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Incidence of entomopathogenic nematodes in soil samples collected from Scotland, England and Wales [1]

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Summary – Between May 1990 and October 1991, 414 soil samples were collected from sites in Scotland (221), England (154) and Wales (39) and were assayed for entomopathogenic nematodes by baiting sub-samples with *Galleria mellonella* larvae at 10, 14 and 18 °C. Nematodes were detected in soil samples from 53 (12.8 %) of the sampling sites, including 41 (18.6 %) sites in Scotland, eleven (7.1 %) in England, and one (2.6 %) in Wales. Nematodes were isolated from 17 sites at 10 °, 13 sites at 14 °C, and 23 sites at 18 °C. A total of six species was distinguished : *Steinernema affinis* Bovien, *S. feltiae* Filipjev, *S. kraussei* Steiner, and three undescribed *Steinernema* species (designated C, D, and E). Conclusions are drawn with respect to the high incidence of *S kraussei* in this survey (24 out of 53 isolates), and the possible adaptation of this species to parasitism of insects at low temperatures. *S. kraussei* is considered to have potential as a biocontrol agent for use in field crops in temperate climates.

Résumé – *Fréquence des nématodes entomoparasites dans des échantillons de sol récoltés en Écosse, Angleterre et Pays de Galles* – Entre mai 1990 et octobre 1991, 414 échantillons de sol récoltés en Écosse (221), Angleterre (154) et Pays de Galles (39) ont été testés pour la présence de nématodes entomopathogènes à l'aide de trois piégeages par *Galleria mellonella,* à 10, 14 et 18 °C. Ces nématodes étaient présents dans 53 (12,8 %) des sites de prélèvements : 41 (26,6 %) en Écosse, onze (7,1 %) en Angleterre et un seul (2,6 %) au Pays de Galles. Au total 47 isolats ont été récupérés : 23 à 18 °C, 13 à 14 °C et 17 à 10 °C. Six espèces au total ont été identifiées : *Steinernema affinis* Bovien, *S. feltiae* Filipjev, *S. kraussei* Steiner et trois espèces de *Steinernema* non encore décrites (désignées par les lettres C, D et E). Des conclusions sont tirées en relation avec la forte prévalence de *S. kraussei* dans les prélèvements (24 sur 53 sites) et l'éventuelle adaptation de cette espèce à l'entomoparasitisme à basses températures. *S. kraussei* est considéré comme un agent de controle biologique à fort potentiel pour un usage au champ en climat tempéré.

Key-words : Entomopathogenic nematodes, soil surveys, *Steinernema affinis, Steinernema feltiae, Steinernema kraussei,* temperature.

Entomopathogenic nematodes (specifically, *Steinernema* spp. and *Heterorhabditis* spp.) have a global distribution and occur in a wide variety of soil types and habitats. They remain in the soil, without feeding or developing, as third stage infective juveniles, and only complete their life-cycle in a killed insect host. They go through several generations in the insect host until resources are depleted and then migrate into the soil as infective juveniles. As infective juveniles, they are able to withstand moderate changes in environmental conditions (Poinar, 1990).

Entomopathogenic nematodes are parasites of a wide range of insects (Klein, 1990; Begley, 1990). This factor has made them the focus of research to exploit their potential for controlling insect pests of horticultural and, more recently, agricultural crops. Currently, there are many products available commercially to control insects in protected crops; however, their use in field crops is limited. In countries with a temperate climate, a major restriction is that the nematodes currently in use are not active at low temperatures (*i.e.,* generally not below 14 °C). In Britain, where the prevailing climate is temperate, the average soil temperature (at 10 cm) between 1982 and 1992, for unprotected crops, was above 14 °C only between mid-May and mid-September (UK meteorological data). Many insects still cause significant damage between September and May. If the use of entomopathogenic nematodes is to be extended to the control of insect pests in field crops in countries with a temperate climate, nematode isolates capable of parasitizing of hosts at the low temperatures found in field conditions are required.

This paper describes the collection and baiting of soil from predominantly northern and upland areas of Scotland, England, and Wales. The results report the incidence of entomopathogenic nematodes, their identity, and their potential to be parasitic on insects at low temperatures.

[1] This work forms part of a thesis by the first author for a PhD at the University of Reading, UK.

