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The role of the host's biomass in the host's localization and recognition by enthomopathogenic nematodes (*Rhabditida: Steinernematidae* and *Heterorhabditidae*)

Rola biomasy żywiciela w jego lokalizacji i rozpoznawaniu przez nicienie entomofilne (*Rhabditida: Steinernematidae* i *Heterorhabditidae)*

SUMMARY

The purpose of the research was to study the mechanisms of localization, recognition and food preferences of enthomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the conditions of the presence of three species of possible hosts with the same biomass, which was composed of different numbers of particular insect species: two larvae of each *Galleria mellonella* and *Tenebrio molitor* and 140 larvae of *Tribolium confusum.* The results showed that the invasive larvae of *S. feltiae* migrated in the greatest numbers and aggregated in the vicinity of *G. mellonella*, while smaller numbers were observed near *T. confusum,* and the smallest near *T. molitor.* The highest intensity of infection was exposed by *G. mellonella,* it was lower for *T. molitor,* and the lowest for *T. confusum.* The invasive larvae *H. bacteriophora* aggregated in the greatest numbers in the vicinity of *T. confusum,* their numbers were smaller near *G. mellonella* and the smallest near *T. molitor.* The greatest infection by *H. bacteriophora* was observed in the case of *G. mellonella*, and the smallest towards *T. molitor.* The results of experiments show that the invasive larvae of enthomopathogenic nematodes of various species are sensitive to a little different other kinds of kaironomes. For *H. bacteriophora,* attractants connected with the number of possible hosts in the environment are important, while for *S. feltiae* the attractants exuded by a given insect species are of greater significance and their importance is not associated with the number of individuals of potential hosts.

STRESZCZENIE

Celem przeprowadzonych badań było zbadanie mechanizmów lokalizacji, rozpoznawania oraz preferencji pokarmowych nicieni entomofilnych *Steinernema feltiae* i *Heterorhabditis bacteriophora*, w warunkach jednoczesnej obecności w glebie trzech gatunków potencjalnych żywicieli

o jednakowej biomasie, na którą składała się różna liczba osobników poszczególnych gatunków owadów: po dwie larwy *Galleria mellonella* i *Tenebrio molitor* oraz 140 larw *Tribolium confusum*. Wyniki doświadczeń wykazały, że larwy inwazyjne *S. feltiae* najliczniej migrowały i kumulowały się w pobliżu *G. mellonella*, mniej w okolicy *T. confusum*, a najmniej w pobliżu *T. molitor*. Z najwyższą intensywnością porażały *G. mellonella,* mniejszą *T. molitor*, a najmniejszą *T. confusum.* Larwy inwazyjne *H. bacteriophora* najliczniej kumulowały się w pobliżu *T. confusum*, mniej w rejonie *G. mellonella*, a najmniej w pobliżu *T. molitor*. Najwyższe porażnie przez *H. bacteriophora* było notowane w przypadku *G. mellonella*, a najniższe w stosunku do *T. molitor*. Wyniki przeprowadzonych doświadczeń wykazują, że larwy inwazyjne nicieni entomofilnych różnych gatunków są wrażliwe na nieco inne rodzaje kairomonów. Dla *H. bacteriophora* istotne są atraktanty związane z ilością potencjalnych żywicieli w środowisku, a dla *S. feltiae* specyficzne atraktanty wydzielane przez dany gatunek owada, których znaczenie nie jest związane z liczbą osobników potencjalnych żywicieli.

K e y w o r d s: enthomopathogenic nematodes, *Galleria mellonella, Tenebrio molitor, Tribolium confusum,* host finding and recognition.

INTRODUCTION

Enthomopathogenic nematodes are a very interesting group of organisms due to their ability to form mutual relations with bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. (1). Bacteria take part in killing the arthropods, mainly insects attacked by those nematodes, and next — multiplying in the dead body of the insect, they make the growth of nematodes possible. Bacteria are carried to the insect by the invasive larvae of nematodes, the only stage of free living nematodes. Those larvae have an ability of active host finding (10, 16) and host recognition in the environment, its localization and — when a few insect species are available — choosing the more attractive host (23, 24).

