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## Survey of *Steinernema* and *Heterorhabditis* (Rhabditida: Nematoda) in Southern Italian soils \*

### ABSTRACT

The occurrence of Steinernematid and Heterorhabditid entomopathogenic nematodes was pointed out in samples of soil collected from cultivated and uncultivated habitats in Southern Italy. Nematodes were isolated from samples of soil by "baiting" with larvae of *Galleria mellonella* L. (Lepidoptera: Galleriidae). Entomopathogenic nematodes were obtained from 26 (13.82%) of 188 soil samples collected: *Steinernema* was located in 16 sites (8.51%), *Heterorhabditis* in 10 sites (5.32%). The *Steinernema* strains were identified as *S. feltiae* (Filipjev, 1934) (50%), *S. anomali* (Kozodoi, 1984) (25%) and as *S. affinis* (Bovien, 1937) (25%). The *Heterorhabditis* strains were identified as *H. bacteriophora* Poinar, 1976. This is the first report of *S. anomali* and *S. affinis* in Italy; the presence of *S. anomali* in the soil of salt pan borders with 3,550 mg of Cl<sup>-</sup> /100 g of soil is extremely interesting.

Key words: Entomopathogenic nematodes, occurrence, *Steinernema feltiae*, *S. anomali*, *S. affinis*, *Heterorhabditis bacteriophora*.

### INTRODUCTION

On biotopes in which research has been carried out on micro and macro organisms of wild pollinators (TRIGGIANI & TARASCO, 1997), soil samples have been collected. Nematodes of genera *Steinernema* and *Heterorhabditis* have been found, which are the subject of the following paper.

Entomopathogenic nematodes of the genera *Steinernema* (Steinernematidae: Rhabditida) and *Heterorhabditis* (Heterorhabditidae: Rhabditida) are obligate and lethal parasites of insects (POINAR, 1990).

Their non-feeding infective juveniles (IJ), usually soil dwelling, hold in their foregut symbiotic bacteria which play an important role in killing susceptible insects. The juveniles enter through the insect's mouth, spiracles,

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anus or through the integument in the case of *Heterorhabditis*, invade the hemocoel through the mid-gut wall and release bacteria which establish suitable conditions for nematode reproduction by providing nutrients and inhibiting the growth of other microorganisms (POINAR, 1979).

The associated bacteria multiply rapidly causing septicemia and death of the host after 24-48 hours, during which time the nematodes feed on the bacteria and reproduce in the cadaver.

The present survey has been conducted with the aim of obtaining information about the types and distribution of entomopathogenic nematodes in Southern Italian soils; only preliminary studies have appeared on this topic (DESEÖ *et al.*, 1988; DESEÖ & MILLER, 1985; EHLERS *et al.*, 1991).

#### MATERIAL AND METHODS

A total of 188 samples was taken during September-October 1996 and March 1997 in the vicinity of Apulia, Basilicata, Molise, Campania and Calabria Regions in Southern Italy. A few samples of soil had been previously collected in June 1990 and in February 1992.

In order to sample ecologically different habitats, such as orchards, fields, uncultivated soils, woodlands, sea coasts, salt pans and grasslands, random soil samples were taken.

Final instars of *Galleria mellonella* L. (Lepidoptera: Galleriidae) were used as bait insects to trap the nematodes in these surveys.

Approximately 2 Kg soil comprised each sample by pooling 3-4 sub samples taken at depths of 10-20 cm from an area of about 50 m<sup>2</sup>.

The soil was transported in sterile polythene bags to the laboratory and six *G. mellonella* larvae were put in a long-handled tea infuser in the middle of each sample (fig. 1).

Afterwards the samples were incubated at 25°C for 7-10 days; if the larvae did not die after 2 weeks, the sample was tested again with *G. mellonella* larvae.

Infected wax moth lar-



Fig. 1 - Long-handle tea infuser used for the "*Galleria mellonella* baiting technique".