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Materials and methods

Soil samples were collected from predominantly northern or upland areas of Scotland (221 samples), England (154 samples), and Wales (39 samples), between May 1990 and October 1991 (Fig. 1). Samples were taken from sites that were relatively undisturbed and/or sites thought to be exposed to temperatures lower than the national average. No specific sampling strategy was used, but clay soils were avoided as Blackshaw (1988) reported that entomopathogenic nematodes are uncommon in these soil types. Sampling was not biased for particular habitats as there were no conclusive records, at the time of sampling, of a correlation between the occurrence of entomopathogenic nematodes and habitat. Records were kept, however, of the habitats from which samples were collected and these were categorised as either field, verge (*i.e.* borders of roads and fields), hedgerow, woodland (decidious or coniferous), or moor (including heath).

At each collection site, sub-samples were taken with a hand trowel from the top 10 cm of the soil profile within an area about 1 × 1 m, until approximately 500 g of soil was collected. In the laboratory, samples were sorted : stones, vegetation, and large invertebrates were removed. Soil samples were stored at 9(± 1) °C until baited.

Nematodes were recovered from the soil using an adaptation of the technique described by Bedding and Akhurst (1975). A final-instar larva of *Galleria mellonella* was placed at the bottom of a 10 × 10 cm Petri dish (Sterilin). A wet disc of milk filter (119 mm diameter, Clares Ltd, Avon, UK) was placed over the larva. Each soil sample was divided into three parts. Each part was used to fill a Petri dish and the lid was replaced and sealed with Parafilm. Petri dishes were incubated at either 10, 14, or 18 °C. Larval mortality was assessed every 3 or 4 days, up to a maximum of 21 days.

Dead *G. mellonella* larvae were removed, washed and placed separately on damp filter paper (Whatman N° 1) in a 55 mm Petri dish and incubated at their respective baiting temperatures. Infective juveniles were extracted using the Baermann technique (Hooper, 1986) which had been modified : a disc of stainless steel gauze was placed inside a glass funnel (10 cm diameter) and a disc of milk filter laid on top. Extracted nematodes were collected after 5 h at room temperature and stored at 6 °C.

To confirm that any nematodes collected were capable of causing insect mortality, a Petri dish assay was done. For each isolate collected, 500 infective juveniles were placed in a Petri dish with ten final-instar *G. mellonella* larvae. The Petri dishes were incubated at 18 °C and emerging nematodes collected using adapted Baermann funnels.

For each site from which nematodes were recovered (positive sites), only the isolate recovered at the lowest temperature was identified and used for further research.

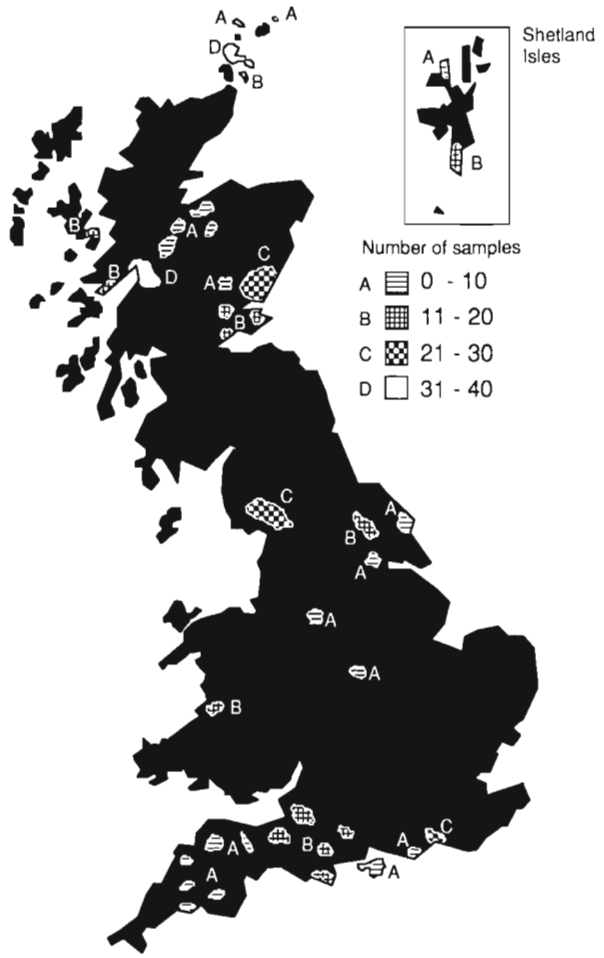

Fig. 1. *Location and number of sites in Scotland, England, and Wales from which soil samples were collected when looking for cold active entomopathogenic nematodes.*

Nematodes for identification were cultured either in *G. mellonella* (as in the Petri dish assay) or monoxenically (Bedding, 1981), at their respective baiting temperatures. Infective juveniles were mounted in Ringer's solution, heat-killed and examined using phase-contrast microscopy. Nematode identification to species was done by Dr A. Reid (International Institute of Parasitology, St. Albans, UK) comparing restriction fragment length polymorphisms (RFLPs) (Reid & Hominick, 1992).

