

ANNA KREFT, HENRYK SKRZYPEK,  
WALDEMAR KAZIMIERCZAK

Department of Zoology and Ecology, Catholic University of Lublin  
al. Kraśnicka 102, 20-718 Lublin, Poland

Susceptibility of *Cameraria ohridella* Deschka & Dimic, 1986  
(Lepidoptera: *Gracillariidae*) pupae to entomopathogenic  
nematodes *Steinernema* sp. and *Heterorhabditis* sp.  
(*Nematoda: Rhabditida*)

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Wrażliwość poczwerek *Cameraria ohridella* (Lepidoptera: *Gracillariidae*)  
na nicienie entomopatogeniczne *Steinernema* sp. i *Heterorhabditis* sp. (*Nematoda*:  
*Rhabditida*)

SUMMARY

Presented experiments were aimed at studying the susceptibility of *Cameraria ohridella* pupae to entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis* sp. The studies examined the effect of the nematode dose, temperature and the season on the efficiency on infection by *Steinernema* sp. and *Heterorhabditis* sp. The experiment was performed using three doses of nematodes, namely 5, 25 and 50 invasive larvae of the insect at the temperatures of 15°C and 20°C. The studies were conducted in August and November of 2003.

Results of the experiments showed that the pupae of *C. ohridella* are infected by *Steinernema* sp. and *Heterorhabditis* sp. Effectiveness of the infection of pupae by entomopathogenic nematodes increased in a statistically significant way together with an increasing of the pathogen dose. Pupae infected in November (over-wintering) turned out to be more sensitive to nematodes. The studies found out slight differences in the effectiveness of infection of *C. ohridella* pupae depending on the applied strains of nematodes.

STRESZCZENIE

Prezentowane doświadczenia miały na celu zbadanie wrażliwości poczwerek *Cameraria ohridella* na entomopatogenne nicienie *Steinernema* sp. i *Heterorhabditis* sp. Badano wpływ dawki nicieni, temperatury oraz pory roku na efektywność porażania przez *Steinernema* sp. i *Heterorhabditis* sp. Doświadczenia przeprowadzono przy użyciu trzech dawek nicieni: 5, 25 i 50 larw inwazyjnych na owada, w temperaturach 15°C i 20°C. Badania przeprowadzono w sierpniu i listopadzie 2003 r.

Wyniki doświadczeń wykazały, że poczwarki *Cameraria ohridella* są porażane przez *Steinernema* sp. i *Heterorhabditis* sp.

Efektywność porażenia poczwarek przez nicienie entomopatogenne wzrastała istotnie statystycznie wraz ze wzrostem dawki patogena. Bardziej wrażliwe na nicienie okazały się poczwarki porażane w listopadzie (zimujące).

Stwierdzono niewielkie różnice w efektywności porażenia poczwarek *C. ohridella* przez zastosowane szczepy nicieni.

**Key words:** *Cameraria ohridella*, horse-chestnut leafminer, *Steinernema*, *Heterorhabditis*, biological control.

## INTRODUCTION

Horse-chestnut leafminer (*Cameraria ohridella*) is a small moth (its body's length is about 3 mm) belonging to the family of *Glacillaridae*. *C. ohridella* larvae feed on the leaves of horse-chestnut *Aesculus hippocastanum* L. (*Hippocastanaceae*). Its caterpillars, while feeding, create the mines, eating out the parenchyma between the upper and lower epidermis of the leaf. Caterpillars are of brown colour. The insects over-winter in dried leaves at the stage of pupa (10, 14). They have a spine on their heads, which makes it easier to hook the mine in this way allowing the moth to leave it. In Poland, the flight of the moths takes place at the end of April and the beginning of May. Three generations occur during the year and the full development of one generation lasts about 2 months. All developmental stages of *C. ohridella* appear in the leaves during the summer period (5).

The first report about the occurrence of *C. ohridella* comes from Macedonia from 1985. This insect spreads very fast and during a period of 15 years it has invaded almost all the continent. In Poland it probably appeared in the second half of the 1990's, and after the year 2000 it was found in all southern and central Poland. Its fast expansion in Europe was favoured by the lack of natural enemies in this area which would be able to limit the population of *C. ohridella* in an effective manner (9, 10, 11). Nowadays 20 species of hymenoptera are known. They are parasites of caterpillars, mainly belonging to the family *Eulophidae*, and two species parasitic towards the pupae: ectoparasite *Minotetrastichus frontalis* and endoparasite *Pediobius saulius* (6). The natural enemies of *C. ohridella* also include other insects, the most effective of which are *Chrysopidae* and *Tettigoniidae*, mites and birds, especially *Parus major* L., *P. caeruleus* L. and *P. palustris* L.

