Laboratory Bioassay of Selected Entomopathogenic Nematodes as Mortality Factors of *Oulema melanopus* (Coleoptera: Chrysomelidae)[1]

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Abstract The biological activity of entomopathogenic nematodes (EPNs) against the larvae and adults of the economically important *Oulema melanopus* (L.) (Coleoptera: Chysomelidae) was assessed under laboratory conditions. The EPNs assessed were five native isolates of the species *Steinernema feltiae* (Filipjev) and *Heterorhabditis megidis* (Poinar, Jackson, and Klein). *Steinernema feltiae* (isolate ZAG15) demonstrated the highest biological activity, causing 100% of mortality of larvae and beetles. The lowest activity occurred with *H. megidis* (isolate Wipsowo), with mortality that did not exceed 58%. At a concentration of 100 infective juveniles/adult, greatest mortality of beetles was observed at 25°C as opposed to lower temperatures for all EPN isolates except the Wipsowo isolate, which showed no statistical significance of mortality among the temperatures tested. At 15°C, adult beetle mortality was concentration-dependent with the *S. feltiae* isolates ZWO4 and ZAG15. However, neither concentration nor temperature had statistically significant effects on larval mortality.

Key Words *Oulema melanopus*, biological control, entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*

Cereal crops occupy about 70% of the total area of arable lands in Poland (Wenda-Piesik and Piesik 1998). These cereal monocultures are susceptible to economic infestations of pests, including two species of cereal leaf beetles (Coleoptera: Chrysomelidae): *Oulema melanopus* (L.) and *Oulema gallaeciana* (Heyden). Of the two, *O. melanopus* is more prevalent in central and northern Poland, while *O. gallaeciana* prevails in southeastern Poland. *Oulema melanopus* is also the most important pest of wheat in other countries (Dimitrijević et al. 1999, Karić 2003), occurs in central Siberia, Sweden, Great Britain, Spain, and western Africa (Olfert et al. 2004, Tanasković et al. 2012), and is reported in cereal crops in the United States and Canada (LeSage et al. 2007, Olfert et al. 2004). In Europe, apart from winter and spring wheat, cereal leaf beetles are economic pests of

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barley, oats, and triticale (Malschi 2009, Meindl et al. 2001, Ulrich et al. 2004). Damage caused by cereal leaf beetles may be manifested in foliar loss, tillering of young plants, reduction of nutrient assimilation by the host plant, and vectoring of viral diseases (Trzmiel et al. 2015).

Efforts to identify alternatives to chemical control of pests in cereal crop production include the assessment of biological control agents. Natural enemies of cereal leaf beetles include predatory arthropods, parasitoids, and predation by some bird species. Parasitoids thus far used in biological control of cereal leaf beetle larvae include *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), *Diaparsis carinifer* (Thomson), and *Lemophagus curtus* (Townes) (Hymenoptera: Ichneumonidae), while *Anaphes flavipes* (Foerster) (Hymenoptera: Mymaridae) has been assessed as an egg parasite (Evans et al. 2006, LeSage et al. 2007). Entomopathogenic nematodes (EPNs) should also be considered as they may also provide an environmentally friendly management approach for cereal production (Laznik et al. 2010b). Members of the Rhabditidae, Steinernematidae, and Heterorhabditidae families are obligatory generalist insect parasites with a free-living infective stage, that is, infective juveniles (IJs). Although their use in biological control has been traditionally for the control of soil-inhabiting pests (Ishibashi and Choi 1991), some research indicates their potential against foliar pests under special conditions (e.g., high concentrations combined with favorable abiotic factors of high humidity and optimum temperatures) (Laznik et al. 2012). Arthurs et al. (2004), among others, have shown the importance of selection of appropriate EPN species or strains.

