molecular taxonomy of *Steinernema.* In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode - bacterium complexes.* Proceedings of a Symposium & Workshop. (A.M. Burnell, R.U. Ehlers & J.P. Masson. Eds.). pp. 49-58. St. Patrick's College, Maynooth, Co. Kildare, Ireland, E.C. DG XII, Luxembourg.

White, G.F. 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66: 302-303.

Miduturi J. S., Waeyenberge L., Moens M. Природное распределение энтомопатогенных нематод (Heterorhabditidae и Steinernematidae) в почвах Бельгии.

Резюме. С использованием гусениц вощинной огневки *Galleria mellonella* как приманки был проведен анализ 248 образцов почвы, собранных в 72 биотопах из 8 провинций Бельгии, на присутствие почвенных энтомопатогенных нематод. Энтомопатогенные нематоды были обнаружены в 21 образце. Морфометрическое изучение личинок, полученных от погибших гусениц, позволило идентифицировать нематод в 20 пробах как *Steinernema* spp. и в одной пробе как *Heterorhabditis* sp. Анализ ДНК показал, что в 8 пробах присутствовали *Steinernema feltiae* типа A1, в 8 пробах – *S. affinis*, в 4 – *Steinernema* sp. типа B3, а *Heterorhabditis megidis* представлял собой обычную для северо-восточной Европы форму. Энтомопатогенные нематоды были обнаружены в 38,1%; 28,5%; 23,8% и 9,6% образцов, собранных соответственно под пологом леса, в посадках кустов вдоль дорог, в обрабатываемых сельскохозяйственных угодьях и на лугах. При этом нематоды *S. feltiae* A1 были обнаружены во всех биотопах за исключением посадок вдоль дорог, тогда как *S. affinis* не обнаруживались на участках обрабатываемой земли. *Steinernema* sp. B3 была обнаружена в обрабатываемой земле, а *H. megidis* – в луговой почве. *S. feltiae* была доминантным видом в песчаных и супесных почвах при разных уровнях содержания органического вещества. *S. affinis* была отмечена в песчаных почвах и суглинках. *Steinernema* sp. B3 и *H. megidis* были выделены в глинистых супесях и суглинках с содержанием органического вещества соответственно 5,6% и 7,5%. Все нематоды были найдены в почвах с pH от 3,6 до 7,8, причем, большая часть нематод *S. feltiae* была обнаружена в кислых почвах.