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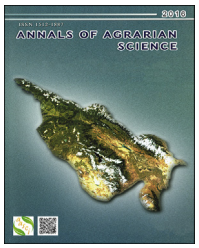

Foraging behavior and virulence of some entomopathogenic nematodes

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ABSTRACT

At present the biological control as a pest control technology is becoming more desirable. Biological formulations on basis of entomopathogenic nematodes are one of the effective means for the protection of agricultural and forest plants from harmful insects. Nowadays, the use of entomopathogenic nematodes as biological control agents is a key component in IPM system. The foraging strategies of entomopathogenic nematodes (EPNs) vary between species. This variation is consistent with use of different foraging strategies between ambush, cruise and intermediate to find their host insects. In order to ambush prey, some species of EPNs nictate, or raise their bodies of the soil surface so they are better poised to attach passing insects, other species adopt a cruising strategy and rarely nictate. Some species adopt an intermediate strategy between ambush and cruise. We compared in laboratory the foraging strategies of the entomopathogenic nematode species: *Steinernema carpocapsae, Heterorhabditis bacteriophora* and the recently described species *Steinernema tbilisiensis* and assessed their virulence against mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). The tests showed that *S. tbilisiensis* adopts both foraging strategies.

Introduction

Insect-pathogenic nematodes of the family *Heterorhabditidae* and *Steinernematidae* have been known for decades as effective biological agents against insect pests. These nematodes can actively locate, infect and kill a wide range of insect species. Only the third-stage infective juvenile (IJs) can survive outside the insect host and move from one insect to another. Insect mortality, due to nematode infection, is caused by a symbiotic bacterium [1]. Heterorhabditid nematodes have a symbiotic association with *Photorabdus* bacteria whereas Steinernematids are associated with *Xenorhabdus* [2]. After gaining access to the host haemocoel, the bacteria multiply, killing the host within 24–48 h, and convert the insect into a suitable environment for development and reproduction of the nematodes' parasitic stages [3].

The foraging strategies of entomo-pathogenic nematodes vary between species, influencing their soil depth distributions and host preferences. Infective juveniles use strategies to find hosts that vary from ambush to cruise foraging [4].

Ambushers – IJs mostly remain in the same spot for a long period of time waiting for the prey to cross the boundary of their strike area. Chemical cues are not important for them. Nematodes belonging to the category of ambushers are also capable to nictate, i.e. to stand on their tails with more than 75% of the body held straight. Nictation is a relatively

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stationary tactic which is applied by infective juveniles [5]. Many *Steinernema* are able to jump by forming a loop with their bodies [6]. Other species adopt a cruising strategy and rarely nictate. Instead, they roam through the soil searching for potential hosts. Cruisers move continuously in the environment in search of hosts hence they may become preys themselves. They largely use long-range chemical cues (carbon dioxide, vibration and other chemical cues) to discover the location of resources [7]. Ambush predators such as *Steinernema carpocapsae* infect more insects on the soil surface, while cruising predators like *Heterorhabditis bacteriophora* infect insects that live deep in the soil.

Based on these data we tested and compared the ability of infective Juveniles of three species of EPNs – *S. carpocapsae, H. bacteriophora* and the autoctone species *Steinernema tbilisiensis* [8] – to find the host insect *Tenebrio molitor* L. on 2-dimensional substrates and in sand columns and determined their virulence against it.

Material and methods

The recently described species of entomopathogenic nematode, *S. tbilisiensis,* was isolated from soil samples of the deciduous forest located in the Tbilisi area. Morphological and morphometric data as well as phylogenetic analysis show that *S. tbilisiensis* belongs to the group *Steinernema affine/intermedium*. *S. tbilisiensis* has been attributed to the group *S. affine/intermedium* on the basis of spicule and gubernaculum structure. The new species differs from other species of the *S. affine/intermedium* group in the following diagnostic characters: the spicule of *S. tbilisiensis* is the smallest; and the gubernaculum of *S. tbilisiensis* is shorter than in other species of the *S. affine/intermedium* group. Infective juveniles of *S. tbilisiensis* are distinguished by having a relatively long body (L = 866 μm), the position of excretory pore (EP = 72 μm), the length of the esophagus (ES = 140 μm), the length of the ABW (25 μm). Infective juveniles of *S. tbilisiensis* have 4 lateral lines like *S. beddingi*, but the number of lines is 6 in *S. affine, Steinernema sichuanense* and *Steinernema intermedium*. Also analysis of rDNA (28S and ITS) gene sequences depict this *Steinernema* species as a distinct and unique entity.