The aim of the study presented here was to assess the biological activity of native isolates of *Steinernema feltiae* (Filipjev) and *Heterorhabditis megidis* (Poinar, Jackson, and Klein) against *O. melanopus* adults and larvae in laboratory assays. Laboratory assays provide information necessary to estimate specificity of species/strains of EPNs to crop pests (Tóth 2006). As yet, no studies have been conducted to compare the potential of controlling the cereal leaf beetle with native isolates of EPNs in Poland. This also represents the first attempt to compare susceptibility of cereal leaf beetle adults and larvae to EPNs.

Materials and Methods

Cereal leaf beetles. *Oulema melanopus* adults used in the laboratory assays were collected in May from winter wheat and winter barley growing on experimental plots at the Institute of Plant Protection, State Research Institute, in Winna Góra, near Poznań, central Poland (N52°12′21.5″; E17°26′51.2″ 83 m elevation). Beetles were captured every second day during a 2-week period until egg laying by females was observed on the host plants.

Larvae used in the assays were obtained from a laboratory colony established by placing 11 pairs of field-collected adult beetles captured during the period of mating and egg laying on each of nine pots of tillering wheat plants. These were maintained in environmental chambers at an illumination of 53,000 lux (lamp SON-T Agro 400 W) on an 18/6-h photo regime at a temperature of 20°C ± 1°C and a relative humidity of 50–60%. After an egg-laying period of 7–10 d, living beetles

Table 1. Isolates used in experiments.

Species	Isolate	Sampling Site	Geographic Coordinates
Steinernema feltiae	ZWO23	Meadow, the Zwolenka River valley (Kozienicka Forest)	N51°23′11.2″, E21°33′10.1″
Steinernema feltiae	ZAG15	Meadow, the Zagożdżonka River valley (Kozienicka Forest)	N51°23′10.4820″, E21°33′15.5412″
Steinernema feltiae	K13	Field (*Miscantus giganteus* crop) (Silesia Region)	N50°15′58.68″, E19°5′52.08″
Steinernema feltiae	ZWO4	Meadow, the Zwolenka River valley (Kozienicka Forest)	N51°23′10.5″, E21°33′33.7″
Heterorhabditis megidis	Wipsowo	Wheat field (Olsztyn Lakeland)	N53°54′32.0″, E20°47′54.4″

were removed from the pots. Egg hatching and larval development (1 mm, 2 mm, 4 mm, and >4 mm in length) was monitored daily.

Nematodes. Five native isolates of EPNs representing either *S. feltiae* or *H. megidis* were assayed (Table 1). These were isolated from soil samples in 2010 and 2011 using the Egner's cane sampling device. Each soil core was 2.5 cm diameter and 30 cm deep. Four cores were obtained at each site, mixed, and placed in a plastic bag for transport to the laboratory. EPNs were isolated from these samples using *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae as "nematode traps" (Bedding and Akhurst 1975). White (1927) traps were used to collect IJs of each nematode isolate by placing the traps in an incubator at 25°C for 2 weeks, after which IJs were pipetted into tissue culture bottles which were then maintained at 4°C. IJs were then used to infect additional *G. mellonella* larvae to maintain cultures of each isolate. Species identification was performed based on morphometric criteria (Adams and Nguyen 2002, Poinar 1990) and genetic methods conducted at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Wroclaw, Poland). Nematode cultures were subsequently maintained on *G. mellonella* larvae as per Kaya and Stock (1997).

Assays. The two concentrations of EPNs assayed against *O. melanopus* adults were 100 and 500 IJs per beetle. The concentrations assayed against larvae were 50 and 100 IJs per larva. Each of these concentrations for each insect stage was assayed at two temperatures (15°C and 25°C). In the assays, five adult or larval (3-mm length) beetles were placed in individual 9-cm-diameter petri dishes lined with moist filter paper along with several pieces of wheat leaves. Appropriate concentrations of IJs of the nematode isolates were then applied to the filter paper in the petri dishes. Controls were treated with distilled water only. Treated dishes were placed in chambers held at either 15°C or 25°C. Each of the 60 treatment combinations (isolate × insect stage × nematode concentration × temperature) was replicated 11 times. Insect mortality in each of the treatments was determined 48, 72, 96, and 120 h after initial exposure. Parasitism by the EPNs was confirmed by microscopic examination of dead insects.

