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SENSITIVITY OF *Agrotis exclamationis* L. (LEPIDOPTERA: NOCTUIDAE) LARVAE TO NATIVE STRAINS OF ENTOMOPATHOGENIC NEMATODES

Anna Mazurkiewicz[1], Magdalena Jakubowska[2], Dorota Tumialis[1], Elżbieta Pezowicz[1], Iwona Skrzecz[3]

[1] Warsaw University of Life Sciences – SGGW

[2] Institute of Plant Protection National Research Institute, Poznan, Poland

[3] Department of Forest Protection, Forest Research Institute, Raszyn, Poland

Abstract. Noctuinae are a challenge for effective control in vegetable crops. The species most dangerous for agricultural crops in central Europe are: *Agrotis segetum*, *A. exclamationis*, *Xestia c-nigrum*. The aim of presented study was to assess the sensitivity of *A. exclamationis* to 8 native strains of entomopathogenic nematode srepresenting two species: *Steinernema feltiae* (Filipjev) and *Heterorhabditis megidis* (Poinar, Jackson & Klein) in laboratory conditions. Presented study showed markedly higher effectiveness of strains Steinernematidae over the strain of *H. megidis*. Obtained results show that in the attempts to control *A. exclamationis*, selection of strain and nematode dose are equally important.

Key words: *Agrotis exclamationis*, biological control, native strains of *Steinernema* and *Heterorhabditis*

INTRODUCTION

Despite their well known biology, Noctuinae are still a challenge for effective control in vegetable crops. The species most dangerous for rooted plant crops are the turnip moth (*Agrotis segetum* Den. et Schiff.) and heart and dart moth (*A. exclamationis* L.). Moreover, the caterpillars of both species and of the lesser black-letter dart moth (*Xestia c-nigrum* L.) may cause large losses in agricultural crops, especially in beets, potatoes and cereals. Because of a hidden lifestyle, prolonged egg lying and caterpillar hatching, these pests are difficult to control and the use of chemical methods do not always bring

Corresponding author: Dorota Tumialis, Faculty of Animals Sciences, Department of Animal Environment Biology, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8 Street, 02-786 Warsaw, Poland, e-mail: dorota_tumialis@sggw.pl

expected effects [Kowalska and Jakubowska 2006]. The main problem to be solved is to estimate the optimum term to perform control measures [Garnis and Dąbrowski 2008, Jakubowska and Ławiński 2011]. The alternative to chemical crop protection might be the use of biological control methods. Biopreparations based on granulosis virus are successfully used worldwide [Jakubowska et al. 2005], moreover, studies on the application of bacteria, fungi and nematodes have been carried out for years [Yun et al. 2004, Sevim et al. 2010]. Insect control with nematodes is broadly used in North America, Europe and Japan [Ehlers 1996, Ansari et al. 2009]. Infective potential of entomopathogenic nematodes (EPNs) is great. For example *Steinernema carpocapsae* is associated with soil pests, particularly with those feeding on potato like: Coleoptera – Elateridae, Scarabaeidae and Lepidoptera – Noctuidae including the Noctuinae subfamily [Dzięgielewska and Erlichowski 2010]. Various studies are carried out to use of *Steinernema kraussei* and *S. websteri* against the turnip moth [Gökce et al. 2013, 2015] and *S. carpocapse* and *Heterorhabditis bacteriophora* against the black cutworm (*Agrotis ipsilon* Hufnagel) [Kunkel et al. 2004, Saleh et al. 2015].

The aim of presented study was to assess the sensitivity of *A. exclamationis* to 8 native strains of EPNs representing two species: *Steinernema feltiae* (Filipjev) and *H. megidis* (Poinar, Jackson and Klein) in laboratory conditions.

MATERIAL AND METHODS

Caterpillars of the heart and dart moth (*Agrotis exclamationis*) in various growth stages from L_4 to L_6 were used in laboratory tests. Caterpillars of this species were bred in two phytotrons at a constant daylight of 53 000 lux (SON-T Agro 400 W lamp), 6 : 12 hours N : D, temperature of 20 ±1ºC and relative air humidity about 70%. Larvae were bred from eggs of moths obtained in the field.

