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Insect infection by entomogenous nematodes (*Rhabditida: Steinernematidae* and *Heterorhabditidae*) in the conditions of competition

Porażenie owadów przez nicienie entomofilne (*Rhabditida: Steinernematidae* i *Heterorhabditidae*) w warunkach konkurencji

SUMMARY

The purpose of the studies was to analyse the way in which a competitor's presence in the environment affects host infection by *Steinernema feltiae* and *Heterorhabditis bacteriophora.* The experiment was performed in the conditions of simultaneous occurrence of three insect species in the soil (*Galeria mellonella, Tenebrio molitor, Tribolium confusum*). Insects were infected by one species or two nematode species at the same time (competitive conditions). The results point out that in no-competitor conditions both nematode species infect *G. mellonella* larvae with the greatest intensity, and *T. confusum* larvae with the lowest. In the presence of *H. bacteriophora* nematodes in the soil, *S. feltiae* infects insects like in the situation when there is no competitor in the environment. On the other hand, in competitive conditions, *H. bacteriophora* infects less attractive hosts with less intensity than *S. feltiae.*

STRESZCZENIE

Celem przeprowadzonych badań było przeanalizowanie, w jaki sposób obecność konkurenta w środowisku wpływa na porażanie żywicieli przez *Steinernema feltiae* i *Heterorhabditis bacteriophora*. Doświadczenia przeprowadzono w warunkach jednoczesnego występowania trzech gatunków owadów w glebie (*Galeria mellonella, Tenebrio molitor, Tribolium confusum*). Owady porażano jednym gatunkiem nicienia lub dwoma równocześnie (warunki konkurencji). Wyniki doświadczeń wykazują, że w warunkach braku konkurenta oba gatunki nicieni porażają najsilniej

G. mellonella, a najsłabiej *T. confusum.* W obecności larw inwazyjnych *H. bacteriophora* w glebie, *S. feltiae* poraża owady podobnie jak przy braku konkurenta w środowisku. Natomiast *H. bacteriophora* w warunkach konkurencji poraża mniej atrakcyjnych żywicieli, z mniejszą intensywnością niż *S. feltiae*.

K e y w o r d s: *Steinernematidae, Heterorhabditidae,* infection, competition.

INTRODUCTION

Entomogenous nematodes *Steinernematidae* and *Heterorhabditidae* infect a large spectrum of insects, and in laboratory conditions, where there are no ecological or behavioural barriers for infection and the contact with a possible host is made easier, entomogenous nematodes infect much more hosts than in natural conditions (15). Poinar (30) states that *S. carpocapsae* infect 250 species belonging to 75 families. W e i s e r and M r a č e k (36) pointed to 13 insect orders susceptible to infection by *Steinernematidae* and 5 susceptible to infection by *Heterorhabditidae*. It was found out that infection by entomogenous nematodes also refers to harmful isopodans, millipedes, wireworms and slugs (12, 31, 32). Butterfly caterpillars are most sensitive to infection by entomogenous nematodes, while the dipterous, homopterons and orthopterans — the least (7, 25, 26). On the other hand, larvae of ladybirds, golden-eyed flies, earth worms and shell snails are insensitive to nematodes (5, 30).

In natural surroundings, entomogenous nematodes develop specialisation towards the infected hosts. To give an example, *S. feltiae* in New Zealand infects butterflies *Noctuidae* and *Hepialidae*, which are plant pests, but they do not infect larvae of cockchafers living in the same environment. The same species of nematode, but of Danish variety, adjusted itself to infecting the dipterous of genus *Bibionidae* (4, 38).

Insects' sensitivity to infection by entomopathogenic nematodes is related not only to the insect species but also to the developmental stage and the age of an individual within a given stage. The final larva stage of *Galleria mellonella, Spodoptera exigua* and *Pseudaletia unipuncta* was characterised by greater sensitivity to infection by *S. carpocapsae* than the pupal stage. The pupa of *G. mellonella* was most susceptible of the pupae of these three insect species (16). Likewise, *Hylobius abietis* larvae were less resistant to infection than pupae and adults (33). Pupae are less attractive hosts for nematodes for example because of mechanic barriers making invasion by invasive larvae more difficult. Pupae infection by nematodes takes place through 6 pairs of uncovered places devoid of cuticle and situated on abdomen segments of insect pupae belonging to *Lepidoptera* (16). Differences in pupal sclerotisation of particular insect species affect the differences in infection by entomopathogenic nematodes. *G. mellonella* pupa, which has the smallest degree of sclerotisation, is most susceptible to nematode invasion (11, 16). Laboratory studies found out that the sensitivity of some insect species increases with the age of larvae (9). The first, second and third larva stages of *Simulium vittatum* were not infected by entomogenous nematodes, while the seventh was highly sensitive. Likewise, the first and second stages of *Culex pipiens* are insensitive to nematode invasion. The resistance of early mosquito larvae to infection by entomogenous nematodes is caused by physical barriers, which make it impossible for too large invasive larvae of the parasite to enter the insect's hemocell (9). The degree of attractiveness of a given host for a given nematode species can also be related to whether it is already infected by other nematode species or not (10).

