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Effectiveness of infection of *Cameraria ohridella (Lepidoptera: Gracillariidae)* larvae by entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis* sp. (*Nematoda: Rhabditida*)

Efektywność porażania larw *Cameraria ohridella* (*Lepidoptera*: *Gracillariidae*) przez nicienie entomopatogeniczne *Steinernema* sp. i *Heterorhabditis* sp. (*Nematoda: Rhabditida*)

SUMMARY

The purpose of the present studies was to evaluate the susceptibility of *Cameraria ohridella* larvae to infection by entomopathogenic nematodes *Steinernema sp.* and *Heterorhabditis* sp. in laboratory conditions. The experiments were done at the temperatures 20°C and 25°C with the use of three doses of the pathogen, namely 5, 25 and 50 nematodes/insect. The results showed that *Cameraria ohridella* larvae were susceptible to infection by entomopathogenic nematodes. Out of two strains of entomopathogenic nematodes used in the studies, the one that was more effective in infecting *Cameraria ohridella* at the temperature 20°C was *Steinernema* sp., while at the temperature of 25°C *Heterorhabditis* sp. infected the insects more intensively. Results of the experiments showed that with lower doses of invasive larvae of nematodes (5 larvae/insect, 25 larvae/insect) *Steinernema* sp. PIS81 was a more pathogenic strain towards *Cameraria ohridella* as compared to *Heterorhabditis* sp. PIH81.

STRESZCZENIE

Prezentowane badania miały na celu ocenę podatności larw *Cameraria ohridella* na porażanie przez nicienie entomopatogeniczne *Steinernema* sp. i *Heterorhabditis* sp. w warunkach laboratoryjnych. Doświadczenia przeprowadzono w temperaturach 20°C i 25°C oraz przy zastosowaniu trzech dawek patogena: 5, 25 i 50 nicieni/owad. Wyniki doświadczeń wykazały, że larwy *Cameraria*

ohridella są podatne na porażenie przez nicienie entomopatogeniczne. Z dwóch wykorzystanych do badań szczepów nicieni entomopatogennych, szczepem efektywniej porażającym larwy *Cameraria ohridella* w temperaturze 20°C był *Steinernema* sp., natomiast w temperaturze 25°C z wyższą intensywnością porażał owady *Heterorhabditis* sp. Wyniki prezentowanych doświadczeń wykazały, że przy niskich dawkach larw inwazyjnych nicieni (5 larw/owad, 25 larw/owad) bardziej patogennym szczepem w stosunku do *Cameraria ohridella* jest *Steinernema* sp. PlS81 niż *Heterorhabditis* sp. PlH81.

K e y w o r d s: horse-chestnut leafminer, *Cameraria ohridella, Steinernema, Heterorhabditis,* biological control.

INTRODUCTION

Horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimic, 1986 is a small butterfly (the length of its body is about 3 mm) belonging to the family *Gracillaridae.* The caterpillar is yellow-green, legless with a triangularly shaped head and a body divided into distinct sections, marked with skin folds. The larvae of *Cameraria ohridella* feed on the leaves of horse-chestnut *Aesculus hippocastanum* L. (*Hippocastanaceae*). Caterpillars form mines while feeding, eating out the parenchyma tissue between the upper and lower cuticles of the leaf, which damages the photosynthesis apparatus of plants, and in the case of mass infection, causes their complete necrosis. The insect overwinters in dried leaves in the pupa stage (8, 11). The butterflies' departure in Poland falls at the end of April and at the beginning of May.

In recent years mass infection of the leaves of *Aesculus hippocastanum* by *Cameraria ohridella* has been observed in Poland. This insect probably appeared in Poland in the late 1990's, and after 2000 it was found in all southern and central Poland. The population of this butterfly is increasing rapidly. It arrived in Poland from the south of Europe, where it had been observed a few years earlier. However, the primary area of its occurrence is unknown. The first observations come from Macedonia from 1985. *Cameraria ohridella* spreads very fast and it was already in 1989 when it reached the area near Linz in Austria, and four years later its was found in the Czech Republic and in Slovakia (11). It conquered the whole continent within 15 years. The lack of natural enemies, which could limit the population of *Cameraria ohridella* in that area, was favourable for its fast expansion in Europe. Intensive work on effective and safe methods of controlling this pest is undertaken in many countries. Biological methods are an alternative to the chemical ones which are toxic to the environment and which have little efficiency.