Cultures of caterpillars from the stage L_1 (newly hatched) to L_4 were carried out in plastic containers (10 individuals/container) with ventilation caps and lined with slightly wet circles of filter paper. From the stage L_4 the containers were filled in half with pure wet sand. Larvae were fed with sugar beet leaves and lettuce. The scale according to Kosobudzki [1949] was used to determine the growth stage of caterpillars.

In the experiment eight strains of EPNs were used representing 2 species (*Steinernema feltiae* and *Heterorhabditis megidis*) isolated in autumn 2011 from a soil sampled with the Egner probe (2.5 cm in diameter) to a depth of 30 cm. Each soil sample was composed of 4 probe contents mixed to obtain a sample uniform for a given site. Taken soil was placed in foil bags and brought to the laboratory.

Entomopathogenic nematodes were isolated with the soil trap method using a live bait (larvae of the greater wax moth *Galeria mellonella* L.) [Bedding and Akhurst 1975]. Invasive larvae of nematodes (IJs) isolated from *G. mellonella* were kept in water at 4ºC until their use in the experiment. Nematodes were identified to species based on morphometric criteria [Poinar 1990, Adams and Nguyen 2002] and with the use of genetic methods in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences.

The moth larvae (with a small fragment of sugar beet leaf) were placed in Petri dishes, 5 larvae per dish. Nematodes were applied at a rate of 30 and 50 IJs/larvae of

A. exclamationis and then Petri dishes were placed in the incubation chambers at 17 and 21ºC.

Each of strains was used to infect 30 larvae of *A. exclamationis* (5 individuals in 6 Petri dishes). Each experiment was repeated three times. Seventy two hours after infection, dead larvae were sectioned and the following parameters were determined with the use of binocular (magnification × 3–5):

– extensity of infection (percent of infected larvae in analyzed sample),

– intensity of infection (number of nematodes found in one infected larva).

The experiment was performed in two setups. In the first, the sensitivity of *A. exclamationis* larvae to 8 strains (*S. feltiae* ZAG 11, *S. feltiae* K 13, *S. feltiae* K 10, *S. feltiae* ZWO 21, *H. megidis* Wipsowo, *S. feltiae* ZAG 15, *S. feltiae* ZWO 4, *S. feltiae* ZWO 23) was compared with the use of one initial dose (50 IJs) at two temperatures (17 and 21ºC). In the second setup, the sensitivity of *A. exclamationis* larvae to 3 strains of EPNs (*S. feltiae* ZWO 21, *S. feltiae* K 13, *H. megidis* Wipsowo) was compared with the use of the lower dose (30 IJs) at two temperatures (17 and 21ºC).

A Chi-squared test was used to estimate the effect of temperature and initial dose of EPNs on the extensity of infection. To compare the numbers of EPNs found in one larva infected in different temperatures and with two doses of nematodes, the Mann-Whitney *U* test was applied. The results were statistically processed using STATISTICA 8.0 and PQStat 1.4.

RESULTS

No statistically significant of temperature effect was found ($p > 0.05$) on the extensity of infection within each of the studied strains (tab. 1). The temperature was significant for the intensity of infection ($p < 0.05$) in only one strain (ZWO 21), which showed higher intensity at 17ºC (tab. 1). Statistically significant differences ($p < 0.05$) were found among strains in the extensity of infection at both temperatures applied. At both 17 and 21ºC the most pathogenic strains were ZWO 21 (extensity 100 and 92.6%, respectively) and K 13 (92.6 and 85.2%, respectively). The lowest percent of extensity at 17ºC was noted for strain Wipsowo (4.8%) and at 21ºC for ZWO 23 (4.5%).

