*Nematol. medit. (1996), 24*: 287-293

*C.L.O. Research Station for Nematology and Entomology - 9820 Merelbeke, Belgium*

# NATURALLY OCCURRING ENTOMOPATHOGENIC NEMATODES IN THE PROVINCE OF EAST-FLANDERS, BELGIUM

by

J. S. MIDUTURI, G. J. M. MATATA[1], L. WAEYENBERGE and M. MOENS

**Summary**. A survey of naturally occurring entomopathogenic nematodes was undertaken in the province of East-Flanders, Belgium. A total of 180 samples were taken from 31 sites of different agronomic characteristics. Using the *Galleria* larva bait technique, eleven soil samples were found positive for entomopathogenic nematodes. Morphometrics identified seven populations as *Steinernema feltiae* and four as *Steinernema affinis*. These identifications were confirmed biochemically. The entomopathogenic nematodes were detected in woodland (3.3%), roadside verges (2.2%) and in grassland (0.5%) but not in cultivated fields. They occurred in soils with 63-95% sand fraction and 3.7-9.3% organic matter. Neither species was pathogenic to the blackvine weevil *Otiorhynchus sulcatus* but showed different levels of infectivity to *Galleria mellonella*.

Entomapathogenic nematodes of the Steinernematidae and Heterorhabditidae (Nemata: Rhabditida) families have great potential for the biological control of many important insect pests. However, they differ in their pathogenicity to a particular insect pest (Poinar, 1990). Many populations have been isolated using the *Galleria mellonella* bait technique (Bedding and Akhust, 1975) and surveys using this method have revealed that these nematodes are distributed worldwide (Poinar, 1990).

Environmental conditions affect the level of control by entomopathogenic nematodes in the field. Therefore, collection of indigenous nematodes may be more suitable for inundative release against local insect pests because of their adaptation to local climate and population regulators (Bedding, 1990).

In Belgium, the only survey of entomopathogenic nematodes was undertaken mainly in the West-Flanders province (Miduturi *et al.*, 1995). The survey reported here was carried out in the East-Flanders province of Belgium with the aims of isolating indigenous entomopathogenic nematodes, studying their distribution in relation to soil type and habitat and their pathogenicity to *Otiorhynchus sulcatus* Fabricicus and *G. mellonella* L.

## Materials and methods

A total of 180 soil samples were collected from 31 locations of varied habitat types (grassland, woodland, cultivated land and roadside verge) from the East-Flanders province of Belgium (Table I). At each sampling site, four or five random sub-samples of approximately 200 ml were collected to a depth of 10 cm over an area of 40-50 $m^2$. A representative sample of

[1] Present address: Sugarcane Research, P.O. Box 30031, Kibaha, Tanzania.