The organisms that are effective in controlling the numerous pests of fields and forests, especially insects, include entomopathogenic nematodes (4, 7, 10, 25, 26, 28), which are a very interesting group of organisms due to their specific biological properties. Entomopathogenic nematodes form mutualistic compounds with bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. (2); their larvae, living feely in the soil, acquired an ability of active host search (14). Entomopathogenic nematodes kill their hosts in a very short time, within about 48 hours (23). They are safe to vertebrates, plants and numerous invertebrates (1, 24). After being introduced into the environment, the invasive larvae can sustain there for a longer time, and there is no need to repeat the treatment. They constitute an important factor regulating the insect population in natural conditions. In Poland, entomopathogenic nematodes from the genus *Steinernema* are common, while those from the genus *Heterorhabditis* are rather scarce.

The purpose of the present paper is to estimate the susceptibility of caterpillars *Cameraria ohridella* to infection by entomopathogenic nematodes in laboratory conditions. Entomopathogenic

nematodes, due to remarkable abilities to infect different insect species, especially butterfly larvae (3, 16, 17, 19, 22), can be treated as a potential factor in the biological control of *Cameraria ohridella.*

MATERIAL AND METHODS

The larvae of *Cameraria ohridella* Deschka & Dimic (*Lepidoptera: Gracillariidae*) collected from the leaves of *Aesculus hippocastanum* L. in September and October 2002 in the area of Lublin were used in the experiments.

The insects were infected by the invasive larvae of *Steinernema* P1S81 and *Heterorhabditis* sp. P1H8. The invasive larvae of nematodes are in permanent culture at the Chair of Zoology and Ecology of the Catholic University in Lublin. Nematodes were multiplied on the larvae of *Galleria mellonella,* and before they were used in the experiments they were stored at a cool room at the temperature 6°C.

The effect of temperature on the infection of larvae of *Cameraria ohridella* by *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81 as well as the effectiveness of insects' infection by entomopathogenic nematodes with various doses were studied. The infections were performed on Petri dishes laid with filter paper.

In order to study the effect of temperature on the infection of larvae *Cameraria ohridella* by *Steinernema* sp. PIS81 and *Heterorhabditis* sp. P1H81 the experiments were done at the temperatures of 20°C and 25°C, in both variant applying the dose of 50 invasive larvae of nematodes per one insect.

In order to study the relation between the nematode dose and the effectiveness of the infection of *Cameraria ohridella* larvae by *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81, the experiments were done at the temperature of 20°C, with three doses: 5 invasive larvae per one insect, 25 invasive larvae per one insect, and 50 invasive larvae per one insect.

The effect of temperature and various doses of nematodes on the effectiveness of infection of *Cameraria ohridella* larvae was evaluated on the basis of such parasitological indexes as infection extensiveness (the proportion of infected insect larvae by nematodes) and infection intensiveness (the number of entomopathogenic nematodes penetrating into an insect). The infection extensiveness was observed on the fourth and eighth day of contact, while the infection intensiveness was established on the fourth day of contact on the basis of the infected insects' dissection.

The statistical analysis of the results was conducted at the Computer Centre of the Catholic University of Lublin by means of SPSS 8.0 P1 for Windows. Z test for proportion was applied in order to analyze the effect of temperature, dose, period of contact on the extensiveness of infection of *Cameraria ohridella* by *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81 and in order to compare the extensiveness of insect infection by both nematode species. The analysis of the intensiveness of insect infection in relation to the temperature and nematode species was conducted by means of t test for independent samples, while the statistical significance of the results concerning the effect of the dose on the infection intensiveness was checked by oneway analysis of variance (oneway ANOVA) and Dunnett's multiple comparisons test. In all the tests that were applied the data are statistically significant when $p < 0.05$.