Table 1. Extensity and intensity of infection of *A. exclamationis* larvae at a dose of 50 IJs

4	Extensity				Intensity			
	temperature		χ^2	p^{**}	temperature		Z	p^{**}
	17ºC	21ºC			17ºC	21ºC		
ZAG 11	80.8[a*]	58.3[a]	2.99145	0.12394	6.5[a]	8.5[a]	1.4796	0.13896
K 13	92.6[a]	85.2[b]	0.75000	0.38648	6.4[a]	9.4[a]	-0.8505	0.39503
K 10	76.0[b]	81.5[b]	0.23384	0.62869	8.9[a]	11.5[a]	-0.6084	0.54287
ZWO 21	100[a]	92.6[b]	2.07692	1.14954	20.7[b]	15.7[b]	1.9748	**0.04828**
Wipsowo	4.8[c]	25.0[c]	3.49221	0.10117	2.0[c]	2.7[c]	-1.8220	0.06845
ZAG 15	55.2[bd]	39.3[ac]	1.44215	0.22979	8.8[a]	14.5[b]	1.4165	0.15662
ZWO 4	28.6[d]	33.3[c]	0.14595	0.70243	19.4[b]	18.6[b]	-0.4112	0.68085
ZWO 23	25.0[d]	4.5[d]	3.83522	0.06380	18.0[b]	5.0[c]	1.9898	0.50019

* – different letters show significant differences at $p \leq 0.05$

** – significant differences at $p \leq 0.05$

The intensity of infection significantly differed ($p < 0.05$) among strains at 17ºC and 21ºC (tab. 1). The highest intensity at 17ºC was observed for strains: ZWO 21 (20.7) and ZWO 4 (19.4). The strain ZWO 4 (18.6) showed the highest intensity also at 21ºC.

Table 2. Extensity of infection of *A. exclamationis* larvae in relation to the initial dose of EPNs and temperature

Strain	Temperature and dose of EPNs							
	17ºC				21ºC			
	30 IJs	50 IJs	χ^2	p^{**}	30 IJs	50 IJs	χ^2	p^{**}
ZWO 21	55.2[a*]	100[a]	15.7623	**0.00007**	16[a]	92.6[a]	30.8712	**0.00001**
K 13	25.0[b]	92.6[a]	24.3529	**0.00001**	26.9[a]	85.2[a]	17.3912	**0.00003**
Wipsowo	0[c]	4.8[b]	0.9279	0.33539	3.6[b]	25[b]	5.0939	**0.02401**

* – different letters show significant differences at $p \leq 0.05$
** – significant differences at $p \leq 0.05$

Table 3. Intensity of infection of *A. exclamationis* larvae in relation to the initial dose of EPNs and temperature

Strain	Temperature and dose of EPNs							
	17ºC				21ºC			
	30 IJs	50 IJs	Z	p^{**}	30 IJs	50 IJs	Z	p^{**}
ZWO 21	12.2[a*]	20.7[a]	-4.3007	**0.00002**	12.5[a]	15.7[a]	-4,9648	**0.00001**
K 13	5.2[b]	6.4[b]	-4.4255	**0.00001**	8.1[a]	9.4[b]	-3.7269	**0.00002**
Wipsowo	0[c]	2[c]	-0.9011	0.36752	2[b]	2.7[c]	-2.2127	**0.02691**

* – different letters show significant differences at $p \leq 0.05$
** – significant differences at $p \leq 0.05$

With the exception of strain Wipsowo at 17ºC in all setups the initial dose of nematodes exerted significant effect ($p > 0.05$) on the extensity and intensity of infection at two temperatures (tab. 2, 3). At a dose of 50 IJs, both analyzed parameters were higher.

DISCUSSION

Because of a few data on the sensitivity of *Agrotis* moth larvae, obtained results were also referred to other species of the family Noctuidae. Presented study showed higher effectiveness of strains Steinernematidae the strain of *H. megidis*. In the latter, the extensity of infection was 25% while higher values were obtained for strains *S. feltiae* ZWO 21 and K 13, whose extensity was 100 and 92.6%, respectively.

