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MAGDALENA DZIĘGIELEWSKA*, ANDRZEJ SKWIERCZ**

THE INFLUENCE OF SELECTED ABIOTIC FACTORS ON THE OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES (*STEINERNEMATIDAE*, *HETERORHABDITIDAE*) IN SOIL

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Abstract. Original research confirms a significant impact of abiotic factors, such as soil type and physicochemical properties, on the biological activity and infectivity of entomopathogenic nematodes from the *Steinernematidae* and *Heterorhabditidae* families. Nematodes were found to prefer sandy loam soils; however, the highest species diversity was found in sandy soils. Some species of nematodes were associated with a specific type of soil. For example, *Steinernema silvaticum* and *Heterorhabditis bacteriophora* were found only in sands, and *H. megidis* predominantly in clay. Nematodes were found in soils of varying pH levels, although individual species preferred a certain degree of acidity. *S. bicornutum* and *H. megidis* were found only in alkaline soils, while others, such as *S. silvaticum,* only in acidic environments (pH<4.5).

Keywords: entomopathogenic nematodes, soil texture, soil moisture, soil salinity, pH, environment interactions

INTRODUCTION

Nematodes of the family *Steinernematidae* Chitwood & Chitwood (1937) and *Heterorhabditidae* Poinar (1976), that parasitize insects, known as entomopathogenic nematodes (EPNs) (*Adenophorea: Rhabditida*), are widely distrib-

* Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology in Szczecin.

** Department of Phytopathology, Entomology and Molecular Diagnostics, University of Warmia-Mazury, entomology@zut.edu.pl

uted all over the world (Hominic 2002, Adams *et al.* 2006). These animals are widely used because of their special attributes such as their wide range of potential hosts, effectiveness at limiting populations of noxious insects, possibility of massive production through solid and liquid growth mediums, and overall environmental safety (Grewal *et al.* 2005, Grewal 2012). High nematode activity in the soil is a necessary condition to maintain a robust nematode population in the environment. One of the most important conditions for nematode development in the soil is the presence of their favored hosts (Peters 1996, Mráček *et al.* 1999, Mráček and Bečvář 2000). The other important conditions are: the type and structure of the soil, physicochemical properties, such as soil moisture, temperature, aeration and acidity (Kaya 1990, Kung *et al.* 1991, Thurston and Kaya 1994, Koppenhöfer *et al.* 1995, Brown and Gaugler 1997, Shapiro *et al.* 2000, Glazer 2002, Millar and Barbercheck 2002, Georgis *et al.* 2006, Siegel *et al.* 2006, Koppenhöfer and Fuzy 2008). The goal of this research is to describe the influence of some abiotic factors on entomopathogenic nematodes in the soil.

MATERIALS AND METHODS

Field studies were carried out from 2010 to 2015 in north-west Poland, in diverse ecosystems and habitats, including forests, agro-ecosystems (farmlands, extensive meadows, and orchards), coastal dunes, xerothermic grasslands and urban greenery. The soil samples from most of the selected sites were taken three times during the high season (spring, summer and autumn). Each research surface was approximately 100 m^2 and 20 cm deep; 50 individual samples were taken using Egner's stick, making the bulk sample approximately 600 cm^3 in volume. In total, 384 soil samples were collected from 80 research plots (Table 1); the soil was transported to the laboratory in perforated bags. The presence of entomopathogenic nematodes in the soil samples were determined using a standard *Galleria mellonella* baiting technique (Bedding and Akhurst 1975, Mráček 1980). Each sample was distributed among 6 pots of a volume of 100 cm^3 each. Then, 3 larvae of *G. mellonella* (the last stage of development, c. 20 mm) were placed in every pot. Pots were placed in an incubator at 20ºC. After 3 days, the first control was performed, dead insects were removed and replaced by live ones (Bedding and Akhurst 1975). Dead larvae of *G. mellonella* were placed in modified White nematode traps (White 1929). The traps were kept in an incubator at 22ºC for c. 1 week until obtaining the invasive larvae from dead larvae of *G. mellonella*.

The mortality of *G. mellonellla* insects infested with nematodes was assessed 5 days after the experiment was established. Isolated nematodes were preserved in 4% formalin and then identified based on morphological and morphometric features of invasive larvae (J3) and second generation adults (Hominik *et al.* 1997, Nguyen 2007).