A lot of countries conduct intensive research on working out effective and, at the same time, safe methods of controlling this pest (17). Biological methods are an alternative for chemical ones, which are toxic to the environment and ineffective. The organisms effectively reducing the population of a number of agricultural and forest pests include for example entomopathogenic nematodes (4, 7, 8, 13, 25, 26, 27). They create mutualistic connections with bacteria *Xenorhabdus* sp. and *Photorhabdus* sp. (2) and their free living invasive larvae created an ability to look for the host in an active way (16). Nematodes kill their hosts very fast, in the period of about 48 hours (23). They are safe for plants, numerous invertebrates and vertebrates (1, 24). After being introduced to the environment, invasive larvae can sustain there for a longer time without a need to repeat this activity. They are an important factor regulating the population of insects in natural conditions. Entomopathogenic nematodes from the genus *Steinernema* and, more rarely, *Heterorhabditis* commonly occur in Poland. The present paper estimates the sensitivity of *C. ohridella* pupae to the infection by entomopathogenic nematodes in laboratory conditions. Due to considerable abilities to infect various insect species, especially larvae of butterflies (3, 18, 19, 21, 22), nematodes from the genera *Steinernema* and *Heterorhabditis* can be treated as a potential factor in biological control of *C. ohridella*.

## MATERIAL AND METHODS

Pupae of *Cameraria ohridella* collected from the leaves of *Aesculus hippocastanum* L. in August and November of 2003 in the area of the city of Lublin were used in the experiment.

The pupae were infected by invasive larvae of entomopathogenic nematodes *Steinernema* sp. PLS81 and *Heterorhabditis* sp. PLH81. Invasive larvae of entomopathogenic nematodes are found in a permanent culture at the Chair of Zoology and Ecology at the Catholic University of Lublin. Nematodes were bred on the larvae of *Galleria mellonella*, and before they were used in the experiments they were kept in a cool room at the temperature of 6°C. The infection of *C. ohridella* pupae was performed on Petri dishes filled with sterile earth (2 hrs at 200°C). In order to study the susceptibility of *C. ohridella* pupae to entomopathogenic nematodes, experiments were done using three doses of the pathogen (5, 25 and 50 invasive larvae of nematodes per one insect pupa) and two temperature variants (15° and 20°C). Experiments were done in three repetitions.

The effect of temperature and various doses of nematodes on the effectiveness of infecting *C. ohridella* pupae was estimated on the basis of such parasitological indexes as infection extensiveness (percent of insect larvae infected by nematodes) and infection intensity (number of entomopathogenic nematodes present in the infected insect) observed on the fourth day of contact.

The statistical analysis of the results was performed at the Computer Center of the Catholic University of Lublin by means of program SPSS 8.0 PL for Windows. A one-factor variance analysis (oneway ANOVA) was conducted for the statistical analysis of the effect of nematode dose on the extensiveness and intensity of infection at definite temperatures. t-Student test was performed in order to compare the effectiveness of infecting the pupae by entomopathogenic nematodes depending on nematode species, temperature and the time of year. In all the tests used in the studies the data are statistically significant at  $p < 0.5$ .

## RESULTS

The pupae of *Cameraria ohridella* are susceptible to entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis* sp. The effectiveness of infecting the pupa changes depending on the nematode dose, the time of year and — to a limited degree — the temperature at which the experiments were conducted.

Experiments carried out in August and November showed that the insects were susceptible to the infection by both nematode species in a similar way (Tab. 1, 2, 3, 4). Statistically significant differences in the extensiveness and intensity of infecting *C. ohridella* pupae by *Steinernema* sp. and *Heterorhabditis* sp. occurred only in the experiment conducted in August. At the temperature of 20°C, when the dose of 25 invasive larvae of nematodes was used per one insect, the extensiveness and intensity of infection were 37% and  $7.6 \pm 6.6$  and 10% and  $1.0 \pm 0.0$  for *Steinernema* sp. and *Heterorhabditis* sp., respectively (infection extensiveness  $t = -2.53$ ,  $df = 48.543$ , two-sided significance = 0.015; infection intensity:  $t = -3.362$ ,  $df = 10.0$ , two-sided significance = 0.007) (Tab. 1, 2).