TABLE I - *Survey of entomopathogenic nematodes in East-Flanders, Belgium.*

| Site | Habitat | Number of Samples | pH | Organic matter content % | Sand fraction % | Result |
|---|---|---|---|---|---|---|
| Aalter | roadside verge | 5 | 6.1 | 9.7 | 92 | + (j44) |
| Bazel | cultivated field | 5 | 3.6 | 6.4 | 69 | – |
| Belsele | woodland | 5 | 3.9 | 5.9 | 85 | – |
| Brakel | roadside verge | 5 | 7.0 | 9.3 | 40 | – |
| Daknam | cultivated field | 5 | 5.8 | 12.6 | 93.2 | – |
| Deinze | woodland | 5 | 4.0 | 6.3 | 88.2 | + (j71) |
| Drongen | woodland | 5 | 5.6 | 5.6 | 77.6 | – |
| Eke | roadside verge | 5 | 7.2 | 4.5 | 89.2 | + (j77) |
| Gontrode | woodland | 20 | 3.7 | 11.9 | 66.6 | + (j1, j8) |
| Gijzegem | roadside verge | 5 | 4.2 | 11.9 | 73.6 | – |
| Kallo | shrubland | 5 | 7.2 | 9.4 | 77 | – |
| Kaprijke | woodland | 5 | 4.3 | 8.0 | 93 | – |
| Kemzeke | woodland | 5 | 3.9 | 4.2 | 91.2 | – |
| Knesselare | grassland | 4 | 5.5 | 11.5 | 80.6 | – |
| Landskouter | woodland | 5 | 3.6 | 8.8 | 92 | + (j33, j35) |
| Maldegem | grassland | 5 | 4.2 | 9.4 | 94.4 | + (j127) |
| Meigem | roadside verge | 5 | 7.6 | 5.8 | 79 | + (j70) |
| Melle | woodland | 10 | 3.6 | 12.3 | 82 | + (j24) |
| Melle | shrubland | 5 | 5.7 | 7.3 | 74.2 | – |
| Mere | roadside verge | 5 | 6.0 | 9.2 | 41 | – |
| Nevele | roadside verge | 5 | 7.1 | 8.4 | 78 | – |
| Ninove | roadside verge | 5 | 5.3 | 5.3 | 34.2 | – |
| Oudenaarde | shrubland | 5 | 6.1 | 6.5 | 39.4 | – |
| Ophasselt | woodland | 5 | 5.9 | 10.7 | 56.4 | – |
| Overmere | roadside verge | 5 | 6.6 | 7.0 | 93 | – |
| Vinkt | grassland | 5 | 7.0 | 6.5 | 66.2 | – |
| Waasmunster | woodland | 5 | 3.9 | 3.7 | 85 | – |
| Wachtebeke | roadside verge | 5 | 5.7 | 9.9 | 90.6 | – |
| Wetteren | roadside verge | 6 | 6.0 | 8.2 | 75 | + (j57) |
| Wetteren | woodland | 10 | 4.7 | 6.3 | 77 | – |
| Zelzate | grassland | 5 | 5.6 | 3.8 | 93 | – |

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

250 ml was placed in a plastic box and five last instar larvae of *G. mellonella* were added. The boxes were incubated at 20-25 °C. After 3-5 days dead larvae were transferred to a White (1927) trap to extract the infective juvenile nematodes. All the samples were baited three times with *Galleria* to obtain the maximum number of positive samples. The infective juveniles collected from the White trap were checked for their pathogenicity to *Galleria* larvae. Nematodes were stored in aerated water at 5 °C until required.

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 a light microscope. Identification of nematodes was made using morphological criteria described by Poinar (1990).

To mesure pH 20 g of soil was suspended in 100 ml of 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). The 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).

DNA was extracted (Joyce *et al.*, 1994) from five to ten infective juveniles from the same population. Nematodes were cut into two or more pieces in a drop of double distilled water on a glass slide under a microscope. Ten µl of water containing the nematode pieces were transfered into an Eppendorf tube with 8 µl WLB (worm lysis buffer: 125 mM KCl, 25 mM Tris-HCl pH 8.3, 0.375 mM $MgCl_2$, 2.5 mM DTT, 1.125% Tween 20 and 0.025% gelatin) and 2 µl proteinase K (600 µg/ml) was added. Each Eppendorf tube was put in a freezer at -80 °C overnight. The next day the DNA extraction mixture was incubated at 65 °C for one hour and then heated at 95 °C for 10 minutes. After centrifugation (5 minutes; 14,000 rpm) 5 µl of the supernatant was used in a PCR reaction.

PCR amplifications were performed in 50 µl volumes containing the following constituents: 5 µl 10 x PCR buffer, 5 µl $MgCl_2$ (25 mM), 1 µl dNTP mixture (10 mM each), 0.5 µl AB 28 (18S forward primer), 0.5 µl TW 81 (26S reverse primer), 0.8U goldstar Taq DNA polymerase (Eurogentec), 5 µ worm lysate and $ddH_2O$ to makeup to 50 µl. This mixture was placed in a thermocycler already heated to 94 °C and subjected to a hot start of 5 minutes at 94 °C followed by 40 cycles (denaturation at 94 °C for 1 minute, annealing at 55 °C for 1.5 minutes and polymerisation at 72 °C for 2 minutes). A five minute polymerisation period at 72 °C followed the last cycle in order to complete any partially synthesized second strands. PCR products were stored at -20 °C until required.