The mealworm beetle *T. molitor* like all holometabolic insects has four life stages: egg, larva, pupa, and adult. The larva of this species has 9 to 20 instars. After the final one it becomes a pupa. Larvae of *T. molitor* were maintained in laboratory condition at the Ilia State University at room temperature (20–22 °C).

This insect is typically fed on cereal bran or flour (wheat, oats, maize) supplemented with fresh fruits and vegetables (carrots, potatoes, lettuce) for moisture together with protein sources such as soybean flour, skimmed milk powder or yeast. Also larvae of *T. molitor* are able to utilize the small amounts of water contained in dry feeds but the productivity of water-deprived is low (one generation per year). It is preferable to provide them with a source of water for better productivity (up to 6 generations per year) and to prevent cannibalism. Relative humidity is linked positively with fertility and adult activity. It is necessary to monitor fresh feeds as they may turn mouldy [9,10].

For cultivation of *T. molitor* at all stages of their development, larvae were placed in the vessels (60 × 40 cm) with a wide bottom. Dishes were filled with bran and wheat flour mix and with fresh carrot/apple pieces offered at least three times a week. All dishes with larvae were maintained in an environmental chamber at 27 C, 75% RH. Pupae and larvae with sizes from 1.5 to 3.2 cm were used for the experiments.

Nematodes were reared at 25 °C in last instar larvae of the wax moth, *Galleria mellonella,* according to procedures described by Woodring and Kaya [11]. The IJs that emerged from cadavers were recovered using modified White traps and stored at 7 °C for 7–14 days before use [12].

Host location and parasitism. Ambushing nematodes would be more effective inseeking hosts on 2-dimensional substrates allowing nictation, whereas cruising species are more effective in a sand column [13].

We compared the proportion of infective juveniles that located and established in a host on a filter paper, sand surface and at the bottom of sand columns. For the filter paper assay dewyfilter paper discs were placed on 10 cm diameter Petri dish whereas for sand substrate 8 g sand with 15% moisture was equally distributed on the same size Petri dish. On each substrate 10 host insects' pupae and larvae were individually placed.

For cruiser nematodes sand columns were prepared by placing three *T. molitor* pupae and three larvae individually at the bottom of a 2-cm diameter and 18-cm high sand columns which were then filled up to 15 cm with sand.

In all situations insects in the stage of pupa and larva were individually exposed to only one nematode species. The dose of nematode suspension for the 10 insects placed on 2-dimensional nictation substrate made 1000 IJ/ml water, while for the three insects placed in sand columns –300 IJ/ml water (i.e. 100 IJ per insect).

Polyethylene was placed on Petri dishes and glass vials in order to protect them from drying out and two-winged insects. Then they were placed in the incubator at 25 °C for 24 h.

Insects invaded by nematodes were collected after 24 h. Before identifying the number of nematodes invading insects, they were incubated for 48 h at 25 °C. The average number of nematodes was determined on the basis of the host insect. The behavior of all three species of nematodes was also observed on two-dimensional substrate for 20, 30 and 50 min after the start of the test.

Insect's mortality was recorded, and the number of nematodes established in each insect was determined by dissection. Presence of nematodes inside the insects was checked as indicator of nematode infection.

Control variants were identical to the treatments except that no IJs were added. Each treatment was replicated five times and included untreated control dishes. All experiments were carried out under laboratory conditions at temperature 23 °C and 80% RH. Much of this work has been focused on nematode species behavioral interactions with hosts.

Results and analysis

H. bacteriophora and *S. tbilisiensis* caused 100% insects mortality on both surface and at the bottom of sand column after 72 h