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Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic

Zdeněk Mráček [a,*], Stanislav Bečvář [a], Pavel Kindlmann [b,c], Jana Jersáková [c]

[a] Department of Insect Pathology, Institute of Entomology CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic
[b] Institute of Landscape Ecology CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic
[c] Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic

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Abstract

Steinernematids and heterorhabditids are widespread soil nematodes. The dependence of their distribution on habitat type, soil texture, soil pH, and altitude has been studied in some detail, while much less is known about how their occurrence depends on the abundance and habitat preference of their insect hosts. Here we surveyed the entomopathogenic nematode fauna of the Czech Republic and evaluated the impact of ecosystem type, habitat, soil, season, altitude, and insect host species on their prevalence. We also examined the effect of temperature on their isolation rates in the laboratory. Nine species of the genus *Steinernema* (*S. kraussei*, *S. feltiae*, *S. affine*, *S. carpocapsae*, *S. intermedium*, *S. arenarium*, *S. bicornutum*, *S. weiseri*, and *S. silvaticum*) and two of the genus *Heterorhabditis* (*H. bacteriophora* and *H. megidis*) were recorded for the Czech Republic. Nematodes occurred in all ecosystems and habitats tested. They were more abundant in tree habitats and light soils and in sites with abundant suitable insect hosts; seasonality and altitude had no significant impact on their occurrence. At two laboratory temperatures (15 and 22 °C) different numbers of isolates were obtained from the *Galleria* bait traps. Abundance of entomopathogenic nematodes in soil samples varied considerably and there were at most five baiting replicates (in habitats with many suitable insect hosts).

Keywords: Entomopathogenic nematodes; Steinernematidae; Heterorhabditidae; *Steinernema kraussei*; *S. feltiae*; *S. affine*; *S. carpocapsae*; *S. intermedium*; *S. arenarium*; *S. bicornutum*; *S. weiseri*; *S. silvaticum*; *Heterorhabditis bacteriophora*; *H. megidis*

1. Introduction

Entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae are widespread, recorded from all continents excluding Antarctica (Hominick et al., 1996; Hominick, 2002). Some species are endemic to islands (e.g., *Steinernema cubanum* Mráček, Hernandez & Boemare, and *Steinernema puertoricensis* Roman & Figueroa), whereas some species seem to be ubiquitous [e.g., *Steinernema carpocapsae* (Weiser), *Steinernema feltiae* (Filipjev), *Heterorhabditis bacteriophora* Poinar)]. They are soil organisms, adapted to most climatic conditions in hot, temperate, and cold zones, distributed from lowlands to high alpine altitudes (Steiner, 1996). They parasitize insect developmental stages living in soil and some humid cryptic microhabitats, e.g., bark crevices or trunk tunnels.

The dependence of the distribution of these nematodes on factors like habitat type, soil texture, soil pH, and altitude has been studied in some detail (e.g., Hominick and Briscoe, 1990a,b; Yoshida et al., 1998). Individual species of EPNs differ in their habitat preference. For example, *S. feltiae* and *Steinernema affine* (Bovien) are more common in open habitats, such as fields and meadows (Sturhan and Lišková, 1999), whereas *Steinernema*

* Corresponding author. Fax: +420 38 5310354.
E-mail address: mracek@entu.cas.cz (Z. Mráček).

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kraussei is mostly found in woodlands (Mráček et al., 1999; Sturhan, 1995). Type of soil also seems to be important. EPNs are more prevalent in light (especially sandy or organic) soils (e.g., Miduturi et al., 1996). Decrease in soil pH may reduce the host-finding ability and prevalence of *S. kraussei* (Steiner) (Fisher and Führer, 1990). Steiner (1996) reported selection in the EPN pH preference, which was found relatively low for *S. kraussei*, avoiding pH extremes for *Steinernema intermedium* (Poinar), and *S. feltiae* and close to neutral for *S. affine*. Soil texture plays an important role in the EPNs' dispersal (Georgis and Poinar, 1983) and persistence (Kung et al., 1990). Surprisingly, much less is known about the impact of prevalence, abundance, and habitat preference of their insect hosts, which may significantly affect the occurrence of EPNs.