Following the PCR, each sample and a suitable control product was digested with *Alu* I and *Hinf* I (1 µl 10x enzyme buffer, 6 µl PCR product, 2 µl $ddH_2O$ and 1 µl restriction enzyme). The mixture was incubated at 37 °C for 4 hours. Later the products were run on a 1.5% agarose gel in 1 x TAE at 100 V for 3.5 hours. The gel was viewed on a UV transilluminator and photographed. The populations were identified by comparing their RFLP patterns to the pattern database of known isolates (Reid, 1994).

For each isolate, a nematode suspension (100 infective juveniles) was pipetted onto the surface of a moistened filter paper in 9 cm diameter Petri plates. Five larvae of *G. mellonella* or *G. sulcatus* were placed on the paper. The Petri plates were then sealed with Parafilm to maintain high humidity and kept in an incubator at 23 ± 1 °C. Each entomopathogenic nematode strain was inoculated four times. After four days the cadavers were opened and the number of fourth stage juvenile or pre-adult nematodes which entered were counted. After log-transformation, data analysis was performed using analysis of variance (ANOVA) (Snedecor and Cochran, 1978).

## Results

Entomopathogenic nematodes were recovered from nine out of the 32 sampling sites (Table I). Among the 180 samples collected, eleven samples (6.1%) were positive for entomopathogenic nematodes. By their ability to multiply on *G. mellonella* all the isolates proved to be entomopathogenic.

The results of the morphometric observations on the isolates are summarised in Table II. Features distinguished on infective juveniles were body length less than 1000 µm and body

width between 18 and 31 µm. Lateral lines were visible on the cuticle surface, the mouth and anus were closed and the intestine was collapsed. The excretory pore was positioned anterior to the nerve ring and bacteria were present in a sac positioned adjacent to the pharyngeal bulb. The tail was short (less than 100 µm). In some individuals a small refractile spine was present at the tail tip. These features indicated that all the isolates were *Steinernema* spp.

Morphometric identification to species level was carried out on all of the eleven isolates and by comparison with the revised descriptions of Poinar (1990). The overall average length and average width of all isolates were 825 µm (518-996 µm) and 25 µm (18-31 µm), respectively.

Among the eleven populations seven were comparable to *Steinernema feltiae* Filipjev. For all these isolates total length, tail length and ratios B and C were consistent with the revised descriptions (Poinar, 1990). However, mean values were overlying the range of these descriptions for the following characters (population): distance head to excretory pore (j 127), distance head to nerve ring (j57), distance head to pharyngeal base (j71, j127), ratio A (j57 and j127), ratio D (j24) and ratio E (j24, j44, j71 and j127).

The mean values of the remaining four isolates fit into the descriptions of *S. affinis* Bovien by Poinar (1990) for the following characters: greatest width, distance head to excretory pore and ratios A, C and E. Differences were found for total length (j70, j77), distance head to nerve ring (j77), distance head to pharynx base (j1, j70, j77), tail length (j70) and ratios B and D (j1). The inconsistency of some morphometric characters led to the biochemical characterization using PCR-RFLP.

All of the isolates yielded a 800-900 bp fragment upon PCR amplification (Fig. 1). Upon digestion with *Alu* I and *Hinf* I, the isolates were classified as follows (Fig. 2): j8, j24, j35, j44, j57, j71 and j127 as *S. feltiae* and j1, j33, j70 and j77 as *S. affinis*. The PCR amplification yielded fur-

TABLE II - *Morphometrics of infective juveniles of isolates of entomopathogenic nematodes from East-Flanders-Belgium comparable to* Steinernema feltiae *and* Steinernema affinis.