Localization, an active movement of enthomopathogenic nematodes towards a potential host and finding it is possible thanks to the sensitivity of the invasive larvae of nematodes to kaironomes emitted to the environment by the hosts (21). Their recognition is made easier for nematodes by amphidia, which fulfil the role of chemoreceptors, and — to a lesser degree — by the papilla on the heads of invasive larvae. Some researchers are of the opinion that the role of kaironomes is played for example by carbon dioxide. Nematodes react in a positive way to its presence in the environment, the effect of which is their migration and accumulation in the area of its sources such as tracheae of insects, plant roots and other soil organisms (7, 12, 14, 15, 19, 41). The hosts' excrements consisting of such compounds as uric acid, xanthine, ammonia and organic acids make it easier for nematode invasive larvae to find the host and have a stimulating effect on nematodes (2, 37–40). An insect cuticle also brings about chemotaxis of nematodes (27, 37). Invasive larvae show an ability to move towards and gather near some cations and anions, while avoiding others. For example, *S. carpocapsae* gathers in places with higher concentration of sodium, magnesium, calcium, chloride and carbonate ions. On the other hand, ammonia repels nematodes. Also, too low soil pH has a repellent effect on nematodes (36). Invasive larvae of nematodes also gather in the vicinity of gram-negative bacteria symbiotic for them (20). The gradient of temperature can be an indicator that makes host finding easier, especially when the host stays in a close contact with nematodes (8, 9).

Localization and migration of enthomopathogenic nematodes towards the potential host frequently takes place under an influence of nonspecific stimuli. That is why the ability of nematodes to recognize the host, which can occur as a result of contact between the invasive larvae

and excrements, cuticula, the content of the fauces (17, 18) and other materials characteristic of the host, is a very important issue.

The purpose of the present paper is to broaden the knowledge on the mechanisms of host finding and recognition by invasive larvae of enthomopathogenic nematodes, and especially to find out the importance of insect biomass in the process of host recognition by enthomopathogenic nematodes.

MATERIALS AND METHODS

The experiment used the invasive larvae of enthomopathogenic nematodes *Steinernema feltiae* Filipjev 1934 and *Heterorhabditis bacteriophora* Poinar 1976. Both species of nematodes are Polish isolates obtained by means of Galleria trap (3). *S. feltiae* was isolated from the soil taken from the Białowieski National Park, while *H. bacteriophora* was isolated from the soil taken from a tree belt on the Bystrzyca valley in Lublin.

Before the invasive larvae of nematodes were used in the experiment, they were stored from one to three weeks at the temperature of 6–7°C in a water solution of 0.001% formaldehyde, and the cultivation was aerated at 1-week intervals. Before beginning the experiment, the vitality of the invasive larvae of nematodes was checked under a microscope.

The experiment used the larvae of the last developmental stage of *Galleria mellonella* L. (Lepidoptera), *Tenebrio molitor* L. (Coleoptera) and *Tribolium confusum* Duv. (Coleoptera) from a permanent laboratory cultivation. All the insect larvae used in the experiments were weighed and selected according to earlier established weight criteria. The mean larvae biomass of *T. confusum* ranged from 2.7 to 3.1 mg, of *T. molitor* from 170 to 190 mg, while of *Galleria mellonella* from 180 to 200 mg. The experiments used the same biomass of insect larvae, which is about 400 mg for each insect species in each repetition. The biomass of 400 mg consisted of a varying number of insect larvae of particular species. In the case of *G. mellonella* two caterpillars made the weight of 400 mg, for *T. molitor* — two larvae, and for *T. confusum* — about 140 insect larvae.

