HABITAT CHARACTERIZATION OF ENTOMOPATHOGENIC NEMATODES IN NORTH LEBANON

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ABSTRACT

Entomopathogenic nematodes are soil organisms, adapted to most climatic conditions in hot, temperate, and cold zones, distributed from lowlands to high alpine altitudes (Steiner, 1996). During a previous survey of entomopathogenic nematodes-EPNs in Lebanon (Noujeim Abi Nader et al., in review), 3 out of 19 sites were estimated positive in EPNs. The reasons for the presence of EPNs in some sites in Lebanon rather than others, are still not well established. Even less is known about the correlation between EPNs distribution in land and soil texture, soil pH, insect hosts, and vegetation cover. In the current study, assessment of habitat preference of EPNs is conducted in a positive site previously sampled for EPNs occurrence. The relationship between EPNs, entomofauna, vegetation cover and soil characteristics is determined using a gridded method and baiting with Galleria mellonella tubes introduced in situ into soil. The method used allows precision sampling with minimal soil disturbance. Results showed a correlation between EPNs and some soil characteristics (humidity, organic matter, texture, porosity) and also communities of invertebrates. No significant linkages were demonstrated between the presence of EPNs and the vegetation nor with the soil pH or any specific entomofauna order.

Keywords: habitat, soils, entomopathogenic nematode, invertebrate fauna, *Steinernema, Heterorhabditis,* herbaceous zones, wooden zones

INTRODUCTION

The ecology of entomopathogenic nematodes from the families *Steinernematidae* and *Heterorhabditidae* was previously studied in attempts to improve their activity as biological control agents. Even though many national surveys gave valuable data on both EPNs

distribution around the world (Glazer *et al.*, 1991; Shamseldean & Abd-Elgawad, 1994; Hazir *et al.,* 2003; Canhilal *et al.,* 2006; Stock *et al.*, 2008; Salame *et al.*, 2010) and their potential use as biological control agents (Parkman *et al.,* 1994; Duncan & McCoy, 1996; Shapiro-Ilan *et al.,* 2002; Koppenhöfer & Fuzy, 2003a), habitat preferences remain inadequately known for entomopathogenic nematodes. This slow progress is not due to lack of interest or importance, but is likely associated with the difficulties of sampling (Brown & Gange, 1990) and studying the specific niche of entomopathogenic nematodes. The low percentage of EPNs recovered in Lebanon (Noujeim Abi Nader *et al*., in review) is comparable to other surveys in the Mediterranean basin such as in Jordan (Stock *et al*., 2008) where 10 of the 1080 soil samples were positive in EPN (0.9%), in Turkey (Hazir *et al*., 2003) where entomopathogenic nematodes have been recovered from 6 out of 7 regions sampled, with 22 positive sites (2%) out of 1080 sites sampled. EPNs incidence in Lebanon is also similar to the surveys conducted in Syria where 3 out of 14 sites sampled were positive, corresponding to five soil samples (2.37%) out of 211 in three sites (Canhilal *et al*., 2006). EPNs were recovered from field crops with 5.08% of the samples positive followed by orchard/vineyard with 2.27%, and forest with 2.04%. Nevertheless, if further surveys show a higher percentage in this region, then a comprehensive understanding of their distribution and the various biotic and abiotic factors influencing their presence is required. The only free-living stage of entomopathogenic nematodes in the soil is the non-feeding infective juvenile or "dauer" larva (IJ). Their intestines host symbiotic bacteria essential for parasitic success. Mobility and persistence of the infective juvenile in the soil may be influenced by numerous factors such as soil moisture, soil texture, vegetation cover, and insect hosts (Molyneux & Bedding, 1984; Kung *et al*., 1991; Koppenhofer *et al*., 1995; Grant & Villani, 2003a; 2003b). Although many of these factors have been explored in laboratory experiments (Barbercheck & Kaya, 1991), there is a lack in comprehensive understanding of how these factors influence the presence of entomopathogenic nematodes under natural conditions. The success of nematode applications as biological control agent and the persistence of EPNs in natural areas depend on the dauer larvae's ability to disperse and persist until it can locate a host. It has been shown that numerous intrinsic factors (*e.g*., behavioral, physiological) and extrinsic factors (*e.g*., temperatures, soil moisture, soil texture, pH, UV radiation) (Kaya, 1990; Smits, 1996) affect their dispersal and persistence. Soil moisture is probably the most important factor affecting nematode performance and survival in the soil (Molyneux & Bedding, 1984; Kung *et al.,* 1991; Koppenhöfer *et al.,* 1995; Grant & Villani, 2003a; 2003b) since EPNs need a water film for effective locomotion (Wallace, 1958). Soil moisture is closely linked with other important factors such as the organic matter and the soil particle size (Koppenhöfer & Fuzy, 2006). Nematode mobility generally decreases as soil pores become smaller (Kaya, 1990). Small soil pores, particularly in combination with higher soil moisture, will also limit oxygen levels and therefore the activity and survival (Kung *et al*., 1990a) of the aerobically respiring (Burman & Pye, 1980) entomopathogenic nematodes. The effects of abiotic and biotic factors on the nematode populations cannot be studied within a large scale in the field, due to technical difficulties. Studying the correlation of these factors with the presence of entomopathogenic nematodes and their spatial distribution therefore require a small sampling area in order to identify the favorable habitat for EPNs. This study addressed correlation of EPNs presence with vegetation formation, soil characteristics, and fauna hosts within positive sites previously sampled for EPNs occurrence by Noujeim Abi Nader *et al*. (in review). This study aims to answer some questions regarding the EPNs occurrence in the Lebanese soils: what are the preferred habitats of EPNs in Lebanon? Does soil texture influence the presence of EPNs in soil? Do vegetation ecosystems affect the presence of EPNs in natural areas? How does hosts' fauna affect their presence in soil?