Statistical analyses. Mortality data were statistically analyzed using of Statistica, version 12, of StatSoft® Polska (Kroków, Poland). A Pearson chi-square test was used to determine the relationship between two variables.

Results

Adult mortality response. At a dose of 100 IJs/adult, temperature significantly affected adult mortality caused by all EPN isolates tested, with the exception of *H. megidis* Wipsowo, with higher mortality levels at 25°C (Table 2). At 500 IJs/adult, the higher temperature resulted in higher adult mortality caused by the *S. feltiae* ZWO4, ZAG 15, and K13 isolates after 48 and 72 h of exposure, and the higher temperature increased adult mortality caused by the *S. feltiae* ZAG15 and K13 isolates 96 and 120 h after exposure. Higher temperature did not increase adult mortality by the *S. feltiae* ZWO23 isolate, with the exception of mortality after 96 h, which was higher at 25°C (Table 2).

At 15°C, adult mortality of beetles was found to be dose-dependent with the *S. feltiae* ZWO4, ZAG15, and K13 isolates regardless of the length of time after exposure, while the dose-dependent mortality response to the *S. feltiae* ZWO23 isolate was observed only at 48 h after application but with no effect observed at 72, 96, and 120 h (Table 3).

Variable results were obtained at 25°C. With the *S. feltiae* ZWO23 isolate, the higher dose had no effect on adult mortality after 48 and 72 h, but caused significantly higher mortality after 96 and 120 h. No dose-mortality effect was found with the *S. feltiae* ZWO4 isolate. With the *S. feltiae* ZAG15 isolate, the higher dose caused significantly higher mortality 96 h after application, but had no effect on mortality at 120 h. Likewise, the higher dose of the *S. feltiae* K13 isolate resulted in higher mortality of insects at 48 h but had no effect at 72 to 120 h. The higher dose of the *H. megidis* Wipsowo isolate resulted in higher adult mortality regardless of length of time after initial exposure (Table 3).

Overall, of the EPN isolates tested, the most pathogenic appeared to be the *S. feltiae* ZAG 15 isolate, which caused 100% mortality of *O. melanopus* adults at a temperature of 25°C and a dose of 500 IJs/adult 48 h following initial exposure. A relatively high level of mortality (84.4%) was also caused by exposure to a lower dose of IJs. A similar level of mortality (81.3%), but at higher dose, occurred following exposure to the *S. feltiae* K13 isolate.

At 15°C, mortality caused by the *S. feltiae* ZWO4 was only 6.5% at a dose of 100 IJs/adult and 30% at a dose of 500 IJs/adult at 48 h after exposure. At 72 h after application at 15°C, the *S. feltiae* ZWO4 isolate remained as the lowest performer at 100 IJs/adult, causing only 16.1% mortality, while the highest mortality of 79.3% was achieved with the *S. feltiae* ZAG15 at a dose of 500 IJs/adult. At 96 h, the *S. feltiae* ZAG15 isolate caused 82.8% mortality at the higher dose, while the *S. feltiae* ZWO23 isolate caused only 33.3% at the lower dose. The highest mortality observed at 120 h after application was 86.2% caused by the *S. feltiae* ZAG15 isolate at the higher dose, while the *S. feltiae* ZWO23 and K13 isolates caused 32.3% mortality applied at lower doses.

At 25°C, the *S. feltiae* K13 isolate was most pathogenic, causing 84.8% mortality at the higher dose. The weakest performer was the *H. megidis* Wipsowo isolate,

Table 2. The effect of temperature on the mortality (%) of cereal leaf beetle adults by entomopathogenic nematodes (EPNs) applied at two doses. IJs = infective juveniles.