Tab. 1 - Characteristics of the sites with *Steinernema* and *Heterorhabditis*

Nematode	Locality	Altitude (m a.s.l.)	Time	Habitat	Soil texture	pH	Org. cont.%	Org. Carb.%	Strains
<i>S. feltiae</i>	Corato (BA)	230	February 1992	Uncultivated land	Silt	7.6	3.23	1.88	IS-CO1
<i>S. feltiae</i>	Sammichele (BA)	280	June 1990	Orchard	Loamy sand	7.9	2.41	1.40	IS-SA1
<i>S. feltiae</i>	Grassano (MT)	300	November 1995	Woodland	Clay	7.5	1.40	0.81	IS-GR1
<i>S. feltiae</i>	Cerignola (FG)	120	October 1996	Orchard	Loamy sand	7.8	1.79	1.04	IS-CE2
<i>S. feltiae</i>	Melfi (PZ)	500	September 1996	Orchard	Sand	7.9	2.17	1.26	IS-MO1
<i>S. feltiae</i>	Mignano del Cardinale (AV)	550	October 1996	Orchard	Clay	7.8	3.60	2.09	IS-MU1
<i>S. feltiae</i>	Montevergine (AV)	700	October 1996	Orchard	Sand	7.3	2.31	1.34	IS-MV1
<i>S. feltiae</i>	Celsi (AV)	650	October 1996	Orchard	Loamy sand	8.0	5.33	3.10	IS-CL2
<i>S. affinis</i>	Forino (AV)	650	October 1996	Orchard	Loamy sand	7.0	5.00	2.91	IS-FO2
<i>S. affinis</i>	Forino (AV)	650	October 1996	Orchard	Sand	7.5	1.95	1.13	IS-FO1
<i>S. affinis</i>	Ariano Irpino (AV)	650	October 1996	Orchard	Clay	7.5	2.34	1.36	IS-AR1
<i>S. affinis</i>	Trivigno (PZ)	350	October 1995	Woodland	Clay	7.6	5.15	2.99	IS-TR1
<i>S. anomali</i>	Margherita di Savoia (FG)	0	October 1996	Uncultivated land	Sand	7.4	0.46	0.27	IS-MS3
<i>S. anomali</i>	Castellaneta (TA)	0	September 1996	Sea coast	Sand	7.4	0.40	0.20	IS-CL5
<i>S. anomali</i>	Metaponto (MT)	50	October 1996	Woodland	Sand	7.9	0.58	0.3	IS-LD3
<i>S. anomali</i>	Margherita di Savoia (FG)	30	October 1996	Salt pan	Silt	7.9	1.98	1.15	IS-MS10
<i>H. bacteriophora</i>	Mellitto (BA)	350	September 1996	Uncultivated land	Silt	9.4	1.67	0.97	IH-ME2
<i>H. bacteriophora</i>	Castellaneta (TA)	0	September 1996	Sea coast	Sand	8.4	0.44	0.25	IH-C11
<i>H. bacteriophora</i>	Castellaneta (TA)	50	September 1996	Woodland	Sand	7.8	1.66	0.97	IH-C6
<i>H. bacteriophora</i>	Castellaneta (TA)	50	September 1996	Orchard	Silt	8.0	2.04	1.21	IH-C13
<i>H. bacteriophora</i>	Lavello (PZ)	650	September 1996	Field	Silt	7.7	2.72	1.58	IH-LA3
<i>H. bacteriophora</i>	Margherita di Savoia (FG)	0	October 1996	Orchard	Clay	7.4	1.11	0.65	IH-MS6
<i>H. bacteriophora</i>	Cerignola (FG)	120	October 1996	Field	Loamy sand	8.0	2.64	1.53	IH-CE1
<i>H. bacteriophora*</i>	San Severo (FG)	90	March 1997	Orchard	Loamy sand	7.6	2.34	1.36	IH-SS1*
<i>H. bacteriophora</i>	Castellaneta (TA)	50	September 1996	Field	Clay	7.4	2.03	1.18	IH-C3
<i>H. bacteriophora</i>	Lucera (FG)	70	October 1996	Wildland	Clay	7.9	2.94	1.71	IH-LU1

\* New variety

vae from each sample were placed in White traps (WHITE, 1927) and kept at room temperature (ca. 23°C). Infective juveniles were harvested and stored in distilled water at 8°C. These nematodes were used to infect fresh *G. mellonella* larvae and the progeny were used for identification and the establishment of cultures.