Mráček et al. (1999) published limited results on survey, but in this paper we posed different questions with a substantially extended dataset. We report further findings of a survey, which allows some questions to be asked regarding species occurrence in the Czech territory. For example, how do ecosystems and habitat preference (close-forested as opposed to open habitats), and additional questions how soil type (texture, organic content), season, and altitude affect their occurrence. In addition, what is the impact of insect hosts on EPN prevalence? Does the laboratory baiting method influence the success of nematode isolation? What is the approximate EPN abundance in soil samples?

2. Materials and methods

2.1. Field collection

During the years 1996–1998, we collected 587 soil samples (each at an approximate volume of 3 dm^3) from the territory of the Czech Republic. In each of these localities, one or more sampling sites (different habitats) were chosen at random or targeted if any high occurrence of insects or outbreaks was recorded. Each sampling site was characterized by the type of ecosystem, habitat, soil, latitude, longitude, and altitude. We selected habitats, in which only one potential insect host species was dominant, such as the web-spinning sawfly in spruce monocultures or group of target pests, such as a noctuid and geometrid moth complex in orchards. Thus, habitats with dense shrub undergrowth, which usually has a diverse and abundant insect fauna that may significantly influence the occurrence of nematodes, were eliminated from our sampling. The presence of EPNs in each sampling site was evaluated by baiting soil samples with wax moth larvae (Bedding and Akhurst, 1975; Mráček, 1980).

We sampled from March to December, but less frequently in winter. Each soil sample was obtained by using an iron core (3.6 cm diameter, 20 cm depth, approximate volume 200 cm^3) five times at each sampling site (to get a constant volume of soil) and transported to the laboratory in a plastic bag. The texture of soils was estimated by feel as the sand/silt and silt/clay with a low or high organic content http://ltp-www.gsfc.nasa.gov/globe/tbf/txtbyfel.htm.

2.2. Laboratory tests

For the laboratory tests each soil sample was mixed, divided into two subsamples, and a part of these set in the "*Galleria* trap" (Mráček, 1980) with 5 *Galleria* per trap, which were kept at 15 and 22 °C, respectively. The later instar of the greater wax moth, *Galleria mellonella* (L.), was used. To prevent the *Galleria* larvae from escaping, they were placed in a small steel mesh pocket situated in the center of a petri dish (15 cm diameter). Mortality of *G. mellonella* was assessed after 5 days. Dead *Galleria* larvae were divided into two batches; one was dissected to obtain adult nematodes of the second generation (more accurate identification) and the other one was cultured at a laboratory temperature on a water trap to obtain infective juveniles (IJs). Both adults and IJs were fixed in 4% formalin and stored for identification.

We assessed the abundance of nematodes in 27 randomly chosen positive sites by the number of adult nematodes recovered from the soil samples by baiting. In each "*Galleria* trap," all larvae (dead and alive) were replaced by a new batch of five living larvae. This was repeated as long as at least one *Galleria* larva died. The number of repeat baits, dead larvae, and adult nematodes in all cadavers was counted.

2.3. Statistical analysis

Statistical significance of differences between percentages of recovered nematodes was tested by means of the log-likelihood ratio for contingency tables (G test—Zar, 1984). The multivariate data were analyzed by means of the linear method of direct gradient analysis (RDA, Redundancy analysis) in the program Canoco v. 4.02. We used the presence of nematodes as dependent variable and the environmental data (type of ecosystem, habitat, and soil) as independent categorical variable. Data were scaled on inter-sample distances and centered by species. The differences in nematode species composition of various soil samples were tested by the Monte Carlo Permutation Test (MCPT). Based on the significant results we constructed ordination diagrams [program CanoDraw v. 4.0; ter Braak and Šmilauer (1998)], which expresses the similarities in species composition between soil samples. The gradient of the highest variability in the data coincides with the first ordination axis.