| Character | j1 (Gontrode) | j8 (Gontrode) | j24 (Melle) | j33 (Landskouter) | j35 (Landskouter) | j44 (Aalter) | j57 (Wetteren) | j70 (Meigem) | j71 (Deinze) | j77 (Eke) | j127 (Maldegem) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Total length | 667(518-824) | 913(714-930) | 917(836-984) | 711(480-780) | 869(848-996) | 802(587-906) | 774(669-801) | 827(778-931) | 876(704-911) | 897(606-936) | 824(700-902) |
| Greatest width | 26(22-31) | 26(22-31) | 28(26-31) | 25(21-29) | 26(24-29) | 23(20-31) | 23(19-26) | 26(23-30) | 23(21-31) | 26(25-30) | 24(18-28) |
| Distance head to excretory pore | 56(48-60) | 66(56-75) | 55(33-60) | 55(42-62) | 58(55-74) | 55(47-59) | 55(34-61) | 58(52-75) | 61(58-71) | 57(39-63) | 52(35-60) |
| Distance head to nerve ring | 87(76-88) | 99(90-110) | 90(74-98) | 83(66-98) | 92(79-112) | 95(90-100) | 87(80-90) | 80(84-111) | 95(90-113) | 98(90-113) | 89(80-94) |
| Distance head to pharynx base | 145(132-163) | 139(131-163) | 142(122-163) | 129(110-146) | 137(122-153) | 137(124-142) | 136(121-153) | 135(131-142) | 130(125-146) | 134(125-151) | 129(120-140) |
| Tail length | 62(50-72) | 85(69-87) | 87(79-100) | 63(41-72) | 83(76-91) | 85(72-90) | 72(69-77) | 80(78-87) | 95(81-103) | 78(72-85) | 75(69-89) |
| Ratio a | 25(20-34) | 31(28-35) | 32(26-36) | 28(22-34) | 33(31-38) | 30(29-34) | 27(25-35) | 25(21-32) | 34(31-38) | 30(26-34) | 26(20-34) |
| Ratio b | 4.7(3.1-6.4) | 6.0(5.4-6.3) | 6.3(4.1-7.5) | 5.5(4.3-6.2) | 6.7(5.6-8.0) | 5.8(5.2-6.0) | 5.5(5.2-5.5) | 5.9(5.5-6.0) | 6.3(5.8-6.7) | 6.1(5.9-6.2) | 6.2(6.0-6.5) |
| Ratio c | 11.1(9.0-15.5) | 10.7(9.7-11.1) | 10.5(9.2-11.9) | 11.3(9.0-12.6) | 10.8(10.2-11.9) | 9.4(9.2-9.6) | 10.7(10.1-11.1) | 10.3(9.9-10.6) | 9.2(9.0-10.1) | 11.5(10.6-11.9) | 10.9(9.7-11.1) |
| Ratio D | 0.38(0.31-0.44) | 0.40(0.31-0.45) | 0.38(0.22-0.45) | 0.43(0.36-0.47) | 0.43(0.39-0.56) | 0.41(0.39-0.56) | 0.46(0.38-0.56) | 0.45(0.31-0.56) | 0.51(0.49-0.56) | 0.43(0.36-0.47) | 0.5(0.48-0.52) |
| Ratio E | 0.85(0.77-1.1) | 0.74(0.72-0.77) | 0.61(0.59-0.71) | 0.89(0.75-1.1) | 0.7(06-9.1) | 0.6(0.58-0.64) | 0.73(0.71-0.79) | 0.69(0.64-0.74) | 0.61(0.58-0.69) | 0.7(0.65-0.74) | 0.64(0.61-0.71) |
| n | 23 | 21 | 20 | 15 | 20 | 21 | 15 | 24 | 20 | 20 | 21 |
| Species (Poinar, 1990) | S. affinis | S. feltiae | S. feltiae | S. affinis | S. feltiae | S. feltiae | S. feltiae | S. affinis | S. feltiae | S. affinis | S. feltiae |

All measurements are in micrometers, range is given in brackets and follows the average; D = distance from head to excretory pore divided by distance from head to pharynx base; E = distance from head to excretory pore divided by tail length.