The experiment was performed in the conditions of simultaneous occurrence of larvae of three insect species in the soil. The dose of nematodes was 2,000 per one crystallizer. The experiment was conducted in three time variants differing with the period of contact between nematodes and insects, which was 24, 48 and 72 hours. Each experiment was carried out in three repetitions. Infection was performed in glass crystallizers, with the diameter of 23 cm and the height of 7 cm, filled with a layer of sterilized earth.

The experiments were conducted in the samples of soil, which was light loam low sandy (42). Every time before the experiment the earth was roasted twice at 24-hours' intervals, at the temperature of 200°, for 12 hours. Next, it was moistened with distilled water.

The larvae of the last stage of development were placed in cages made of copper net with the dimensions of $1 \times 1 \times 3$ cm, filled with earth. One cage contained the larvae of only one insect species. Next, the cages with insects were uniformly placed in the soil on the circuit of crystallizers, at the depth of 2 cm. Crystallizers were placed in a climatic chamber at the temperature of 23°C and the air relative humidity of 99.8% RH. After 24 hours the invasive larvae of nematodes were introduced in the central part of the crystallizers in a dose of 2,000 nematode larvae per one crystallizer.

Next, after 24, 48 and 72 hours, respectively for particular "time variants", the insect larvae were taken out of the crystallizers, rinsed with distilled water and placed on petri dishes lined with pads of filter paper soaked with 0.001% water solution of formaldehyde. Alive and dead larvae of insects were placed on separate dishes. Next, petri dishes were transferred to thermostats, where

they stayed at the temperature of 23°C. Four days after the infection in the case of *S. feltiae,* and five days after the infection in the case of *H. bacteriophora,* the dead insect larvae were submitted to dissection in order to determine the numbers of 1st generation of nematode population.

After the insect larvae were removed from the crystallizer, dispersion of the other invasive larvae of nematodes was determined by means of trap method by Bedding and Akhurst (3). The earth from the area in the vicinity of the insects and from the regions where insects did not occur as well as from the regions of nematodes' introduction was taken to Petri dishes. Next, five larvae of *G. mellonella* were placed on each dish. The infected insect larvae were sorted out in order to determine the numbers of the first generation of nematodes. The directions of migration of the invasive nematode larvae in the soil in the presence of three hosts were established on the basis of the results of all dissections of the infected insect larvae.

The statistical analysis of the results of dispersion of the invasive larvae of nematodes was carried out by means of Pearson's χ^2 test, a method of hierarchical-logarithmic-linear analysis. A comparison of host infection by *S. feltiae* and *H. bacteriophora* was statistically presented by means of t-Student test. The calculations were performed using program SPSS/P$^+$ 4.0 at the Computer Centre of the Catholic University of Lublin.

RESULTS

Results of the experiments point out that the larvae of enthomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* recognize the presence of three different hosts in the environment, they localize them and migrate towards the preferred host. However, a difference is seen in the directions of migrations of both nematode species.

After 24 hours of exposition of insects to nematodes, the invasive larvae *S. feltiae* migrated in the greatest numbers towards *T. confusum* (308 invasive larvae, which is 38.6% of the recovered invasive larvae *S. feltiae*) and *G. mellonella* (301 invasive larvae, which is 37.8%), while five times less to the vicinity of *T. molitor* (58 invasive larvae, which is 7.3%). The longer the contact between nematodes and insect was, the more nematodes moved in the direction of caterpillars *G. mellonella,* and after 72 hours' contact 723 nematodes were recovered in the vicinity of *G. mellonella* (which constituted 50.0% of the recovered nematodes). 350 nematodes were recovered in the vicinity of *T. confusum* (21.4%), and 309 in the vicinity of *T. molitor* (21.4%) (Fig.1). Differences in the number of invasive larvae accumulated in the vicinity of particular species are statistically significant ($\chi^2 = 428.955$, DF = 2, level of significance 0.000).