Table 1. The occurrence of entomopathogenic nematodes (*Steinernematidae*, *Heterorhabditidae*) in selected ecosystems with different soil moisture levels and salinity

Ecosystem	Number of research position	Total number of the soil samples/number of identified samples to species	Soil moisture [%]*		Salinity g NaCl/l*		*S. feltiae*	*S. silvaticum*	*S. affine*	*S. bicornutum*	*H. bacteriophora*	*H. megidis*
			average value	range	average value	range						
Urban greenery	16	91/32	14.25	4.1–38.5	0.2	0.08–0.75	11	2	5	1	0	13
Forest	18	93/24	12.91	9.9–17.5	0.07	0.03–0.13	11	5	7	0	0	1
Xerothermic grassland	4	12/4	17.77	12.3–26	0.11	0.02–0.16	0	0	0	0	4	0
Coastal dunes	16	112/10	1.53	0.1–3.9	0.09	0.03–0.27	5	0	0	0	1	4
Agrocoenoses:												
- orchards	5	43/26	10.49	8.77–12.1	0.19	0.11–0.27	14	1	2	2	0	7
- farmlands	16	18/6	10.93	8.2–16.5	0.07	0.05–0.1	4	1	1	0	0	0
- extensive meadows	5	15/4	18.5	15.4–24.9	0.10	0.04–0.2	1	0	2	0	0	1
Total	80	384/106	-	-	-	-	46	9	17	3	5	26

* The differences were not statistically important ($p<0.05$)

To evaluate how some abiotic factors can influence entomopathogenic nematodes and their biological activity in the soil, the following analyses were done:

1) potentiometric analysis of soil pH active acidity (in H_2O) and cation exchange acidity (in 1N KCl) with proportions between soil and water/potassium chloride 1:2,5 after 24 hours of extraction;

2) determination of soil moisture in fresh soil samples with a dry-weight method using a Radwag WPE 30S meter with automatic results showing in % at 106ºC temperature;

3) conductometry analysis of electrical conductivity with a CPC-501 ELMETRON meter with a EC-60 conductometry sensor;

4) carbon, nitrogen, sulphur indication (CNS) analysis with an elemental Costech analysis instrument;

5) soil fraction analysis using Casagrande's method as modified by Prószyński (PN 04032: 1998).

Statistical analyses were performed with STATISTICA 6.0 software (StatSoft 1999) using: statistical significance tests on differences between structural factors (frequency of nematodes present in different ecosystems) and significance tests on differences between means, assuming a normal distribution of variables ($p<0.05\%$). Pearson's chi square test was used to verify the relationship between nematode occurrence and biotic factors studied. The data is presented in Table 1, including mean values of variables and their standard deviations and ranges.

RESULTS AND DISCUSSION

The soil was taken from different types of ecosystems and categorised into three groups: sand, loamy sand and clay (Figure 1). Clay provided the lowest percentage of samples (78%) with nematodes, while nematodes were found in 100% of the loamy sand samples. The difference was not statistically important ($p<0.05$). Entomopathogenic nematodes were the most present in sand: four were identified as *Steinernematidae* and two as *Heterorhabditidae* (Figure 1). *Steinernema feltiae* nematodes were present in every type of soils, but were most common in sand (64% of samples), while appearing in only 23% of clay and 13% of loamy sand samples. Some species, such as *Heterorhabditis bacteriophora,* were only present in sand; however, related *H. megidis* were predominant in clay (68%). Soil pH levels ranged from very acidic to alkaline (Figure 2); almost 50% of the soil samples were acidic (pH=4.5–6.5), mainly from forests and crop fields; 37% were neutral, comprising the majority of samples from urban greenery, orchards and xerothermic grasslands; and 13% of all samples were alkaline, mainly from sand dunes. Alkaline soil had the lowest percentage of samples with nematodes (approximately 14%; Figure 2). The most flexible

species with regard to habitat were *S. feltiae* and *H. megidis*, which were present in acidic, neutral and alkaline soil. However, the largest percentage (67%) of isolate *S. feltiae* was from acidic soil, and more than 78% of *H. megidis* came from neutral soil. *S. silvaticum*, a species rarely observed in Poland, was present in acidic soil samples (pH<4.5; Figure 2). Neutral soil was preferred by *S. bicornutum*. *H. bacteriophora* was present in completely different soil environments, both acidic and alkaline (Figure 2).