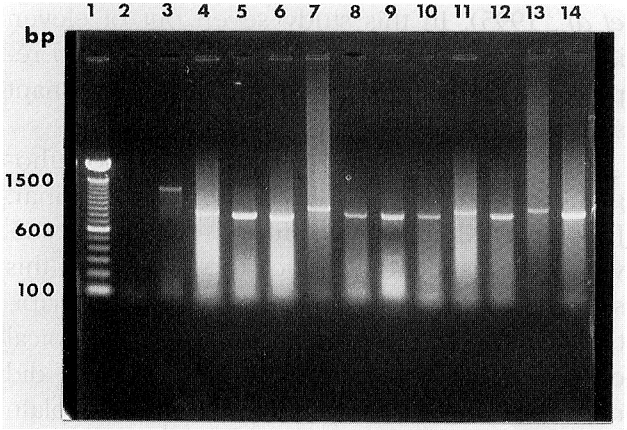


Fig. 1 - Amplification of rDNA (5-10 infective juveniles) for ITS region fractionated on an ethidium-bromide stained 1.5% agarose gel. Lane 1 indicates 100 bp marker (Gibco), lane 2 negative control, lane 3 positive control (*C. elegans*) and lane 4 to 13 *Steinernema* isolates (j1, j8, j24, j33, j35, j44, j57, j70, j71, j77 and j127).

ther differentiation among the *S. feltiae* populations: j44, j57, j71 and j127 as Al type and j8, j24 and j35 as B3 type (Reid, 1994).

Entomopathogenic nematodes were detected most frequently in samples taken from woodland (3.3%) and roadside verges (2.2%); they were found less frequently in samples taken from grassland (0.5%). No nematodes were recovered from cultivated fields. The nematodes were recovered in soil with pH 3.3-7.7. The organic matter content of the positive samples varied between 3.7 and 9.3% and the sand fraction (particles > 63 µm) ranged from 63% to 95% (Table I).

In the conditions of the experiment, no isolate penetrated and killed larvae of *O. sulcatus*. However, different levels of infection to *G. mellonella* were observed (Table III). An average infectivity of more than 10% (14.7-17.4%) was observed with three isolates j1 and j77 (*S. affinis*) and j57 (*S. feltiae*). This infectivity was significantly different from other isolates. The box plots of Fig. 3 show that the intra-specific results were not consistent; some isolates (*S. affinis*: j1 and j77 and *S. feltiae* A1 type: j44 and j57) showed a greater variability in their infectivity than others.

## Discussion

Entomopathogenic nematodes were detected in 6.1% of the 180 soil samples collected in East Flanders. The incidence was lower than in an other Belgian survey conducted in the West Flanders province where nematodes were detected in 12.3% of the samples (Miduturi *et al.*, 1995). In both surveys repeated baiting of the soil samples was practised. Hominick and Briscoe (1990), however, partially attributed higher detection rates of entomopathogenic nematodes to repeated soil baiting. Griffin *et al.* (1991) also used double baiting and reported an incidence of only 10.5%. In a survey conducted in Nor-