RESULTS

The experiments show that the effectiveness of infection of *Cameraria ohridella* larvae by entomopathogenic nematodes varied considerably depending on the temperature at which the experiments were done. The extensiveness of insect infection by *Heterorhabditis* sp. P1H81 increased from 60% at 20°C to 94.4% at 25°C, and those differences were statistically significant ($z = 3.206$, $p = 0.01$) (Table 1). Such a significant increase of infection extensiveness was not observed in the case of *Steinernema* sp. P1S81, where it remained at similar levels at temperatures of 20°C and 25°C (100%, 96.6%).

On the other hand, the intensiveness of insect infection by both strains of entomopathogenic nematodes increased significantly at the temperature 25% as compared to the infections at 20°C (Table 1). The intensiveness of insect infection by *Steinernema* sp. P1S81 increased from 3.13 at the temperature 20°C to 7.51 at 25°C ($t = 4.346$, $df = 18$, $p < 0.05$), and by *Heterorhabditis* sp. P1H81 from 2.30 at the temperature of 20°C to 29.89 at 25°C ($t = 10.552$, $df = 49.187$, $p < 0.05$).

Table 1. The effect of temperature on the effectiveness of infection of *Cameraria ohridella* larvae by entomopathogenic nematodes *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81 (mean values)

Parameter	Nematode species	Infection temperature	
		20°C	25°C
Infection Extensiveness	*Steinernema* sp. PlS81	100%	96.6%
	Heterorhabditis sp. PlH81	60% *a*	94.4% *a*
Infection Intensiveness	*Steinernema* sp. PlS81	3.10 ±2.64 *b*	7.51 ±4.26 *b, d*
	Heterorhabditis sp. PlH81	2.30 ±2.30 *c*	29.89 ±14.88 *c, d*

a, b, c, d — two-tailed probability ($p < 0{,}05$).

Comparing the effectiveness of infection of *C. ohridella* by two strains of entomopathogenic nematodes it should be emphasized that at the temperature of 25°C the invasive larvae *Heterorhabditis* sp. P1H81 infected insects with significantly higher intensiveness (29.89) than *Steinernema* sp. P1S81 (7.51). ($t = 9.662$, $df = 48.667$, $p < 0.05$). On the other hand, the strain that was more effective at the temperature of 20°C was *Steinernema* sp. P1S81 since it caused higher death rate of *C. ohridella* larvae (100%) than *Heterorhabditis* sp. P1H81 (60%) and it infected the insects with higher intensiveness (Table 1).

The death rate of *C. ohridella* grew together with increased doses of entomopathogenic nematodes: in the case of *Steinernema* sp. P1S81 from 50% with the dose of 5 invasive larvae/insect up to 100% with the dose of 50

invasive larvae/insect ($z = 3.206$; $p = 0.001$), and in the case of infections by *Heterorhabditis* sp. P1H81 from 30% with the lowest dose to 60% with the maximum one (Table 2).

Insect infection extensiveness also increased with the time of contact. An especially significant effect of increased time of contact from four to eight 24 hours' periods on the increase of the number of infected insects was found out for the lowest dose, i.e. five nematodes/insect. The extensiveness of infection of *C. ohridella* by *Steinernema* sp. P1S81 grew from 50% with the contact time of four 24 hours' periods up to 100% after eight 24 hours' periods ($z = 2.974$; $p = 0.003$), while in the case if infections by *Heterorhabditis* sp. P1H81 it grew from 30% after four 24 hours' periods to 100% after eight 24 hours' periods ($z = 3.282$; $p = 0.001$) (Table 2).

Table 2. Extensiveness of infection (in %) of *Cameraria ohridella* larvae by entomopathogenic nematodes *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81 at the temperature of 20°C (mean values)

	Time of contact (in 24 hours' periods)	Dose of nematodes (number of invasive larvae/insect)		
		5	25	50
Steinernema sp. PlS81	4	50 *a, c*	90	100 *c*
	8	100 *a*	100	100
Heterorhabditis sp. PlH81	4	30 *b*	70	60
	8	100 *b*	90	90

a, b, c — two-tailed probability ($p < 0.05$).