Measurements were carried out on fresh specimens; identification was based on infective juveniles and male morphology (POINAR, 1990).

For each sampling location, soil texture, pH, content of organic matter and organic carbon, altitude, time and type of culture were recorded (tab. 1). The pH, % organic carbon and % organic content of soil were tested by the Walkley et Black method. The soil structure was determined by texture. Only the soils with *S. anomali* were tested for % NaCl.

## RESULTS

Entomopathogenic nematodes were recovered from 26 (13.82%) of 188 soil samples collected: *Steinernema* was located in 16 sites (8.51%), *Heterorhabditis* in 10 sites (5.32%) (fig. 2; graf. 1, 2).

Among *Steinernema*, 50% were identified as *S. feltiae* (Filipjev, 1934), 25% as *S. anomali* (Kozodoi, 1984) and 25% as *S. affinis* (Bovien, 1937).

No sample yielded more than one nematode species.

The *Heterorhabditis* isolates were identified as *H. bacteriophora* Poinar, 1976. Among these 10 isolates, IH-SS1 is a new variety of *H. bacteriophora* (Poinar, pers. com.). IH-C3, IH-C13 and IH-C6 are *Heterorhabditis bacte-*

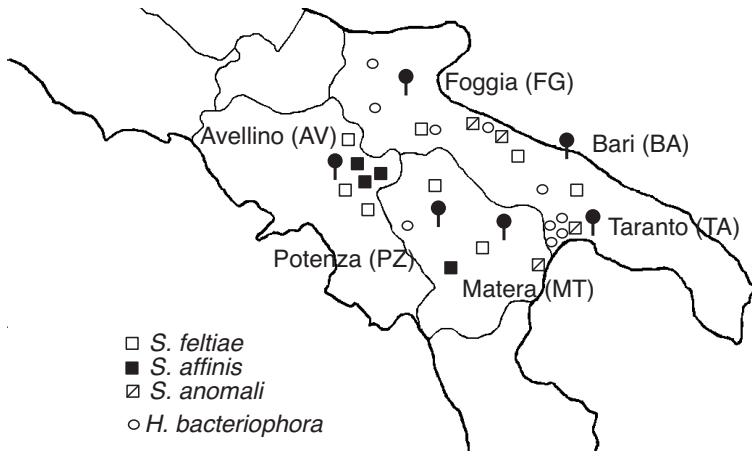
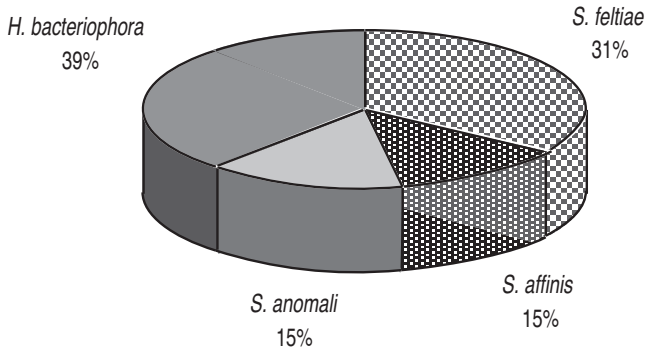
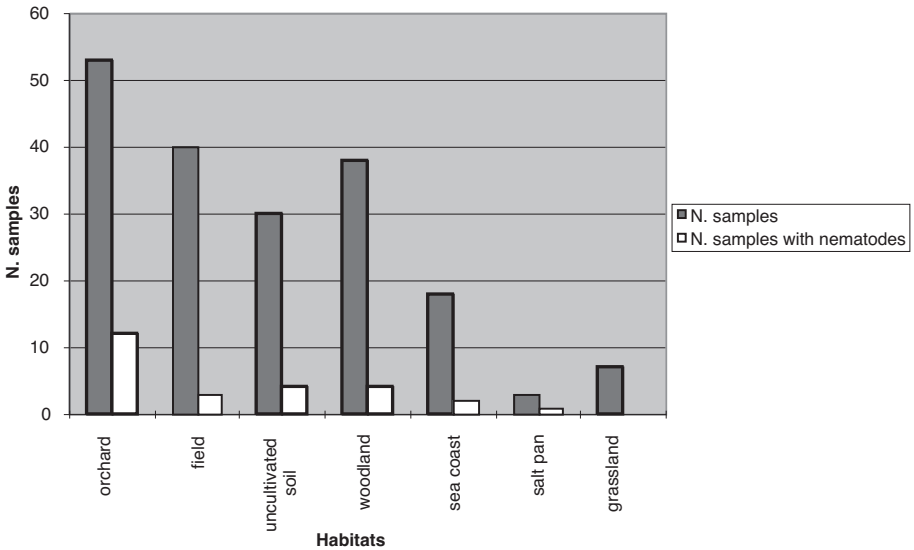