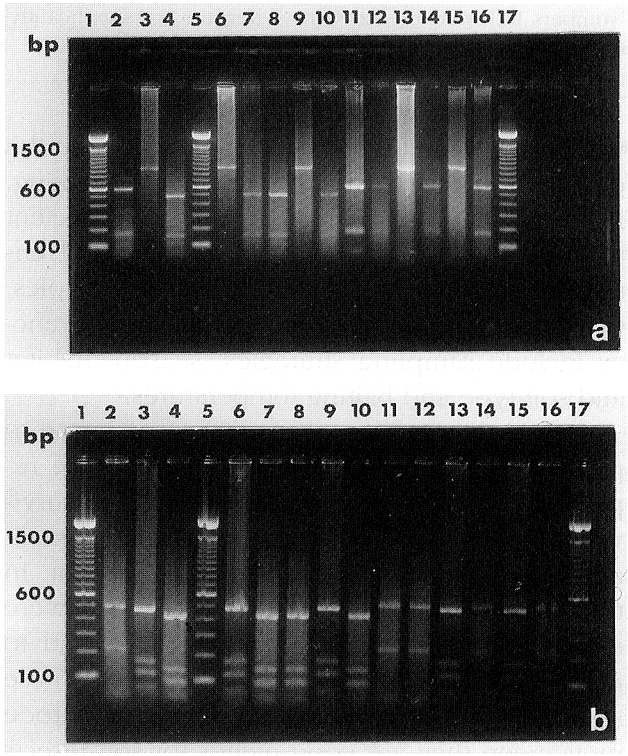


Fig. 2 - Restriction digests of amplified rDNA ITS region of *Steinernema* isolates separated on an ethidium-bromide stained 3% agarose gel: panel a, RFLPs obtained by digestion with *Hinf I*; panel b, RFLPs obtained by digestion with *Alu I*. Lane 1, 5 and 17: 100 bp marker; lanes 2-4 positive controls of *S. feltiae* A1, *S. affinis* and *Steinernema* sp. B3. Lanes 6, 9, 13 and 15 *S. affinis* (j1, j33, j70 and j77); lanes 7, 8 and 10 *Steinernema* sp. B3 (j8, j24 and j35); lanes 11, 12, 14 and 16 *S. feltiae* A1 (j44, j57, j71 and j127).

TABLE III - *Mean percent infectivity of isolates of* Steinernema *spp. to* Galleria mellonella *larvae.*

| Isolate | % infectivity |
|---|---|
| j57 (*Steinernema feltiae*) A1 type | 17.4 a |
| j77 (*Steinernema affinis*) | 15.5 a |
| j1 (*Steinernema affinis*) | 14.7 a |
| j70 (*Steinernema affinis*) | 9.2 b |
| j44 (*Steinernema feltiae*) A1 type | 8.7 b |
| j127 (*Steinernema feltiae*) A1 type | 5.3 bc |
| j24 (*Steinernema feltiae*) B3 type | 5.0 bc |
| j71 (*Steinernema feltiae*) A1 type | 4.7 bc |
| j33 (*Steinernema affinis*) | 4.5 bc |
| j35 (*Steinernema feltiae*) B3 type | 3.5 c |
| j8 (*Steinernema feltiae*) B3 type | 2.6 c |
| Control | 0 d |

Numbers followed by the same letter are not significantly different (P = 0.05, SE = 2.548).

way, Haukeland (1993) was able to increase the detection rate by 7 to 18% after double baiting. Differences in baiting method are not sufficient to explain all variations in the detection of entomopathogenic nematodes from soil samples. Other factors that may relate to these differences include sampling strategies, season, habitat and soil type and baiting temperatures.

Soil texture affects the survival of entomopathogenic nematodes. Kung *et al.* (1990), reported that entomopathogenic nematode survival is highest in soils with higher sand content and lowest in soils with higher clay content. In this study, entomopathogenic nematodes were mostly found in sandy soils with light acid to neutral pH and medium organic content. These soils are well aerated and facilitate nematode movement; they are good niches for soil insects which serve as hosts for entomopathogenic nematodes. These insects, however, were not always present and most of the sampled soils with similar characteristics were without entomopathogenic nematodes.

In a previous Belgian survey *S. feltiae* was the most commonly detected species (Miduturi *et al.*, 1995). In this study seven out of eleven isolates were *S. feltiae.* Based on these two reports, *S. feltiae* appears to be the predominant species in Belgium.

The other important genus of entomopathogenic nematodes, *Heterorhabditis* sp. Poinar, Jackson *et* Khan was not found during our survey. There is some evidence to suggest that this species occurs more commonly in coastal habitats (Griffin *et al.*, 1991) or warm and tropical climates (Hara *et al.*, 1991). These habitats did not occur in the surveyed area and may explain why *Heterorhabditis* species were not detected.

In addition to Steinernematidae other entomopathogenic organisms like fungi and bacteria were found associated with *G. mellonella* cadavers. This suggests that there may be other entomopathogenic organisms in the soil that warrant further investigations as biocontrol agent.