In the experiments carried out in August, the extensiveness of infection by *Steinernema* sp. increased in a statistically significant way together with

the increase of the nematode dose, both at the temperature of 15°C and 20°C. For the temperature 15°C it was as follows: 7%, 13% and 40%, respectively ( $F=6.479$ , significance=0.002) and for the temperature 20°C: 10%, 37% and 20%, respectively ( $F=3.274$ , significance=0.043) (Tab. 1, 2).

A statistically significant increase of the pathogenic character of *Steinernema* sp. together with the increase of infection temperature was observed at the dose of 25 invasive larvae. Infection extensiveness increased from 13% at the temperature 15°C to 37% at 20°C ( $t=-2.131$ ,  $df=52.133$ , two-sided significance=0.038), whereas infection intensity increased from  $1.7 \pm 0.9$  at 15°C to  $7.6 \pm 6.6$  at 20°C ( $t=-2.898$ ,  $df=11.1$ , two-sided significance=0.014) (Tab. 1, 2).

Table 1. Extensiveness of infecting *Cameraria ohridella* pupae by entomopathogenic nematodes in August (in percent)

Nematode	Dose	Temperature	
		15°C	20°C
<i>Steinernema</i> sp.	5	7*	10*
	25	13 <sup>*a</sup>	37 <sup>*a, b</sup>
	50	40*	20*
<i>Heterorhabditis</i> sp.	5	20	23
	25	30	10 <sup>b</sup>
	50	27	20

\* statistically significant data.

<sup>a, b</sup> two-sidedly statistically significant data.

Table 2. Intensity of infecting *Cameraria ohridella* pupae by entomopathogenic nematodes in August ( $\pm$  standard deviation)

Nematode	Dose	Temperature	
		15°C	20°C
<i>Steinernema</i> sp.	5	$2.0 \pm 1.4$	$2.7 \pm 1.5$
	25	$1.8 \pm 0.9^a$	$7.6 \pm 6.6^{a, b}$
	50	$4.6 \pm 4.7$	$3.0 \pm 3.2$
<i>Heterorhabditis</i> sp.	5	$3.8 \pm 4.4$	$1.0 \pm 0.0$
	25	$4.6 \pm 7.3$	$1.0 \pm 0.0^b$
	50	$1.8 \pm 1.8$	$4.7 \pm 5.3$

<sup>a, b</sup> two-sidedly statistically significant data.

The experiments showed that the pupae of *C. ohridella* collected from the chest-nut trees at the end of the vegetation period were more susceptible to entomopathogenic nematodes. Both the extensiveness and intensity of pupa

infection by *Steinernema* sp. and *Heterorhabditis* sp. clearly increased together with the increase of the applied dose of invasive pathogens, which was true for both temperatures, namely 15°C and 20°C. The extensiveness of infecting the insects by *Steinernema* sp. at the temperature of 15°C grows from 10% with the dose of 5 invasive larvae per one insect to 43% with the dose of 50 invasive larvae per insect ( $F=4.6$ , significance = 0.013), while at the temperature 20°C it increases from 13% with the minimum dose to 43% with the maximum ( $F=3.749$ , significance = 0.027). Increased extensiveness of infection by *Heterorhabditis* sp. together with the nematode dose was especially marked at the temperature of 20°C. With the dose of 5 invasive larvae per insect the established extensiveness of infection by *Heterorhabditis* sp. was 10%, with the dose of 25 invasive larvae per insect it was 40%, while with the dose of 50 invasive larvae it reached 47% ( $F=4.053$ , significance = 0.021). A statistically significant growth of the intensity of infecting the insects together with the increased dose of invasive larvae of nematodes was found in the case of *Steinernema* sp. at the temperature 20°C. In the present experiment, the intensity of infection grew from  $1.5 \pm 1.0$  with the lowest dose to  $12.7 \pm 5.6$  with the dose of 25 invasive larvae per one insect and to  $29.4 \pm 20.3$  with the highest dose ( $F=5.807$ , significance = 0.009) (Tab. 3, 4).

Table 3. Extensiveness of infecting *Cameraria ohridella* pupae by entomopathogenic nematodes in November (in percent)

Nematode	Dose	Temperature	
		15°C	20°C
<i>Steinernema</i> sp.	5	10*	13*
	25	27*	23*
	50	43*	43*
<i>Heterorhabditis</i> sp.	5	23	10*
	25	40	40*
	50	40	47*

\* statistically significant data.