MATERIALS AND METHODS

Study area and site description

The chosen site for sampling consists of abandoned agriculture terraces located in North Lebanon at an altitude of 1500 m, covering an area of approximately 1000 m^2. Soil humidity within this region is maintained since it is located in the bottom of a valley in proximity with a river and it benefits from 1000 to 1500 mm of precipitation per year in addition to spring thaw. This region is a mixture of herbaceous and wooded zones where two cohabiting EPNs species were previously recovered, *Steinernema feltia* and *Heterorhabditis bacteriophora.*

Preliminary soil study and vegetation cover

A preliminary study for 2 soil profiles with 4 sub-units was first conducted. For these purposes and in order to study the different layers of the soil, a pit of 2x1x1 meters was done. Humidity, porosity, consistence, root abundance, soil texture, organic matters and pH were evaluated for soil layers. The main ecosystems present in the site were described as the herbaceous ecosystem, the woody ecosystem (walnut and oaks zones) and the rocky cover ecosystem.

Spatial distribution of entomopathogenic nematodes according to vegetation zones

Sampling was conducted during the humid season, in March and April, previously demonstrated as the favorable period to identify EPNs within the chosen site (Noujeim Abi Nader *et al*., in review). The site was divided in 4 categories (herbaceous, oak, walnuts, rocky) according to the ecosystems. Within each ecosystem, representative areas were chosen to evaluate the presence of EPNs in the soil by baiting soil samples with larvae of the greater wax moth or honeycomb moth *Galleria mellonella.* Trapping was conducted in each chosen area using a gridded method of 1x1m spread out on a defined surface for each chosen area as shown in Figure 1. For example, if the surface of a sampling area is 6x1m, 6 traps were introduced in the soil. A total of 321 traps were introduced in the soil at a depth of 0-20 cm. Traps consisted of a perforated tube attached to a stick with a color flag and containing 2 *Galleria mellonella* larvae introduced in the soil. An empty duplicate trap was also placed next to each trap permitting the localization of each positive trap for further analysis. Traps were then removed after 3 days from the soil, transported to the laboratory (22-24°C) where the assessment of larva mortality is conducted during 6 days. On the 7^{th} day, one of the cadavers (when the two *Galleria* larvae are dead) is dissected to confirm the infestation by EPNs, the other one is placed on white traps to allow the emergence of the infective-stage juveniles. When only one *Galleria* is dead, the cadaver is used for desiccation.