On the other hand, in all the time variants the invasive larvae of *H. bacteriophora* most often migrated in the direction of *T. confusum*, where — after a 24-hours' contact between nematodes and insects — the studies found out 26.7% *T. confusum,* after 48 hours there were 64.1%, while after 72 hours there were 86.5%. A significantly lower number of *H. bacteriophora* moved towards *G. mellonella* (10.5% of the recovered nematodes after 74 hours), and a slight

Fig. 1. Percentage of recovered invasive larvae *Steinernema feltiae* in particular fields in the conditions of simultaneous presence of three hosts in the environment

number of invasive larvae *H. bacteriophora* moved to the vicinity of *T. molitor* (Fig. 2), ($\chi^2 = 925.472$, DF = 2, level of significance 0.000).

When the time of contact of the host with the parasite grew, the migration of invasive larvae of both species of nematodes got more intensive. The number of nematodes leaving the introduction region and migrating towards the potential host grew (*H. bacteriophora*: $\chi^2 = 617.448$, DF = 2, level of significance 0.000, *S. feltiae:* $\chi^2 = 227.198$, DF = 2, level of significance 0.000).

Results of dissection of the infected insect larvae show that invasive larvae *S. feltiae* infect the host present in the environment more effectively that *H. bacteriophora. S. feltiae* infected more insect larvae with greater intensity, and the differences in the extensiveness and intensity of insect infection by *S. feltiae* and *H. bacteriophora* were statistically significant (Tab. 1).

When three insect species were present in the soil, both *S. feltiae* and *H. bacteriophora* infected *G. mellonella* caterpillars in the most effective manner. Poorer infection by *S. feltiae* was observed towards *T. molitor*, and almost none towards *T. confusum.* On the other hand, *H. bacteriophora* infected *T. confusum* better than *T. molitor.*

The invasive larvae of *S. feltiae* infected all the three insect species with higher mean extensiveness and intensity than *H. bacteriophora,* and those differences were in most cases statistically significant (Tab. 1).

In both species of entomopathogenic nematodes the effect of the time of contact between the hosts and the invasive larvae was seen on the intensity and extensiveness of infection. When the period of contact was longer, the number

Fig. 2. Percentage of recovered invasive larvae *Heterorhabditis bacteriophora* in particular fields in the conditions of simultaneous presence of three hosts in the environment

of the infected insects and the number of penetrating invasive larvae *S. feltiae* and *H. bacteriophora* increased. However, the period of the host exposition to nematodes was more significant in infection by *H. bacteriophora* since the extensiveness and intensity of infection increased gradually with the time of contact with the host, reaching the highest level in the longest, 72-hours' time variant. On the other hand, the intensity and extensiveness of *S. feltiae* increased significantly when the time of contact grew from 24 to 48 hours, while in the 72-hours' variant the further growth was insignificant. In all the time variants the ability of insect infection by *S. feltiae* was higher than by *H. bacteriophora*, which is proved by the differences in the extensiveness and intensity of insect infection by both species of nematodes (Tab. 2).

DISCUSSION

Results of experiments show that invasive larvae of enthomopathogenic nematodes of different species are susceptible to slightly different kinds of kaironomes. Attractants connected with the number of potential hosts in the environment are significant for *H. bacteriophora.* On the other hand, the invasive larvae of *S. feltiae* are susceptible to specific attractants exuded by a given insect

Tab. 1. A comparison of mean extensiveness and intensity of insect infection by *Steinernema feltiae* and *Heterorhabditis bacteriophora* taking into consideration the species of the infected insect

		Galleria mellonella	*Tribolium confusum*	*Tenebrio molitor*
Infection extensiveness	*H. bacteriophora*	33.00%	11.59%	11.11%
	S. feltiae	100.00%	35.49%	88.89%
	t	–2.92	–3.97	–5.84
	DF	9.99	10.95	13.88
	Level of significance	0.0036	0.002	0
Intensity of infection	*H. bacteriophora*	10.33	2.27	1
	S. feltiae	93.11	2.17	45.69
	t	–5.5	0.15	–2.095
	DF	9.99	6.89	9.9
	Level of significance	0	0.882	0.0361

t — student value; DF — degrees of freedom; statistically significant at the level of significance <0.05.