Invasive larvae of entomogenous nematodes show an ability to recognise the hosts in the environment and to pick up the most attractive one in the conditions of a choice of a few possible hosts (8, 22, 24, 34).

Koppenhofer et al. (20), Choo et al. (6), Koppenhhofer and Kaya (18, 19) studied the activity of entomogenous nematodes in the conditions of the occurrence of invasive larvae of another species of entomogenous nematodes, which differed with the strategy of host seeking. The results suggest that the presence of invasive larvae of entomogenous nematodes with different strategies of host seeking in the environment does not increase their effectiveness. The use of two nematode species differing with the strategies of host seeking can be effective in controlling two different insect species occurring in two different parts of the soil profile (14). The activity of particular species of entomogenous nematodes varies in relation to the depth on which invasive larvae occur in the soil (17).

Studying inter-species interactions of entomogenous nematodes is also the subject of the present paper. The experiments aimed at finding out in what way a competitor's presence in the environment affects the infection of possible hosts by invasive larvae *S. feltiae* and *H. bacteriophora,* that is the nematodes actively seeking the host by means of a cruise strategy.

MATERIAL AND METHODS

The experiments used the invasive larvae of entomogenous nematodes *Steinernema feltiae* Filipjev 1934 (*Nematoda: Steinernematidae*) (strain PLSf81, isolated from forest soil in Białowieża, 1981), and *Heterorhabditis bacteriophora* Poinar 1976 (*Nematoda: Heterorhabditidae*) (strain PLHb81, isolated from soil under grass, weeds and trees near the Bystrzyca river in Lublin, 1981), from a continuous laboratory cultivation. Since the moment of isolation the nematodes have remained in continuous cultivation in laboratory of Zoology and Ecology Department.

Before being used in experiments, the invasive larvae of nematodes were kept from one to three weeks at the temperature of 6–7°C, in water solution of 0.001% formaldehyde, the cultivation being aired at one-week's intervals. Before the experiment was set, the vitality of invasive nematodes was checked under a microscope.

In the experiment there were used laboratory cultivation larvae of the final developmental stage of the following insect species: *Galleria mellonella* L. (*Lepidoptera: Pyralidae*), *Tenebrio molitor* L. (*Coleoptera: Tenebrionidae*) and *Tribolium confusum* Duv. (*Coleoptera: Tenebrionidae*).

All the insect larvae used in the experiments were weighed and selected according to the formerly established weight criteria. The mean biomass of larvae *T. confusum* ranged from 2.7 to 3.1 mg, *T. molitor* from 170 to 190 mg, while those of *Galleria mellonella* from 180 to 200 mg.

All the experiments were conducted in the conditions of simultaneous occurrence of larvae of three insect species. Individual and cross infections were performed. In particular repetitions of individual variants a water suspension of only one nematode species, namely *S. feltiae* or *H. bacteriophora,* was introduced to the soil, while in cross variants both species of entomogenous nematodes were introduced to the soil at the same time. The experiments were carried out in three time variants differing with the period of contact between nematodes and insects, which was 24, 48 and 72 hours. The number of nematodes was 100 invasive larvae per one insect larva, and the number was made up of 50 invasive larvae of *S. feltiae* and 50 invasive larvae of *H. bacteriophora.*

Six repetitions of experiments were performed. Tests were performed in glass crystallizers, with the diameter of 23 cm and height of 7 cm, filled with a 4.5 cm-deep layer of roasted earth, light silty loam sandy (35). Each time before the experiment the earth was roasted twice in 24 hours' intervals at the temperature of 200°C, for 12 hours, and then it was moistened with distilled water.

Larvae of the final developmental stage of three insect species were placed in copper net cages with the dimensions of $1 \times 1 \times 3$ cm, formerly filled with earth. One cage contained the larvae of

only one insect species. Next, the cages were evenly placed on the circuit of the crystallizers, in the soil at the depth of 2 cm.