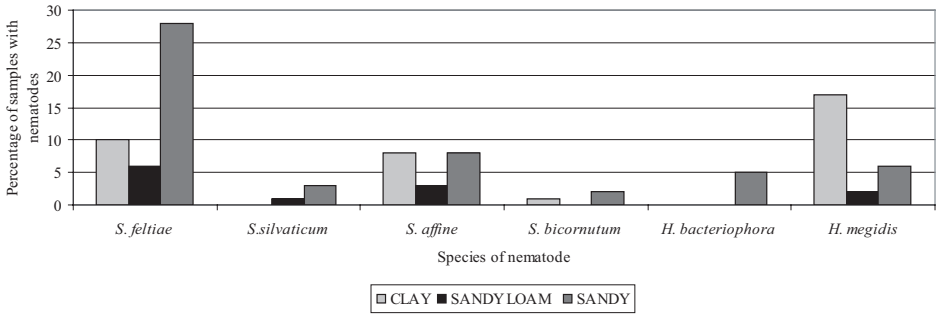

Fig. 1. The occurrence of entomopathogenic nematodes *Steinernematidae* and *Heterorhabditidae* in three types of soil

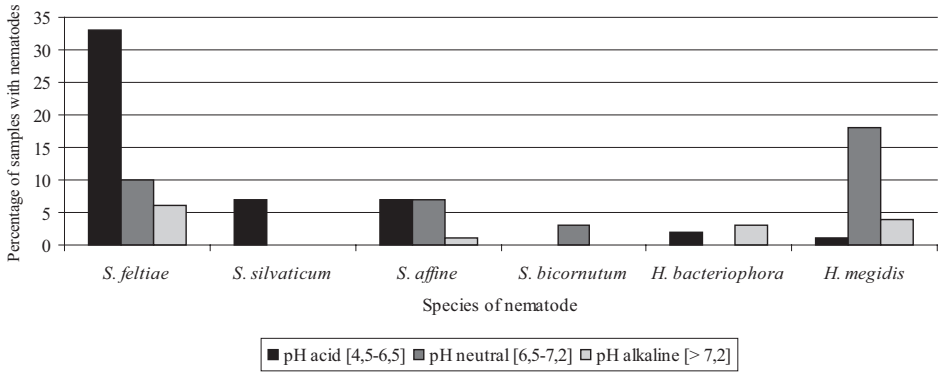

Fig. 2. The occurrence of entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) in soil with different pH levels (scale: Mocek *et al*. 1997)

Most of the soil in the research had low humus content (about 1–5%), wherein the percentage of samples with nematodes was the highest (36%) in the soil with moderate humus content (approximately 2–5%; Table 2). 29% of the samples with nematodes were isolated from the soil with a strong presence of humus. Four species of nematodes (*S. feltiae*, *S. silvaticum*, *S. affine* and *H. megidis*) were present in soils with extremely different organic content (Table 2). The highest species diversity was recorded in medium-humus soils,

mainly from forests, orchards and meadows, which particularly favored *S. feltiae* and *H. megidis* nematodes (Table 2). *S. bicornutum* was only isolated from soils with an average humus content of 2 to 5% (Table 2).

Table 2. The occurrence of entomopathogenic nematodes (*Steinernematidae*, *Heterorhabditidae*) in selected ecosystems with different humus content

Humus content [%]*	Total number of samples	Number of samples with nematodes	Percentage of samples with nematodes	*Steinernema*				*Heterorhabditis*	
				feltiae	*silvaticum*	*affine*	*bicornutum*	*bacterio-phora*	*megidis*
Soils with low humus content 1–2%	139	25	18	11	1	6	0	3	4
Soils with moderate humus content 2–5%	152	55	36	26	5	4	3	2	15
Soils with moderate humus content >5%	93	26	29	9	3	7	0	0	7
Total	384	106	-	46	9	17	3	5	26

* Mocek *et al.*'s scale (1997)

All the research plots had comparably low-salinity soil, ranging from 0.01 to 0.75 g NaCl/l. Average salinity was from 0.06 to 0.2 g NaCl/l, a factor which was not significant for the presence of nematodes and species diversity in different ecosystems (Table 1).

Entomopathogenic nematodes were present in dune sand with very low moisture, averaging 1.53%, and in soil from meadows and pastures with more than 22% moisture (Table 1).

Overall, less nematode species diversity was observed in soils with higher moisture. In these soils, the most common species of *S. feltiae* was not found; however, it was isolated, along with two other species of the *Heterorhabditidae* family, from the soil with the very low moisture average of 1.53% (Table 1). On dunes, with extremely low water content in the surface up to 30 cm, *S. affine* was not present; it preferred soil with a moisture content of more than 10%. Other species, such as *S. bicornutum* and *S. silvaticum*, were found in soils with an average moisture of 10.32 to 14.25% (Table 1).

Overall, loam soil samples had the largest percentage of samples with nematodes, but the greatest species diversity was found in sand soil.

Some nematodes were present in only one type of soil. For example, *S. silvaticum* and *H. bacteriophora* were present only in sand. In the clay, *H. megidis* was predominant, appearing in 47% of the samples. In all soil types, the presence of *S. feltiae* was recorded, though the species was most prevalent in sand soil.