Fig. 2 - Distribution of Rhabditid entomopathogenic nematodes in Southern Italian soils.



Graf. 1 - Species representation of Rhabditid entomopathogenic nematodes in Southern Italian soils.



Graf. 2 - Nematodes found in different habitats.

*riophora* with non-chromatic strain of *Photorhabdus luminescens*, (*Xenorhabdus luminescens*; Boemare *et al.* 1993) (Enterobacteriaceae) (Poinar, pers. com.); the *G. mellonella* larvae infected with these *Heterorhabditis* turn gray-green.

Nematodes were found to be widely distributed in Southern Italy from 0 to 700 m on s.l. and were collected in all sampled habitats. *S. feltiae* was isola-

ted from most diverse habitats and type of soil textures; with a pH between 7.3 and 8.0 and organic content from 1.40 to 5.33%.

*S. anomali* was isolated from four locations, three of them characterized by sandy soil and low organic content (from 0.40 to 1.98%). The four isolates of *S. anomali* were collected in uncultivated land, pinewood, sandy beaches and salt pan borders with sand in the first three soils and silt in the last one. The presence of  $\text{Cl}^-$  in the salt pan border was 3,550 mg/100 g while it was 0.25 mg/g in uncultivated land, 0.04 mg/g in woodland and 0.09 mg/g in the sandy beach. The pH difference was not significant but the percentage of organic content varied from 0.40 to 1.98%.

*S. affinis* was found in three orchards, in a woodland with clay and salty soil all with nearly the same pH (7.0-7.6), the organic content varied from 1.95 to 5.15%.

*H. bacteriophora* was collected in nearly of all the habitats and soil textures with an extremely variable pH (from 7.4 to 9.4) and organic content ranging from 0.44 to 2.94%.

No nematodes were found in grasslands.

## DISCUSSION

Even though Steinernematid and Heterorhabditid nematodes have been collected throughout Europe, their presence in Northern Europe (less than 20%) appears to be lower than in Central Europe (over 25%) (STEINER, 1996). In the Mediterranean area (i.e. Spain and Italy) entomopathogenic nematodes were found from 23.3% of the sites in Spain with 94.3% of the sites positive for the Steinernematids and 5.7% for Heterorhabditids (DEL PINO & PALOMO, 1996). The entomopathogenic nematodes collected by us in Southern Italian soils are more abundant than those found in Northern Italy (DESEÖ *et al.*, 1988; DESEÖ & MILLER, 1985; EHLERS *et al.*, 1991). In the former they consist in about 14% of the soil samples while in all of Italy they comprise only 6%.

On 545 samples collected by DESEÖ *et al.* (1988) 50% contained *Steinernema carpocapsae* Weiser and *S. feltiae* and 50% *Heterorhabditis*. In Southern Italy 61% were *Steinernema* and 39% *Heterorhabditis*.

Analysis of the survey results show no correlation at  $P < 0.05$  between habitat and nematode occurrence.