Results

Entomopathogenic nematodes were isolated from 53 sites (12.2 %) : 41 from Scotland (18.6 %) and eleven from England (7.1 %) and one from Wales (2.6 %), (Table 1). All the nematode isolates collected were ca-

Table 1. *Entomopathogenic nematode isolates detected in soil samples collected from Scotland, England, and Wales after baiting with* Galleria mellonella *at low temperatures. (+ nematodes detected, – no nematodes detected, * isolates identified from morphometric characters only).*

Species	Baiting temperature (°C)			Habitat	Region and country	Isolate code
	10	14	18			
S. kraussei	+	+	+	Field	Shetland, Scotland	L/108
S. kraussei	+	+	+	Wood	Fife, Scotland	L/169
S. kraussei	+	+	+	Wood	Fife, Scotland	L/137
S. kraussei	+	+	+	Wood	Somerst, England	L/017
S. kraussei	+	+	+	Field	Shetland, Scotland	L/363
S. kraussei	+	–	+	Field	Shetland, Scotland	L/363
S. kraussei	+	–	+	Field	Orkney, Scotland	L/268
S. kraussei	–	+	+	Field	Highland, Scotland	L/220
S. kraussei	+	+	+	Field	Tayside, Scotland	L/154
S. kraussei	–	+	+	Verge	Somerset, England	L/019
S. kraussei	–	+	+	Wood	Grampian, Scotland	L/195
S. kraussei	–	+	+	Wood	Tayside, Scotland	L/135
S. kraussei	–	–	+	Moor	Highland, Scotland	L/249
S. kraussei	–	–	+	Moor	Highland, Scotland	L/252
S. kraussei	–	–	+	Moor	Fife, Scotland	L/165
S. kraussei	–	–	+	Moor	Fife, Scotland	L/180
S. kraussei	–	–	+	Verge	Highland, Scotland	L/230
S. kraussei	–	–	+	Verge	Tayside, Scotland	L/119
S. kraussei	–	–	+	Verge	Fife, Scotland	L/126
S. kraussei	–	–	+	Wood	Orkney, Scotland	L/308
S. kraussei	–	–	+	Wood	Tayside, Scotland	L/120
S. kraussei	–	–	+	Wood	Tayside, Scotland	L/129
S. kraussei	–	–	+	Wood	Tayside, Scotland	L/136
S. kraussei	–	–	+	Wood	Fife, Scotland	L/133
S. kraussei	–	–	+	Wood	Fife, Scotland	L/178
S. feltiae	+	–	+	Field	Tayside, Scotland	L/128
S. feltiae	+	–	–	Field	Yorkshire, England	L/392
S. feltiae	–	+	–	Field	Lothian, Scotland	L/181
S. feltiae	–	+	–	Wood	Cornwall, England	L/339
S. feltiae	–	+	+	Wood	Somerset, Scotland	L/012
S. feltiae	–	–	+	Moor	Dorset, England	L/067
S. feltiae	–	–	+	Verge	Wiltshire, England	L/326
S. affinis	+	+	+	Verge	Lothian, Scotland	L/167
*S. affinis**	+	+	+	Verge	Devon, England	L/343
*S. affinis**	+	+	+	Field	Shetland, Scotland	L/267
*S. affinis**	+	+	+	Moor	Orkney, Scotland	L/320
S. affinis	+	–	–	Verge	Lothian, Scotland	L/179
*S. affinis**	–	+	+	Field	Orkney, Scotland	L/304
*S. affinis**	–	–	+	Verge	Dorset, England	L/069
*S. affinis**	–	–	+	Verge	Orkney, Scotland	L/280
Species C	+	+	+	Hedge	Sussex, England	L/098
Species C	+	–	+	Verge	Highland, Scotland	L/213
Species C	–	–	+	Verge	Highland, Scotland	L/232
Species C	–	–	+	Wood	Dyfed, Wales	L/274
Species D	–	–	+	Field	Highland, Scotland	L/189
Species D	+	+	+	Verge	Tayside, Scotland	L/132
Species E	+	–	+	Wood	Highland, Scotland	L/194
Species E	+	–	+	Wood	Highland, Scotland	L/208
Species E	–	+	–	Field	Tayside, Scotland	L/138
Species E	–	+	+	Verge	Highland, Scotland	L/216
Species E	–	–	+	Field	Highland, Scotland	L/196
unidentified	–	+	+	Field	Fife, Scotland	L/130
unidentified	–	+	–	Moor	Tayside, Scotland	L/149
unidentified	–	–	+	Verge	Cornwall, England	L/336

pable of killing and completing their life cycle in *G. mellonella* larvae in the Petri dish assays.