The isolates were not infective to *O. sulcatus,* one of the major insect pest in West-European horticulture. Probably the insect was not a

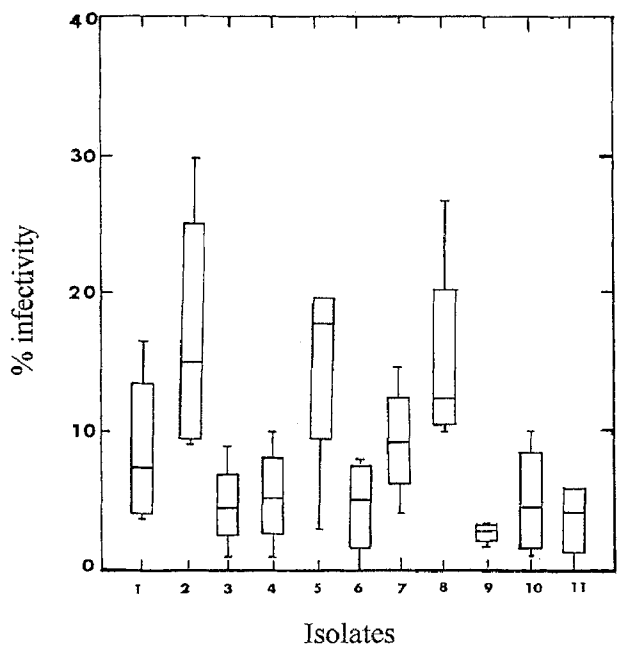


Fig. 3 - Boxplots representing infectivity of eleven isolates of *Steinernema* spp. (Lane 1-4, *Steinernema feltiae* A1 type j44, j57, j71 and j127; lane 5-8, *Steinernema affinis* j1, j33, j70 and j77; lane 9-11, *Steinernema* sp. B3 j8, j24 and j35).

suitable host to the isolates but it is worthwhile confirming these results. These results and the general low infectivity to *G. mellonella* may be attributed to a prolonged storage which decreases their pathogenicity.

**Acknowledgements**. Thanks to Ing. G. Hendrickx for his technical assistance during the survey and to the CLO-Rijksstation voor Nematologie en Entomologie, Merelbeke for providing facilities to conduct this research. Thanks also to ABOS, Belgium, for awarding scholarship to the second author which made this study possible.

## Literature cited

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

BEDDING R. A., 1990. Logistics and strategies for introducing entomopathogenic nematode technology into developing countries, pp. 233-245. *In*: R. Gaugler & H. K. Kaya (Eds). *Entomopathogenic nematodes in Biological control*, Boca Raton, Florida, CRC Press.

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

GRIFFIN C. T., MOORE J. and DOWENS M. J., 1991. Occurrence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematologica, 37*: 92-100.

HARA A. H., GAUGLER R., KAYA H. K. and LEBECK, L. M., 1991. Natural populations of entomopathogenic nematodes (Rhabditida, Heterorhabditidae, Steinernematidae) from the Hawaiian Islands, *Environ. Entomol., 20*: 211-216.

HAUKELAND S., 1993. Entomopathogenic nematodes found in Norway. *Norw. J. Agricult. Scien., 7*: 17-27.

HOMINICK W. M. and BRISCOE B. R., 1990. Occurrence of entomopathogenic nematodes (Rhabditidae; Steinernematidae; Heterorhabditidae) in British soils. *Parasitology, 100*: 295-302.

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

KUNG S. P., GAUGLER R. and KAYA H. K., 1990. Influence of soil pH and oxygen on persistence of *Steinernema* spp. *J. Nematol., 22*: 440-445.

MIDUTURI J. S., MOENS M. and DE GRISSE A., 1995. Occurrence of entomopathogenic nematodes in the west-Vlaanderen province of Belgium. *Nematologica, 41*: 322 (abstract).

POINAR G. O. JR., 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae, pp. 23-58. *In*: Gaugler R. and Kaya H. K., *(Eds) Entomopathogenic nematodes in Biological control*, Boca Raton, USA, CRC Press.

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

SNEDECOR G. W. and COCHRAM W. G., 1978. *Statistical methods.* Ames, Iowa State University Press.

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

Accepted for publication on 12 August 1996.