Crystallizers were placed in a climatic chamber at the temperature of 23°C at relative air humidity of 99.8%. After 24 hours the invasive nematode larvae were induced in the central part of the crystallizers.

The insect larvae were removed from the crystallizers after 24, 48 and 72 hours. In the case of *S. feltiae* dead insects were selected 4 days after their contact with nematodes, while in the case of *H. bacteriophora* after 6 days. The purpose was to determine the numbers of generation I of nematode population.

The experiment analyzed the following:

— infection extensiveness (percent of infected insect larvae);

— infection intensity (quotient of the sum of nematodes of generation I and the sum of dead insects).

The statistical analysis of the results of insect infection by nematodes *S. feltiae* and *H. bacteriophora* was carried out by means of variance two-factor analysis of Annova Calculations using the program SPSS/PC^{+}4.0 at the Computer Centre of the Catholic University of Lublin.

RESULTS

In the conditions of a lack of a competitive nematode species in the environment, the highest death rate of insects resulting from infection by *S. feltiae* was found among the larvae of *G. mellonella.* After 24 hours of contact between nematodes and insects, 97% caterpillars died with signs of infection by *S. feltiae*, and when the contact time was increased to 48 hours the extensiveness of caterpillar infection by nematodes increased to 100%. The death rate was lower for *T. confusum.* After 48 hours 88% of *T. confusum* were infected by *S. feltiae,* and after 72 hours the number grew to 94%. On the other hand, the least extensive infection was found in the case of *T. molitor.* After 24 hours' exposure, 73% of *T. molitor* larvae were dead and after 72 hours the number increased to 94% (Table 1a).

A two-factor variance analysis showed that the extensiveness of infection by *S. feltiae* was statistically and significantly different depending on the species of the infected insect and on the time of contact between the invasive nematode larvae and the host (Table 1b). When the time of contact between *S. feltiae* invasive larvae and the insects grew, the nematodes infected more and more insects. After a 24-hours' exposure, 86% of infected hosts were found, after 48 hours there were 88%, and after 72 hours — 96%.

After a dissection of the infected insects significant differences were found in the numbers of the first *S. feltiae* generation in the dead larvae of particular host species. By far the highest mean intensity of infection, in all time variants, was found in the case of *G. mellonella,* and the greatest number of nematodes penetrated into the caterpillars in the 48-hours' variant (on average, 33.11 *S. feltiae*

Table 1a. Extensiveness and intensity of insect infection by *Steinernema feltiae* in individual infections (mean values)

	Contact time	Infected species			Totally (for contact time)
		Galleria mellonella	*Tribolium confusum*	*Tenebrio molitor*	
Infection extensiveness	24 h	97%	88%	73%	86%
	48 h	100%	88%	77%	88%
	72 h	100%	94%	94%	96%
	Totally (for infected species)	100%	90%	81%	90%
Infection intensity	24 h	21.06	1.97	4.17	9.06
	48 h	33.11	2.94	8.39	14.81
	72 h	27.56	3.33	18.06	16.31
	Totally (for infected species)	27.24	2.75	10.20	13.40

Table 1b. Variance analysis of mean extensiveness and intensity of insect infection by *Steinernema feltiae* in individual infections

	Sources of variability	F	Level of significance
Infection extensiveness	Infected species	11.475	0
	Contact time	4.515	0.016
	Interaction: Infected species X Contact time	1.461	0.23
Infection intensity	Infected species	32.978	0
	Contact time	3.065	0.057
	Interaction: Infected species X Contact time	9.772	0.212

Statistically significant at the level of significance < 0.05.

were found per one infected insect). Infection intensity for *T. molitor* in the 48-hours' variant was nearly six times lower than infection intensity of *G. mellonella* caterpillars. The lowest number of nematodes was found in infected *T. confusum* larvae. Infection intensity ranged from 1.97 after 24 hours to 3.33 after 72-hour--long exposure (Table 1a).

A statistical analysis of the results showed that infection intensity of particular insect species by *S. feltiae* differed in a statistically significant manner (Table 1b).

H. bacteriophora showed the greatest insecticidal activity towards *G. mellonella* caterpillars in all the time variants (mean extensiveness of infection being 74%), it was less effective towards *T. molitor* (53%), and the least effective towards *T. confusum* (28%) (Table 2a). A two-factor variance analysis showed that differences in infection extensiveness of particular insect species by *H. bacteriophora* are statistically significant (Table 2b).