In the case of both studied species of entomopathogenic nematodes increased intensiveness of infection was observed together with increased pathogen doses, but a higher increase was found out in infections by *Steinernema* sp. P1S81, and those differences are statistically significant ($df = 2$; $F = 4.597$, $p = 0.019$) (Table 3). The highest, almost fourfold increase of infection intensiveness was observed in the case of increased dose of *Steinernema* sp. P1S81 from 5/insect to 25/insect (2, 7) ($p = 0.19$).

DISCUSSION

Susceptibility of *Cameraria ohridella* caterpillars to entomopathogenic nematodes from the genera of *Steinernema* and *Heterorhabditis* as well as the development of nematodes in these insects point to the possibility of using these nematodes in controlling them. Nematodes infect the larvae of *Cameraria ohridella* at

Table 3. Intensiveness of infection of *Cameraria ohridella* larvae by entomopathogenic larvae *Steinernema* sp. P1S81 and *Heterorhabditis* sp. P1H81 at the temperature of 20°C (mean values)

	Nematode dose (number of invasive larvae/insect)		
	5	25	50
Steinernema sp. PlS81	0.7 ±0.82 *a*	2.70 ±1.77 *a*	3.10 ±3.10 *a*
Heterorhabditis sp. PlH81	0.7 ±1.25	1.40 ±1.58	2.30 ±4.24

a — statistically significant data ($p < 0.05$).

a short time with relatively high intensiveness in relation to the applied pathogen doses. Numerous studies show that the death rate of hosts and the intensiveness of their infection is correlated with the nematode dose (15, 27), therefore it is necessary to study this relation while evaluating the pathogen effectiveness in controlling a given pest species. The present studies deliberately used relatively low doses of nematodes so that the infection conditions would be similar to the conditions of the natural environment. Results of the studies showed that when the doses of invasive larvae of nematodes were low (5 larvae/insect, 25 larvae/insect) *Steinernema* sp. P1S81 was a more pathogenic strain towards *Cameraria ohridella* than *Heterorhabditis* sp. P1H81.

The temperature had a significant effect on the activity of invasive larvae of entomopathogenic nematodes (5, 13, 20, 29). *S. feltiae* can infect the hosts within the temperature ranging from 2°C to 30°C, and various geographical varieties of *Heterorhabditidae* from 7°C to 35°C (21). Gray and Johnson (9) observed a high death rate of invasive larvae *S. carpocapsae* when the soil temperature exceeded 30°C. However, when discussing the effect of temperature on the occurrence of a given species or a geographical variety, attention should be paid to the origin of nematodes and their adjustment to the physical conditions. For example, *Heterorhabditis* sp. D1, which is adapted to the tropical conditions, sustains at the temperature of 10°C for a short time, causing infection only in a small degree at the temperature that is not lower than 12°C. On the other hand, it shows the highest invasive character at the temperature of 20°C — 33°C (21). When the invasive larvae of nematodes stay in the soil with high temperature, their pathogenic character is decreased (6, 12), followed by greater death rate as a consequence of intensive metabolism (18). Out of the two Polish origin strains of entomopathogenic nematodes used in the present experiments, *Steinernaema* P1S81 infected the larvae of *Cameraria ohridella* more effectively at the temperature of 200%, while *Heterorhabditis* sp. P1H81 was more effective at 25°C.

So far, no studies have been carried out on either the susceptibility of *Cameraria ohridella* to entomopathogenic nematodes or on the possibilities of us-

ing them in controlling the pest. The fact that *Cameraria ohridella* pupas overwinter in dried leaves, often mixed with the soil, creates possibilities for a natural contact of nematodes with the insect. Further studies should show the usefulness of nematodes in controlling *Cameraria ohridella* in field conditions.

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