Tab. 2. A comparison of mean extensiveness and intensity of insect infection by *Steinernema feltiae* and *Heterorhabditis bacteriophora* taking into consideration the time of contact between nematodes and insects

		24 h	48 h	72 h
Infection extensiveness	*H. bacteriophora*	0.00%	19.81%	35.75%
	S. feltiae	65.21%	78.91%	80.18%
	t	–4.61	–3.76	–2.01
	DF	8.01	15.89	14.55
	Level of significance	0.002	0.002	0.02
Intensity of infection	*H. bacteriophora*	1	1.725	7.8
	S. feltiae	22.93	57.69	57.82
	t	–	–3.04	–2.82
	DF	8	8	8.88
	Level of significance	0.018	0.016	0.02

t — student value; DF — degrees of freedom; statistically significant at the level of significance <0.05.

species. The importance of those attractants is not related to the number of individuals of potential hosts. When three species of insects of the same biomass (consisting of different numbers of individuals: two larvae of *G. mellonella,* two of *T. molitor* and about 140 larvae of *T. confusum*) are available in the environment, the greatest number of the invasive larvae of *S. feltiae* migrated and gathered in the vicinity of *G. mellonella*, a smaller number near *T. confusum*, and the smallest near *T. molitor.* In the same experimental conditions, the greatest number of invasive larvae of *H. bacteriophora* accumulated in the vicinity of

T. confusum, a smaller number near *G. mellonella* and the smallest in the region of *T. molitor.* Results of dissection of the infected insects show that *S. feltiae* infects *G. mellonella* with the greatest intensity, *T. molitor* with smaller intensity, and *T. confusum* with the smallest. Infection of particular insect species by *H. bacteriophora* was the greatest in the case of *G. mellonella* caterpillars, smaller for *T. confusum* larvae, and the smallest for *T. molitor.* It is of special interest to compare the results with the experiments carried out by Kreft A. and Skrzypek H. (24, 25), where three individuals from each insect species were placed in the soil, which means that the number of insects of each species was the same but their biomass was different. Then, *S. feltiae* and *H. bacteriophora* larvae migrated in the greatest numbers towards *G. mellonella*. Their numbers were smaller when migrating in the direction of *T. molitor* and the smallest when migrating towards *T. confusum.* When three species of potential hosts were available in the same numbers of individuals (23), both nematode species infected *G. mellonella* with the highest intensity, *T. molitor* with lower intensity and *T. confusum* with the lowest. When three insect species are available in the environment in an equal number of individuals, then the ability of infection of particular insect species by *S. feltiae* and *H. bacteriophora* is consistent with the directions of migration of nematode invasive larvae, i. e. with the initial choice of the potential host (24). A comparison of the results of the presented studies shows that *S. feltiae* invasive larvae are susceptible to specific attractants of an insect, which is more susceptible to infection, even when another species occurring in the environment is more numerous.

Susceptibility of eight species of insects belonging to butterflies and cockchafers to infection by *S. carpocapsae* and *H. bacteriophora* was also studied by Pezowicz (32, 33). After infections in the soil in one-species schemes, the author stated that in the quantity variant (the same number of individuals of the host) butterflies were more susceptible to infection by *H. bacteriophora* than cockchafers. On the other hand, in the weight variant (the same biomass of the hosts), *T. confusum* and *Sitophilus granarius* were infected with higher intensity than *Pieris brassicae* and *Barathra brassicae.* In both experimental variants *G. mellonella* was more attractive for nematodes than *T. confusum* (32, 33).