After examination of the microscope-slides of infective juveniles, it was concluded that all the isolates belonged to the genus *Steinernema*. Of the 53 isolates, 44 were identified to species using RFLP studies, six by morphological examination of infective juveniles (all *S. affinis* Bovien), and three were not identified because there were too few nematodes (Table 1). Of the six species identified, three were described species; *S. affinis* (eight isolates), *S. feltiae* Filipjev (seven isolates) and *S. kraussei* Steiner (24 isolates). The other three species were undescribed species and designated; species C (four isolates), species D (two isolates), and species E (five isolates) after the system described by Reid and Hominick (1992). The new species C and D had been found in other British soil surveys (Hominick *et al.*, 1995). The isolates of species E are the only known record in Britain. None of the isolates comprised a mixture of species.

Steinernema affinis (6/8 isolates), *S. kraussei* (21/24 isolates), species D (2/2 isolates), and species E (4/4 isolates) were detected most frequently in Scotland, whereas *S. feltiae* (4/7 isolates) was detected most frequently in England, and species C was found distributed in all three countries.

Entomopathogenic nematodes were detected most frequently at 18 °C (23/53 isolates), there were fewer at 10 °C (17/53 isolates) and the least at 14 °C (13/53 isolates) (Table 1). At 10 °C, *S. kraussei* (6/17 isolates) and *S. affinis* (5/17 isolates) were the most frequently detected species, while at 14 °C and 18 °C *S. kraussei* was the most frequently detected species, 5/13 isolates and 13/23 isolates, respectively.

Of the habitats sampled, entomopathogenic nematodes were detected most frequently in soil samples taken from verge (16/88 sites); fewer were detected in moor (7/46 sites), field (14/116 sites), and woodland (15/121 sites) habitats. The lowest frequency was found in samples taken from hedgerow habitats (1/37 sites). Six sites were not classified for habitat. *Steinernema affinis* was most commonly associated with samples collected from verge habitats (5/8 isolates), *S. feltiae* with field habitats (3/7 isolates), and *S. kraussei* with woodland habitats (10/23 isolates). Species C, D and E were not found to be associated with any particular habitat (Table 1).

Discussion

The results of this survey show that *Steinernema* spp. are found commonly in soils of northern and upland regions of Britain where temperatures are likely to be lower than the national average. This corroborates the findings of Burman *et al.* (1986) and Haukeland (1993) who surveyed Sweden and Norway, respectively, in areas which were at similar latitudes to Scotland. However, the results contrast with the findings of Boag *et al.* (1992) who collected samples from similar locations to those sampled in this survey but detected *Steinernema* spp. in only 2.2 % of the samples, leading them to conclude that the distribution of *Steinernema* spp. was limited by low temperatures.

The discovery of six entomopathogenic nematode species (three undescribed) indicates that species diversity is not as limited by cold climate as has been suggested (Boag *et al.*, 1992). The number of *Steinernema* spp. discovered in this survey was higher than expected from other surveys of the UK and countries with similar prevailing temperatures (Burman *et al.*, 1986; Blackshaw, 1988; Hominick & Briscoe, 1990; Griffin *et al.*, 1991; Boag *et al.*, 1992; Haukeland, 1993). To obtain accurate identification of isolates to species the RFLP technique was crucial. Identification based on the infective juveniles was not reliable because morphologically, there were no distinct features which could be used to determine the identity of the infective juveniles recovered, except for *S. affinis* (with its characteristic tail spine). The similarity between the infective juveniles of the species collected in this survey may account for the conclusion of some other surveys that *S. feltiae* was the most common species and only two other *Steinernema* spp. (including *S. affinis*) were recorded. Identification of nematodes to species has also provided evidence of the association between some nematode species and particular habitats although no species were exclusively found associated with one habitat.

The selection of sampling sites in northern and upland, hence colder, areas probably accounts for the high incidence of detection of *S. kraussei*. This finding is confirmed by Steiner (1994) who recorded a high incidence of *S. kraussei* in samples from alpine areas (at altitudes between 1000 and 2600 m), implying that *S. kraussei* is adapted to low temperature environments. Further evidence for this conclusion is provided by the ability of isolates of this species to parasitise black vine weevil *(Otiorhynchus sulcatus),* in laboratory assays, at 6 °C and one isolate as low as 2 °C (Gwynn, 1994). This conclusion warrants research to assess the suitability of *S. kraussei* as a biocontrol agent for use in field crops in temperate areas where low temperatures prevail.