Identification of entomopathogenic nematode isolates

Nematode isolates were identified by studying the morphometric traits for IJs and first-generation males. Partial sequences of their 28S rRNA genes (28S, D2-D3 domains) and of their internal transcribed spacer regions (ITS) (Stock *et al*., 2001; 2008) were compared to those available in GenBank.

Analysis of biotic and abiotic soil characteristics

Once EPNs were localized onto the screened areas, samples of 0.5 Kg of soils were taken next to the positive traps at a depth of 20 cm, placed in plastic boxes and transported to the laboratory in a cooler to avoid humidity loss. Humidity, pH, bulk density and soil porosity were then analyzed. Samples were also sent to the soil laboratory of the Ministry of Agriculture in Lebanon for soil texture and organic matters analysis.

Correlation between EPNs and invertebrate fauna

Additional soil samples were taken next to positive traps at the surface of the ground for invertebrate fauna extraction using a Berlèse funnel. Forty three soil samples were collected and invertebrate fauna of each sample were placed in tubes containing alcohol before being morphologically identified.

Statistical analysis

Percentage of positive samples according to the vegetation's distribution on site was calculated and correlation between the presence of EPNs and the vegetation type was then evaluated using Chi^2 statistical test. Chi^2 was also used to calculate the correlation between the occurrence of EPNs and soil texture. ANOVA was used to study the correlation between the occurrence of EPNs and the invertebrate fauna, as much as the correlation between EPNs and the soil according to the following parameters: humidity, real density, porosity, pH and organic matter.

RESULTS

Identification of entomopathogenic nematode isolates

Ten out of the 321 traps (3.12%) were positive in EPNs. Based both on the analysis of their 28S+ITS sequences and their morphometric traits, the nematode isolates recovered were identified as *Steinernema feltiae* and *Heterorhabditis bacteriophora.*

Distribution of EPNs according to vegetation formation

Out of the 10 positive traps, 3 were found in the rocky zones, 2 in the walnuts zones, 4 in the oak zones and 1 in the herbaceous (Table 1), and EPNs traps followed a patchy distribution (Figure 1).

Heterorhabditis were found in herbaceous (1 positive trap) and walnut zones (1 positive trap). *Steinernema* were found in rocky zones (3 positive traps), walnut zones (2 positive traps), and oak zones (3 positive traps). The occurrence of EPNs, with the low number of positive traps analyzed, was revealed not to be correlated with vegetation zones as shown in Table 2.

TABLE 1

Number of Representative Areas and Traps per Ecosystem

Ecosystem	Number of chosen sites	Total number of traps per ecosystem	Number of positive traps per ecosystem	% of positive traps per ecosystem
Rocky	8	34	3	8.8
Walnut	5	37	2	5.4
Oak	13	93	4	4.3
Herbaceous	15	157	1	0.64
TOTAL	41	321	10	

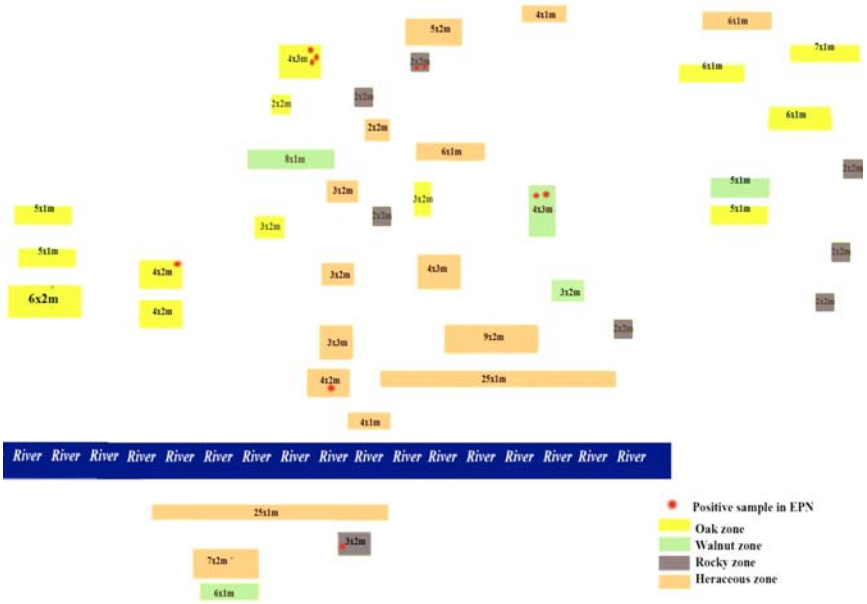

Figure 1. Representation of the site sampled.