An analysis of migration of nematode invasive larvae when different species of hosts with the same biomass are available shows that the density of nematodes in the areas of particular insect species does not directly affect the intensity of the host infection. For example, *S. feltiae* gathered in the vicinity of *T. confusum* after 24 hours, but it infected *T. molitor* with higher intensity. *H. bacteriophora* gathered in the greatest numbers in all the time variants in the vicinity of *T. confusum* but it infected *T. confusum* with greater intensity only after a 24--hours' contact with the hosts. After 48 and 72 hours of contact between nematode

and the host, the highest infection by *H. bacteriophora* was found out for *G. mellonella.* In all the time variants more *H. bacteriophora* penetrated into *T. confusum* than to *T. molitor.*

The early studies point out that infection of particular insects by enthomopathogenic nematodes is influenced by the host's individual resistance (5), the size of insects and their natural openings (30) or the host's biomass (43). The present studies show a slight difference in the intensity of infection of *T. confusum* by both species of nematodes. The intensity of infection of those small cockchafers with small natural openings by *S. feltiae*, whose invasive larvae are bigger than *H. bacteriophora*, was lower. On the other hand, the biomass of the hosts of different insect species does not have a crucial importance in the intensity of infection of particular individuals. *H. bacteriophora* infected *T. confusum* with greater intensity than *T. molitor.*

The studies conducted so far point out differentiated susceptibility of particular insect species to infection by enthomopathogenic nematodes, butterflies being more susceptible, cockchafers less, while the Diptera, homopterons and orthopterans the least (4, 13, 25, 26, 28, 29). Larvae of ladybirds, earth-worms and shell snails are resistant to infection by *Steinernematidae* and *Heterorhabditidae* (11, 35). The presence of another species of a potential host can affect infection by enthomopathogenic nematodes of a given insect species, which is a consequence of different degrees of attractiveness of particular cockchafer species for nematode invasive larvae (22).

The experiments discussed in the present paper show that when the contact between nematodes and insects is longer, the hosts' mortality increases, which is confirmed by the results obtained by Pezowicz (31), who infected *A. grisselia* caterpillars with the same dose of nematode invasive larvae and found out that the final extensiveness of infection was related to the period of contact between the parasite and the insect.

S. feltiae — thanks to its susceptibility to attractants — finds a host more effectively in all the experimental variants. Already in the shortest time variant, after 24 hours of contact, more *S. feltiae* gathered near each insect species than in the areas without any host. Also, *S. feltiae* infects a greater number of hosts with higher intensity during a shorter period of contact than *H. bacteriophora.* Similar relations were observed by Pezowicz and Sandner (34), who infected *Barathra brassicae* caterpillars with *S. feltiae* and *H. bacteriophora* by means of a contact method on petri dishes and by means of a spray method. In infection by a contact method *S. feltiae* caused higher mortality of *B. brassicae* after 24 hours than *H. bacteriophora.* On the other hand, in infection by spraying dead insects which showed the signs of invasion of *S. feltiae* were observed already after 12 hours, while in the case of *H. bacteriophora* the time was 24 hours,

and the mortality of *B. brassicae* caused by *H. bacteriophora* invasion was twice as low as in infection by *S. feltiae. S. feltiae* also infected insects with higher intensity than *H. bacteriophora.* When the period of contact between nematodes and caterpillars got longer, the difference between *B. brassicae* mortality due to infection by *S. feltiae* and *H. bacteriophora* got smaller, and after 96 hours the insects' mortality due to invasion by *H. bacteriophora* was higher than for those which were infected by *S. feltiae.*

So far, *S. feltiae* (=*S. bibionis*) has been considered to be the dominating species among enthomopathogenic nematodes in Poland. Its presence was observed in 75.5% examined sites of agrocenoses, while *H. bacteriophora* was found only in 5% (6). However, such a small number of *H. bacteriophora* sites can result from simultaneous presence of both species of nematodes in the soil.

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