The prevalence of *S. kraussei* in all the habitats sampled (field, verge, wood, hedgerow, and moor) confirms Mráček's (1991) and Steiner's (1994) observations that the distribution of *S. kraussei* is not limited to forest, its type locality (Steiner, 1923; Mracek, 1994). It is unlikely that *S. kraussei* is associated solely with *Cephalcia abietis* and its related species as suggested by Fischer and Führer (1990). In this survey *S. kraussei* was found in habitats well removed from forests; therefore, in its natural habitat, it must be able to complete its life cycle in a wider range of host species.

This survey has shown that the diversity of nematode species is not as limited by cold climates as previously implied, although the abundance of a species may be

limited. The discovery of nematode species with adaptations to particular abiotic conditions, as *S. kraussei* appears to be adapted to low temperature habitats, is important for understanding the ecology of entomopathogenic nematodes and for their exploitation as insect pest control agents.

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References

BEDDING, R. A. (1981). Low cost *in vitro* mass production of *Neoaplectana* and *Heterorhabditis* species (Nematoda) for field control of insect pests. *Nematologica,* 27 : 109-114.

BEDDING, R. A. & AKHURST, R. J. (1975). A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica,* 21 : 109-110.

BEGLEY, J. W. (1990). Efficacy against insects in habitats other than soil. *In :* Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton FL, USA, CRC Press : 215-227.

BLACKSHAW, R. P. (1988). A survey of insect parasitic nematodes in Northern Ireland. *Ann. appl. Biol.,* 113 : 561-565.

BOAG, B., NEILSON, R. & GORDON, S. C. (1992). Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. *Ann. appl. Biol.,* 121 : 355-360.

BURMAN, M., ABRAHAMSSON, K., ASCARD, J., SJÖBERG, A. & ERIKSSON, B. (1986). Distribution of insect parasitic nematodes in Sweden. *In :* Samson, R. A., Vlak, J. M. & Peters, D. (Eds). *Fundam. appl. Aspects Invert. Pathol. :* 316 [Abstr.].

FISCHER, P. & FÜHRER, E. (1990). Effect of soil acidity on the entomophilic nematode *Steinernema kraussei* Steiner. *Biol. Fertil. Soils,* 9 : 174-177.

GRIFFIN, C. T., MOORE, J. F. & DOWNES, M. J. (1991). Occurrence of insect-parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematologica,* 37 : 97-100.

GWYNN, R. L. (1994). *Development of cold active nematodes for insect pest control.* PhD. Thesis, Department of Agriculture, Reading University, UK, 214 p.

HAUKELAND, S. (1993). Entomopathogenic nematodes found in Norway. *Norwegian J. agric. Sci.,* 7 : 17-27.

HOMINICK, W. M. & 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 steinernematid and heterorhabditid nematodes isolated during soil surveys of the U.K. and the Netherlands. *J. Helminth.,* 69 : 27-32.

HOOPER, D. J. (1986). Extraction of free-living stages from soil. *In :* Southey, J. F. (Ed.). *Laboratory methods for work with plant and soil nematodes.* London, UK, HMSO : 5-30.

KLEIN, M. G. (1990). Efficacy against soil inhabiting insect pests. *In :* Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 195-210.

MRÁČEK, Z. (1991). *Steinernema kraussei* (Steiner, 1923) (Nematoda : Rhabditida) : rediscovery of its type locality in Germany. *Folia parasit.,* 39 : 181-182.

MRÁČEK, Z. (1994). *Steinernema kraussei* (Steiner, 1923) (Nematoda : Rhabditida : Steinernematidae) : redescription of its topotype from Westphalia. *Folia parasit.,* 41 : 59-64.

POINAR, Jr. G. O. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. *In :* Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control.* Boca Raton, FL, USA, CRC Press : 23-61.

REID, A. P. & HOMINICK, W. M. (1992). Restriction fragment length polymorphisms within the ribosomal DNA repeat unit of British entomopathogenic nematodes (Rhabditida : Steinernematidae). *Parasitology,* 105 : 317-323.

STEINER, G. (1923). *Aplectana kraussei* n. sp. eine in der Blattwespe *Lyda* sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. *Zentralbl. Bakt. ParasitKde, Abt.* II, 59 : 14-18.

STEINER, W. A. (1994). Distribution of entomopathogenic nematodes in the Swiss Alps. *In :* Smits, P. H. (Ed.). *Proc. 4th Europ. Meet. microb. Control. Pests. IOBC/WPRS Bull.* 17 : 105-107.