Distribution of EPNs according to soil characteristics

ANOVA tests showed dependence of the occurrence of EPNs with the soil's humidity and independence with real density, porosity, pH and organic matter as shown in Table 3. For soil texture, Chi^2 statistical test revealed a dependent relation between the occurrence of EPNs and soil texture as shown in Table 2 with preference for sandy loam (8 of the positive samples) and sandy clay loam (2 of the positive samples) textures.

TABLE 2

χ2 Statistical Test Showing Correlation between EPNs and Vegetation Zones, Soil Texture and Order of Invertebrate Fauna

Correlation between EPNs and	χ2 statistical test				
	χ2	degrees of freedom	critical value	α	results
Vegetation zones *(rocky, walnut, herbaceous, oak)*	2	3	7.82	0.05	χ^2 < critical value of χ^2
Soil texture	17.1	3	7.82	0.05	χ^2 > critical value of χ^2
Order of identified invertebrates	358.67	13	22.36	0.05	χ^2 > critical value of χ^2

Distribution of EPNs according to the occurrence of invertebrate fauna

Morphological identification classified the invertebrate fauna recovery in 14 orders representing 210 invertebrates in the positive samples. ANOVA statistical test showed that EPNs occurred randomly regarding the abundance of the invertebrate fauna (Table 3). Chi^2 was then used to test the relation between EPNs and all the identified invertebrates: This test showed that the occurrence of EPNs depends on the identified invertebrate Orders (Table 2). Therefore, ANOVA statistical test was used to test the relation between the occurrence of EPNs and 3 more abundant orders in this sampling: *Lepidoptera, Coleoptera* and *Hymenoptera*. Statistical tests showed no correlation of EPNs with *Lepidoptera*, *Coleoptera* or *Hymenoptera* (Table 3).

TABLE 3

ANOVA Statistical Test Showing Correlation between EPNs and Some Soil Characteristics, Abundance and Order of Invertebrate Fauna

Correlation between EPNs and			ANOVA statistical test			
			F	F critical value	p-value	results
Soil characteristics	Soil humidity		6.517709	3.963472	0.012632	F> F critical value
	Real density		0.734719	3.963472	0.393984	F< F critical value
	Porosity		0.053662	3.963472	0.817417	F< F critical value
	Ph		0.074833	3.963472	0.785149	F< F critical value
	Organic matter		0.142597	3.963472	0.012632	F< F critical value
	Abundance		1.843883	4.030392	0.180474	F< F critical value
Invertebrate fauna	Order	*Lepidoptera*	4.5	5.987378	0.078141	F< F critical value
		Coleoptera	0.352941	4.543077	0.561307	F< F critical value
		Hymenoptera	0.423764	5.117355	0.531332	F< F critical value