When the time of contact between *H. bacteriophora* nematodes and the insects grew, the percent of infected species also increased: after 24-hours it was 17%, after 48 hours the number grew to 66%, and after a 72-hour-long exposure it was 73%. Differences in the extensiveness of insect infection by *H. bacteriophora* in particular time variants are statistically significant (Tables 2a, 2b).

Table 2a. Extensiveness and intensity of insect infection by *Heterorhabditis bacteriophora* in individual infections (mean values)

	Contact time	Infected species			Totally (for contact time)
		Galleria mellonella	*Tribolium confusum*	*Tenebrio molitor*	
Infection extensiveness	24 h	27%	3%	21%	17%
	48 h	97%	32%	68%	66%
	72 h	100%	48%	71%	73%
	Totally (for infected species)	74%	28%	53%	52%
Infection intensity	24 h	35.25	0	4	21.86
	48 h	27.67	2.33	16.44	18.19
	72 h	56.39	8	14.39	28.54
	Totally (for infected species)	40.33	5.57	13.21	23.22

After 24-hour-long contact between *H. bacteriophora* and the hosts the greatest number of nematodes penetrated into *G. mellonella* (32.25 nematodes on average). Infection intensity was over 8 times lower in the case of *T. molitor.* Although in the discussed time variant dead larvae of *T. confusum* were observed with the signs of infection by *H. bacteriophora,* dissections did not show the presence of the first generation of nematodes in the larvae of this species. When the time of contact between nematodes and insects grew, the numbers in the first generation in the infected host also increased. Like in the 24-hours' variant, after 48- and 72-hour-long exposition of hosts to nematodes, the highest intensity of infection of insects by *H. bacteriophora* was established for *G. mellonella* caterpillars, lower for *T. molitor,* and the lowest for *T. confusum.* Differences in

Table 2b. Variance analysis of mean extensiveness and intensity of insect infection by *Heterorhabditis bacteriophora* in individual infections

	Sources of variability	F	Level of significance
Infection extensiveness	Infected species	17.657	0
	Contact time	29.836	0
	Interaction: Infected species X Contact time	1.275	0.294
Infection intensity	Infected species	6.347	0.005
	Contact time	1.029	0.37
	Interaction: Infected species X Contact time	0.728	0.543

Statistically significant at the level of significance < 0.05.

the numbers in the first generation of *H. bacteriophora* in particular host species are statistically significant (Tables 2a, 2b).

A comparison of host infection by *S. feltiae* and *H. bacteriophora* in no--competition conditions in the environment shows that *H. bacteriophora* infects the insects with the highest intensity after a shorter time of contact.

In infections when two entomogenous nematode species were simultaneously introduced into the soil, invasive larvae of *S. feltiae* were most effective in infecting *G. mellonella,* less effective for *T. molitor*, and the least for *T. confusum.* This means that introducing a competitive species did not have a significant effect on insect infection by *S. feltiae.*

A two-factor variance analysis showed that differences in infection extensiveness of particular insect species and the number of the infected insects by *S. feltiae* in particular time variants are statistically significant (Table 3a, 3b). When the period of contact between *S. feltiae* and insects grew from 24 to 48 hours, the extensiveness of host infection also grew considerably (from 71% to 90%). After a more increased time of contact, up to 72 hours, the extensiveness of infection by *S. feltiae* decreased to 83%.

During a dissection of the dead insects the studies found out the highest numbers in the first *S. feltiae* generation, in all the time variants, in *G. mellonella* caterpillars (mean number of nematodes was 25.81). Lower infection intensity was observed in the case of *T. molitor* (17.29), and the lowest for *T. confusum* (2.66 per one insect). In 24- and 48-hours' variants, the number of *S. feltiae* was

Table 3a. Extensiveness and intensity of insect infection by *Steinernema feltiae* in cross infections *S. feltiae x H. bacteriophora* (mean values)

	Contact time	Infected species			Totally (for contact time)
		Galleria mellonella	*Tribolium confusum*	*Tenebrio molitor*	
Infection extensiveness	24 h	97%	48%	70%	71%
	48 h	100%	84%	84%	90%
	72 h	97%	60%	91%	83%
	Totally (for infected species)	98%	64%	82%	81%
Infection intensity	24 h	14.18	0.75	6.65	7.22
	48 h	34.24	2.67	17.79	18.23
	72 h	29.02	4.56	25.66	19.74
	Totally (for infected species)	25.81	2.66	17.29	15.22