In addition, other researchers have identified an association of *S. silvaticum* with sandy soils and the presence of *S. feltiae* in all of the soil types studied (Mráček *et al.* 2005). The obtained results confirm the fact that entomopathogenic nematodes are present in different types of soils (Tumialis *et al.* 2016) and, as shown in the research in north-west Poland, may also be present in seasonal wetlands (Hominik and Briscoe 1990).

As clay content increases, nematode activity decreases (Kung *et al.* 1990, Koppenhöfer and Fuzy 2006), a result which is related to the size of the intermolecular space and the aeration (Gaugler and Kaya 1990, Hominik and Briscoe 1990). Shapiro-Ilan *et al.* (2002) reported that clay soils restrict nematode movement and are poorly aerated. The literature shows that entomopathogenic nematodes are more frequently found in sandy loam soils (Kary *et al*. 2009, Raheel *et al.* 2015), sandy soils (Kung *et al.* 1990) and loam sand soil (Stock *et al.* 1999), and their frequency increases (effect of infestation intensity) in areas with moderate precipitation (Koppenhöfer *et al.* 1997).

Physicochemical tests determined that pH soil level did not directly influence the presence of entomopathogenic nematodes: They were present in very acid soil (pH<4.5) and alkaline soil (pH>7.2). Their presence in a wide range of pH soil levels (from 4.6 to 8) has also been established by Hara *et al.* (1991), Griffin *et al.* (1994) and Stock *et al.* (1999). Other researchers have shown that the pH range of the soils from 4.5 to 5.7 was most considered tolerable by the EPNs (Nyasani *et al.* 2008).

Other researchers have shown that the vitality of invasive larvae is significantly reduced in very acidic and highly alkaline soils (Kung *et al.* 1990). As observed in the present research, some species of nematode, such as *S. bicornutum*, are only found in neutral soils, and others, such as *S. silvaticum*, only in acidic environments (pH<4.5). The research also shows that nematodes prefer soil with moderate humus content (2–5%).

The observations of other researchers show that organic-rich soils provide a good shelter for entomopathogenic nematodes (Hominik and Briscoe 1990). An important factor for the migration of nematodes in soil is adequate soil moisture (Koppenhöfer *et al.* 1995, Půža and Mráček 2007).

The present study has shown that nematodes can be found in very low moisture soil in coastal dunes and high moisture soil in meadows and floodplains. Other researchers have also noted that low as well as excessive moisture levels have a negative effect on nematodes and their dispersion in the ground (Kondo and Ishibasi 1985). For example, the lowest percentage of nematodes in soil samples was recorded in soils with extreme moisture levels: over 22% moisture content (25% of samples) and less than 1.53% moisture content (10% of samples).

Alekseev *et al.* (2006) recorded that the activity of IJs of *S. carpocapsae* in the soil upper layer (1 cm depth) was strongly affected by the soil type. When the soil moisture was low and the number of nematodes found in the

upper layer correspondingly low. Půža and Mráček (2007) suggest that low water content in the soil may slow the migration of invasive larvae from dead insects to the environment and influence nematode density in the soil. It has also been observed that during periodic droughts, the number of nematodes in the soil decreases significantly (Půža and Mráček 2005). However, Kung *et al.* (1991) and Hass *et al.* (2002) notice that in most soil, the presence of nematodes for a long period of time can cause excessive activity, resulting in the reduction of their energy supply. However, invasive larvae have numerous mitochondria and fat pads that temporarily protect them against starvation and other negative abiotic factors (Poinar 1990, Qui and Bedding 2000a, 2000b).

Considering the population of domestic nematodes predominating in particular habitats, it is appropriate to understand the biology and ecology of individual species of entomopathogenic nematodes, including their relations with the environment and specific hosts, for the effective use of these beneficial animals in integrated pest management programs.

CONCLUSIONS

1. The research has shown that nematodes are specific to the environment. The soil type and its physicochemical properties influence the presence of particular species of nematodes in the environment.
2. Entomopathogenic nematodes can live in soils with a variety of pH levels, from very acidic (pH<4.5) to alkaline (pH>7.2). Some species of nematode have specific habitat requirements. For example, the nematode *Steinernema bicornutum* was found only in neutral soils, and *S. silvaticum* only in acidic environments.
3. Loamy and sandy soils with moderate humidity (10–14%) are the best habitats for the life and biological activity of the *Steinernematidae Heterorhabditidae* insectivorous nematodes.
4. *Steinernema feltiae* has the highest ecological flexibility, predisposing it to a wide application in pest control for a variety of agro- and biocoenoses habitats.

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