Kowalska and Jakubowska [2006] studied the sensitivity of *A. exclamationis* larvae to *H. megidis* and *S. bicornutum* demonstrated the reverse relationship: higher effectiveness of *H. megidis*, whose 2 doses caused 92.5 and 95% mortality while for *S. bicornutum* the effect was 42.5 and 72.5%, respectively. In studies by Salvadori et

al. [2012] on the sensitivity of *Spodoptera frugiperda* to 4 strains of Steinernematidae the mortality varied from 28 to 70% and the sensitivity to 4 strains of Heterorhabditidae manifested itself in 55 to 77% mortality. Maybe a higher effectiveness of nematodes of the genus *Heterorhabditis* noted by the cited authors could be explained by their choice of higher experimental temperatures (25– 30ºC), which is more favorable for the development of *Heterorhabditis*. This conclusion may be supported by studies of Gozel and Gunes [2013], who observed a great temperature-dependent variability in the extensity of infection of *Sesamia cretica* larvae by *H. bacteriophora*. At 15ºC the extensity of infection was 14% but achieved 94% at 30ºC. Saunders and Webster [1999] found a significant effect of temperature on pathogenicity for *H. megidis* and *S. carpocapse*. Both strains showed a higher potential for infection at higher temperatures (20–24ºC) than in the lower ones (8–16ºC). At the higher temperatures there were no statistically significant differences between the strains, while in the lower, a higher pathogenicity was found for of *S. carpcapsae*. The results presented in this paper confirm the higher efficacy of the strains from the genus *Steinernema* at lower temperatures.

Presented study was performed at lower temperatures (17 and 21ºC) because most of control measures against *Agrotis* larvae with the use of EPNs should be undertaken in Poland in spring and autumn, when air temperatures are lower.

In this study statistically significant differences were found in the extensity of infection between nematodes' doses. Despite the fact, that both applied doses were relatively small (30 IJs and 50 IJs) compared with those used by other authors, the difference in the extensity of infection markedly increased at the higher dose. Similar significant effect ($p < 0.05$) of the nematode dose was obtained by Gökce et al. [2013] who studied the sensitivity of *A. segetum* larvae to *S. kraussei*. At the highest dose (500 IJs) larval mortality was 97.8% and at a dose of 100 IJs it was 52.2%. Similar relationships were obtained by Kowalska and Jakubowska [2007] for *A. segetum* and by Abdel-Razek [2006] for *Spodoptera littoralis*.

Obtained results show that in the attempts to control *A. exclamationis*, selection of strain and nematode dose applied at appropriate temperature are equally important.

CONCLUSIONS

1. *Steinernema feltiae* is characterized by higher ability to infect the larvae of turnip moth, than *Heterorhabditis megidis*.
2. In both temperatures (17, 21ºC) there were found statistically significant differences in pathogenicity between the tested strains.
3. *Steinernema feltiae* is very efficient in infecting turnip moth with relatively low initial dose, despite used temperature.
4. The strains of *S. feltiae* ZWO 21 and *S. feltiae* K13 were the most pathogenic for *Agrotis segetum* larvae, regardless of tested temperature.
5. This study showed that some native strains of *Steinernema feltiae* can be successfully used as the agents in biological control of cultivated plants (for instance: beetroot, potatoes, grain, root crops) against pests from Noctuidae family.

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WRAŻLIWOŚĆ LARW *A. exclamationis* L. (LEPIDOPTERA: NOCTUIDAE) NA RODZIME SZCZEPY NICIENI ENTOMOPATOGENICZNYCH

Streszczenie. Skuteczne zwalczanie rolnic w uprawach warzyw stanowi ciągle duże wyzwanie. Najgroźniejszymi gatunkami dla upraw roślin korzeniowych są *Agrotis segetum* i *A. exclamationis*. Celem przedstawionych badań była ocena wrażliwości larw *A. exclamationis* na 8 rodzimych szczepów reprezentujących 2 gatunki: *Steinernema feltiae* (Filipjev) i *H. megidis* (Poinar, Jackson & Klein) w warunkach laboratoryjnych. W wyniku przeprowadzonych badań stwierdzono większą skuteczność szczepów Steinernematidae, niż szczepu *H. megidis.* Na podstawie wyników badań wnioskuje się, że w próbach zwalczania *A. exclamationis* znaczenie ma zarówno wybór szczepu, jak i dawka nicieni zastosowana w odpowiedniej temperaturze.

Słowa kluczowe: *Agrotis exclamationis*, biologiczna ochrona roślin, rodzime szczepy nicieni *Steinernema* i *Heterorhabditis*

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