Table 3b. Variance analysis of mean extensiveness and intensity of insect infection by *Steinernema feltiae* in cross infections *S. feltiae x H. bacteriophora*

	Sources of variability	F	Level of significance
Infection extensiveness	Infected species	14.338	0
	Contact time	4.223	0.021
	Interaction: Infected species X Contact time	1.829	0.14
Infection intensity	Infected species	20.485	0.0
	Contact time	6.637	0.003
	Interaction: Infected species X Contact time	1.551	0.204

Statistically significant at the level of significance < 0.05.

twice lower in infected *T. molitor* than in *G. mellonella* caterpillars, while in the 72-hours' variant the difference was much smaller (Table 3a). In cross infections the differences in infection intensity by *S. feltiae* towards particular insect species and in particular time variants are statistically significant (Table 3b).

In competitive conditions, *H. bacteriophora* did not infect *G. mellonella* caterpillars, the species preferred by *H. bacteriophora* in individual infections. After the first 24 hours of contact between nematodes and insects, only *T. molitor*

larvae died with the signs of infection by *H. bacteriophora.* The extensiveness of *T. molitor* infection after 24- and 48-hour-long contact with nematodes was 3%, and after 72 hours it grew three times. In the 48-hours' variant, four times more *T. confusum* larvae were infected as compared to *T. molitor* larvae, while in the 72-hours' variant another (double) increase of the extensiveness of infection of *T. confusum* larvae by *H. bacteriophora* took place (Table 4a, 4b).

A two-factor variance analysis showed that differences in the extensiveness of infection by *H. bacteriophora* of particular insect species are statistically significant. On the other hand, there were no significant differences in the extensiveness of insect infection by *H. bacteriophora* when the time of contact between the host and nematodes grew (Tables 4a, 4b).

Table 4a. Extensiveness and intensity of insect infection by *Heterorhabditis bacteriophora* in cross infections *S. feltiae x H. bacteriophora* (mean values)

	Contact time	Infected species			Totally (for contact time)
		Galleria mellonella	*Tribolium confusum*	*Tenebrio molitor*	
Infection extensiveness	24 h	0%	0%	3%	1%
	48 h	0%	12%	3%	5%
	72 h	0%	22%	9%	10%
	Totally (for infected species)	0%	11%	5%	6%
Infection intensity	24 h	0	0	1	1
	48 h	0	2	18	7.33
	72 h	0	3.83	1	2.89
	Totally (for infected species)	0	3.22	5.25	4.03

In competitive conditions, in all time variants, more invasive larvae of *H. bacteriophora* penetrated into *T. molitor* as compared to *T. confusum.* The mean intensity of infection of *T. molitor* was 5.25, and 3.22 for *T. confusum* (Table 4a).

The intensity of infection of particular insect species by *H. bacteriophora* changed in a significant way with various times of contact (interaction: infected species x contact time < 0.05) (Table 4b). The highest numbers in the first generation of *H. bacteriophora* were found out in *T. molitor* larvae after a 48-hour-long contact. In the other two experimental variants the intensity of infection of these insects was low. One nematode penetrated into one host. On the other hand, after a 48-hour-long exposition, *T. confusum* was infected with a nine times lower intensity than *T. molitor.* When the time of contact was lengthened to 72

Table 4b. Variance analysis of mean extensiveness and intensity of insect infection by *Heterorhabditis bacteriophora* in cross infections *S. feltiae x H. bacteriophora*

	Sources of variability	F	Level of significance
Infection extensiveness	Infected species	4.414	0.018
	Contact time	2.879	0.067
	Interaction: Infected species X Contact time	1.548	0.205
Infection intensity	Infected species	2.636	0.165
	Contact time	3.533	0.111
	Interaction: Infected species X Contact time	17.516	0.009

hours, the numbers in the first generation of *H. bacteriophora* in *T. confusum* increased rapidly, reaching the value which was four times higher than the number of nematodes found in *T. molitor* (Tables 4a, 4b).