The dominant species in Italy are undoubtedly *S. feltiae* and *H. bacteriophora*. Regarding to *S. feltiae*, unlike the result from Switzerland (STEINER, 1996), it was not found in the grasslands.

This is the first report of *S. anomali* and *S. affinis* in Italy. The presence of *S. anomali* in salt pan soil borders with 3,550 mg of  $\text{Cl}^-$  /100 g of soil is extremely interesting.

## RIASSUNTO

INDAGINI SU *STEINERNEMA* E *HETERORHABDITIS* (RHABDITIDA: NEMATODA) NEI TERRENI  
DELL'ITALIA MERIDIONALE

È stata effettuata una indagine per valutare la presenza e la distribuzione di nematodi entomopatogeni (*Steinernema* ed *Heterorhabditis*) in campioni di terreno provenienti da diversi habitat dell'Italia meridionale. I nematodi sono stati estratti dai campioni di terreno utilizzando larve di *Galleria mellonella* L. (Lepidoptera: Galleriidae), come insetto esca. La presenza di nematodi entomopatogeni è stata accertata in 26 campioni (13,82%) su 188 esaminati: gli *Steinernema* sono stati rinvenuti in 16 campioni (8,51%), *Heterorhabditis* in 10 (5,32%). Tra gli isolati di *Steinernema* sono state identificate 3 specie: *S. feltiae* Filipjev, 1934 (= *S. bibionis*) (Bovien, 1937) (50%), *S. anomali* (Kozodoi, 1984) (25%) e *S. affinis* (Bovien, 1937) (25%). I ceppi di *Heterorhabditis* sono risultati appartenere alla specie *H. bacteriophora* Poinar, 1976. La presenza di *S. anomali* e *S. affinis* viene segnalata per la prima volta in Italia. Di particolare interesse è il ritrovamento di un isolato di *S. anomali* in un terreno con Cl<sup>-</sup> /100 g di 3.550 mg.

Parole chiave: nematodi entomopatogeni, distribuzione, *Steinernema feltiae*, *S. anomali*, *S. affinis*, *Heterorhabditis bacteriophora*.

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## REFERENCES

- DEL PINO G., PALOMO A., 1996 - Natural occurrence of Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soils. *J. Invertebr. Pathol.* 68: 84-90.
- DESEÒ K.V., MILLER L.A., 1985 - Efficacy of entomogenous nematodes *Steinernema* spp. against clerwing moth, *Synanthedon* spp. in North Italian apple orchards. *Nematologica*, 31: 100-108.
- DESEÒ K.V., FANTONI P., LAZZARI G.L., 1988 - Presenza di Nematodi Entomopatogeni (*Steinernema* spp. *Heterorhabditis* spp.) nei terreni agricoli in Italia. Atti giornate Fitopatologiche 1988, 2: 269-280.
- EHLERS R.U., DESEÒ K.V., STACKEBRANDT E., 1991 - Identification of *Steinernema* spp. and their symbiotic bacteria *Xenorhabdus* spp. from Italian and German *Soil. Nematologica*, 37: 360-366.
- POINAR G.O. JR., 1979 - Nematodes for Biological Control of Insects. CRC Press, Boca Raton, Fl.: 277 pp.
- POINAR G.O. JR., 1990 - Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Entomopathogenic Nematodes in Biological Control, Gaugler R., Kaya H.K. (Eds.), Boca Raton Fl, USA. CRC Press: 23-61.
- STEINER W.A., 1996 - Distribution of entomopathogenic nematodes in the Swiss Alps. *Riv. Suisse de Zool.*, 103 (2): 439-452.
- TRIGGIANI O., TARASCO E., 1997 - Rilievi sulla presenza e diffusione del nematode *Sphaerularia bombi* Dufour, 1837 (Nematoda, Tylenchida, Allantonematidae) in Italia Meridionale. *Entomologica*, Bari, XXXI: 77-97.
- WHITE G.F., 1927 - A method for obtaining infective nematode larvae from cultures. *Science*, 66: 302-303.