A comparison of experiments made in August and November shows that entomopathogenic nematodes are more effective in infecting the over-wintering pupae of *C. ohridella*. The November experiments found out much higher extensiveness and intensity of infection. Differences in the parasitological indexes are especially high with the doses of 25 and 50 invasive larvae per insect. The intensity of infecting the insects by *Steinernema* sp. at the temperature of 15°C with the dose of 25 invasive larvae per insect grew from  $1.8 \pm 0.9$  in August to  $7.8 \pm 6.3$  in November ( $t=-2.626$ ,  $df=7.62$ , two-sided significance = 0.032), while

Table 4. Intensity of infecting *Cameraria ohridella* pupae by entomopathogenic nematodes in August ( $\pm$  standard deviation)

Nematode	Dose	Temperature	
		15°C	20°C
<i>Steinernema</i> sp.	5	3.0 $\pm$ 2.6	1.5 $\pm$ 1.0*
	25	7.8 $\pm$ 6.3	12.7 $\pm$ 5.6*
	50	25.9 $\pm$ 21.6	29.4 $\pm$ 20.3*
<i>Heterorhabditis</i> sp.	5	2.7 $\pm$ 2.4	5.0 $\pm$ 5.7
	25	19.5 $\pm$ 20.6	21.3 $\pm$ 23.1
	50	32.4 $\pm$ 34.0	37.2 $\pm$ 26.1

\* statistically significant data.

with the dose of 50 invasive larvae per insect it grew from  $4.6 \pm 4.7$  in August to  $25.9 \pm 21.6$  in November ( $t = -3.46$ ,  $df = 13.223$ , two-sided significance = 0.004). In the experiments conducted at the temperature of 20°C the intensity of infecting the pupae by *Steinernema* sp. clearly increased with the dose of 50 invasive larvae per insect and it was  $3.0 \pm 3.2$  in August and  $29.4 \pm 20.3$  in November ( $t = -4.7$ ,  $df = 13.2$ , two-sided significance = 0.001) (Tab. 2, 4). On the other hand, higher effectiveness of infecting the pupae of *C. ohridella* by *Heterorhabditis* sp. at the end of the vegetation year was marked with a statistically significant increase of the extensiveness and intensity of insect infection at the temperature of 20°C with the doses of 25 and 50 invasive larvae per one insect. The intensity of infecting the pupae by *Heterorhabditis* sp. with the dose of 25 nematodes increased from  $1.0 \pm 0.0$  in August to  $21.3 \pm 23.1$  in November ( $t = -3.03$ ,  $df = 11.0$ , two-sided significance = 0.01), while with the dose of 50 invasive larvae per insect it increased from  $4.7 \pm 5.3$  in August to  $37.2 \pm 26.1$  in November ( $t = -4.452$ ,  $df = 15.264$ , two-sided significance = 0.000). Differences in the intensity of infecting the insects by *Heterorhabditis* sp. in August and November also occurred at the temperature of 15°C, and they were most strongly marked with the highest nematode dose: in August the infection intensity was  $1.75 \pm 1.75$ , and in November  $32.4 \pm 34.0$  ( $t = -3.115$ ,  $df = 11.087$ , two-sided significance = 0.01) (Tab. 2, 4).

## DISCUSSION

The pupae of *Cameraria ohridella* are susceptible to the infection by entomopathogenic nematodes. Slight differences were found in the effectiveness of infecting the pupae by *Steinernema* sp. and *Heterorhabditis* sp. The studies on the

susceptibility of *C. ohridella* larvae showed that they are susceptible to the infection by *Steinernema* sp. and *Heterorhabditis* sp., with *Steinernema* sp. nematodes being more pathogenic (20).

Results of the experiments show that *C. ohridella* chrysalides are more susceptible to infection by entomopathogenic nematodes at the end of the vegetation period, when they are prepared for the over-wintering in the fallen leaves. The pupae of various developmental stages (5) are found in the leaves in summer months, which is due to a big number of generations in a year and unsynchronized developmental cycles of particular insects. Earlier studies showed that both the developmental stage and the age of insects within a given stage have a significant influence on the insects' sensitivity to infection by entomopathogenic nematodes (7, 12, 15). The studies found out no significant differences in the effectiveness of infecting *C. ohridella* pupae by *Steinernema* sp. and *Heterorhabditis* sp. at the temperatures of 15°C and 20°C. On the other hand, when the temperature grew from 20°C to 25°C, a statistically significant increase of the pathogenicity of nematodes *Steinernema* sp. and *Heterorhabditis* sp. was observed in relation to the larvae of *Cameraria ohridella* (20).

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