DISCUSSION

The results point out that when *S. feltiae* and *H. bacteriophora* occur simultaneously, *S. feltiae* nematodes were more effective in insect infection. In all the time variants, *S. feltiae* nematodes infected a greater number of larvae of each insect species than *H. bacteriophora*. In each time variant and in each host species, the numbers of nematodes of the first generation of *S. feltiae* in the infected insect larvae were also higher than the numbers in the first generation of *H. bacteriophora.* This allows us to state that introducing a competitive species did not have a significant effect on insect infection by *S. feltiae.* Both in individual and cross infections, *S. feltiae* showed the highest ability of infection towards *G. mellonella,* lower for *T. molitor,* and the lowest for *T. confusum.*

Analysing the results achieved in both experimental variants, one should pay attention to the doses of entomogenous nematodes. The conclusion can be drawn that the presence of a competitive species *H. bacteriophora* in the environment increases the activity of invasive larvae of *S. feltiae.*

A comparison of individual and cross infections showed significant differences in insect infection by *H. bacteriophora.* In individual infections, *H. bacteriophora* was most effective in infecting *G. mellonella* caterpillars, less effective for *T. molitor* and the least for *T. confusum.* On the other hand, in cross infections

H. bacteriophora did not infect *G. mellonella* caterpillars at all. In individual infection *H. bacteriophora* infected more larvae of *T. molitor* than *T. confusum,* while in cross infections a reverse phenomenon was observed, namely the extensiveness of *T. confusum* infection had a higher value than in the case of *T. molitor.* Both in individual infections and in competitive conditions a higher number of the first generation of those nematodes was found in dead *T. molitor.*

When the time of contact between nematodes and insects grew, in individual and cross infections the insects' death rate and the numbers in the first generation of *S. feltiae* were also increased. In individual infections the maximum activity of nematodes was found after 72-hours' exposition, while in cross infections it was marked as early as after 48 hours.

Differences in individual variants of infecting particular insect species by *S. feltiae* and *H. bacteriophora* confirm the finding that entomogenous nematodes show food preferences (21, 22).

Mraček and Ruzicka (27) found out that infection of particular insects by entomogenous nematodes is related to the size of insects, and especially to the size of their natural openings. Small openings of smaller insects can limit infection by *S. feltiae*, not limiting the number of penetrating *H. bacteriophora* larvae. It cannot be ruled out that this is the cause of differences in the infection of *T. confusum* by *S. feltiae* and *H. bacteriophora* in individual infections. Wójcik (37) found out that the smaller the host's biomass the lower the intensity of infection by entomogenous nematodes. According to Bednarek (2) differences in the intensity of infection are caused by intra-population factors and individual resistance of the host to nematode invasion. The number of invasive larvae of entomogenous nematodes penetrating into an insect is regulated by the number of invasive larvae which earlier managed to penetrate into it (3).

The experiments carried out by the author show that when the time of contact between nematodes and insects grows, then the hosts' death rate also increases, which confirms the results obtained by Pezowicz (28), who infected *A. grissella* caterpillars with the same dose of invasive larvae of nematodes. This author found out that the final extensiveness of infection depended on the time of contact between the parasite and the insect.

Differentiated susceptibility of different insect species to infection by *Steinernematidea* was already noticed by Dutky (7). He found out that *Steinernematidea* were most effective in infecting butterfly caterpillars, less infective towards cockchafer caterpillars, and the least towards the dipterous. Bedding et al. (1), who performed an individual infection of various insects species by nematodes of genera *Steinernema* and *Heterorhabditis* confirmed Dutky's findings. They also showed that *Heterorhabditis* are characterised by similar food preferences as *Steinernema.* Laumond et al. (23) used the method of direct contact in

laboratory condition in studying the susceptibility of 128 insect species belonging to 12 orders of *S. carpocapsae.* They confirmed considerable sensitivity of butterflies to nematode infection, which was the highest in the case of *G. mellonella* and *Pieris brassicae.* Sensitivity of eight insect species belonging to butterflies and cockchafers to infection by *S. carpocapsae* and *H. bacteriophora* was also studied by Pezowicz (29). Having performed infections in the soil in one--species schemes, this author found out that in the quantitative variant butterflies were more susceptible to infection by *H. bacteriophora* than cockchafers, while in the weight variant *T. confusum* and *Sitophilus granarius* were infected with higher intensity than *Pieris brassicaei* and *Barathra brassicae.* In both experimental variants *G. mellonella* was more attractive for nematodes than *T. confusum.*

Kamionek and Sandner (13) carried out experiments in which two insect species were simultaneously exposed to the invasive stadium of *S. carpocapsae.* Infection was performed on filter paper in Petri dishes. In the experiments there were used cockchafers of *Sitophilus oryzae, Tribolium castaneum* and *Trogoderma granarium.* The presence of the other host had a significant effect on the death rate of both insect species. The death rate in two-species schemes was different from that in one-species schemes, which — according to the authors — could have resulted from varying degrees of attractiveness of particular cockchafer species for the invasive larvae *S. carpocapsae (Neoaplectana carpocapsae).*

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