DISCUSSION

The current study highlighted the occurrence of EPNs according to defined biotic and abiotic factors in natural areas, represented here by a positive site in Lebanon previously sampled. Entomopathogenic nematodes are sampled in natural areas worldwide (Garcia Del Pino & Palomo, 1996; Stock *et al*., 2008) and are recovered from a large range of ecosystems such as sandy beaches, meadows, non agricultural fields, organic vines, oak woodlands, and forests (Sturhan & Liskova, 1999; Stock *et al*., 1999; Tarasco & Triggiani, 2005; Stock & Gress, 2006; Emelianoff *et al.*, 2008). In the current study EPNs were found in rocky, walnuts, oak and herbaceous zones of an abandoned agriculture terrace. EPNs presence tend not to be correlated with a specific vegetation habitat, and the limited recovery of EPNs showed a wide range of distribution of these species in the different vegetation ecosystems sampled. Moreover, in other studies, EPNs are not recovered in the same vegetation type, as demonstrated by Emelianoff *et al*. (2008) where no EPNs were recorded in oak forest in the Southern part of France. Habitat heterogeneity and complexity can contribute to population persistence by presenting microhabitats in a mosaic that spatially and temporally separate competitors, predators, and prey (Ettema, 1998). The persistence of EPNs at a particular site is probably due to the presence of suitable hosts (Mráček & Webster, 1993; Peters, 1996; Mráček *et al.,* 1999). That is why Campbell *et al*. (1995) highlighted the presence of EPNs and insects at the same time in the soil. Mráček (1982) established a positive correlation between EPNs and their hosts. Other authors have reported an independent correlation between EPNs density and insects (Campbell *et al.*, 1995; Půža & Mráček, 2005), probably caused by the limitation of host's nutrients. In this study, the occurrence of EPNs tends to be dependent on the presence of a community of insect host and not a specific order; the presence of the community of all the invertebrate orders studied together seems to be important for the presence of entomopathogenic nematodes. In the Czech Republic, dependence of EPNs on insects seems to be elementary for their incidence and abundance (Mráček *et al*., 1999). Later on, Mráček and Sturhan, (2000) found that the presence of *Lepidoptera*, *Hymenoptera* and *Coleoptera* creates an ideal environment for the EPNs, which is in accordance with this study. On the other hand, the coexistence of *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the same site, as shown in this study, is expected since behavioral differences of these species and variability in environmental factors enable strong niche separation and avoidance of competition. Depending on the host seeking strategy, EPNs can be ''cruisers'', ''ambushers'' or ''intermediates'' (Kaya & Gaugler, 1993). *Heterorhabditis bacteriophora* is reported to be a cruiser (Lewis, 2002), and *Steinernema feltiae* is known as a specie with intermediate foraging behavior (Lewis, 2002). Differences in foraging behaviors apparently reduce competition among some EPN species and permit coexistence (Koppenhöfer & Kaya, 1996a; 1996b). Patchy distributions are often an apparent consequence of the distribution of resources and of interactions among species (Stuart *et al*., 2006). Studies addressing these issues have found that EPNs populations are extremely patchy both spatially and temporally, within and among sites (Cabanillas & Raulston, 1994; Stuart & Gaugler, 1994; Spiridonov & Voronov, 1995; Garcia Del Pino & Palomo, 1996; Glazer *et al*., 1996; Koppenhöfer & Kaya, 1996a; Strong *et al*., 1996; Campbell *et al*., 1998; Taylor, 1999).

Soil characteristics represent an additional factor influencing the presence of EPNs in this study. Results showed correlation between the presence of EPNs and soil humidity and texture, even though the analyses were limited to a small number of samples. In fact, moisture plays a key role for mobility and survival of infective larvae and hence their ability to find a host (Mauléon *et al*., 2006). EPNs presence was also dependent in the current study on soil

texture with preference to sandy loam and sandy clay loam textures. As EPNs and soil moisture are statistically dependent, soil texture influence on EPNs is expected since these two factors are closely related, and as the size of composition of soil particles and organic matter strongly influence the availability of water in soil. Moisture may also improve EPNs locomotion and foraging efficacy (Nielsen *et al*., 2008). In California, Canhilal and Carner (2006) recovered some extremophilic and acid tolerating EPNs (pH = 4.3). This indicates that EPNs are adapted to different soil organic content and pH, and explains why no correlation between EPNs, soil organic matter, and pH could be found in the current study.

CONCLUSION

This study of EPNs contributes and extends information concerning these nematodes in relation to their habitat preference in Lebanon. The results of this study extend previous research, by studying EPNs' relation with invertebrate community, as well as their relationship with vegetation type and soil characteristics. Although the data recorded in this area suggest that humidity, soil texture, and invertebrate community influence positively on EPNs, the low number of positive samples in the current study cannot confirm all these hypotheses. Finding other sites with similar conditions would provide more accurate and reliable knowledge on habitat preference and host specificity of EPNs.

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