Hours after EPN Application	Isolate	Temperature and Doses of EPNs							
		100 IJs/Adult				500 IJs/Adult			
		15°C	25°C	χ^2	*P**	15°C	25°C	χ^2	*P**
48	ZWO 23	9.7	34.1	5.86	0.0155	33.3	40.0	0.29	0.5921
	ZWO4	6.5	77.4	32.06	0.0000	30.0	70.6	10.51	0.0012
	ZAG15	43.8	84.4	11.47	0.0007	75.9	100	8.22	0.0042
	K13	22.6	59.4	8.80	0.0030	40.0	81.8	11.65	0.0006
	Wipsowo	30.3	27.3	0.07	0.7857	30.3	54.5	3.97	0.0463
72	ZWO 23	25.8	46.3	3.18	0.0747	33.3	56.7	3.30	0.0693
	ZWO4	16.1	77.4	23.39	0.0000	46.7	73.5	4.83	0.0280
	ZAG15	56.9	87.5	11.98	0.0005	79.3	100	6.91	0.0086
	K13	29.0	68.8	9.94	0.0016	53.3	84.8	7.41	0.0065
	Wipsowo	36.4	33.3	0.07	0.7962	36.4	57.6	2.98	0.0843
96	ZWO 23	29.0	53.7	4.37	0.0367	33.3	80.0	13.30	0.0003
	ZWO4	32.3	77.4	12.76	0.0004	63.3	76.5	1.32	0.2510
	ZAG15	59.4	87.5	6.49	0.0109	82.8	100	5.65	0.0174
	K13	32.3	68.8	8.39	0.0038	60	84.8	4.92	0.0265
	Wipsowo	42.4	33.3	0.07	0.7962	42.4	57.6	1.52	0.2184
120	ZWO 23	32.3	56.1	4.04	0.0004	40.0	80.0	2.86	0.0910
	ZWO4	35.5	77.4	11.09	0.0009	80.0	76.5	0.34	0.5586
	ZAG15	59.4	87.5	5.13	0.0236	86.2	100	4.44	0.0351
	K13	32.3	68.8	8.39	0.0038	63.3	84.8	3.84	0.0501
	Wipsowo	42.4	33.3	0.07	0.7962	42.4	57.6	1.52	0.2184

* Significant differences at $P \leq 0.05$.

which caused only 33.3% mortality at a dose of 100 IJs/adult. Longer exposure of the adults to the EPNs at this temperature did not alter mortality levels observed.

Larval mortality response. Of the EPN isolates tested, the *H. megidis* Wipsowo isolate caused the greatest larval mortality (89.3–100%) after 48 h at 25°C. The *H. megidis* Wipsowo isolate also caused the lowest mortality response (34.4%) at a temperature of 15°C and a dose of 50 IJs/larva after 48 h of exposure, but the same isolate caused 96.7% larval mortality at a dose of 100 IJs/larva after 72 h of exposure at the same temperature.

Table 3. The effect of entomopathogenic nematode (EPN) doses applied at two temperatures on the mortality (%) of cereal leaf beetle adults. IJs = infective juveniles.

Hours after EPN Application	Isolate	Temperature and Doses of EPNs							
		15°C				25°C			
		100 IJs/Adult	500 IJs/Adult	χ^2	*P**	100 IJs/Adult	500 IJs/Adult	χ^2	*P**
48	ZWO 23	9.7	33.3	5.09	0.0241	34.1	40.0	0.26	0.6131
	ZWO4	6.5	30.0	5.72	0.0168	77.4	70.6	0.39	0.5314
	ZAG15	43.8	75.9	6.49	0.0109	84.4	100	5.10	0.0240
	K13	22.6	40.0	2.16	0.1419	59.4	81.8	3.96	0.0467
	Wipsowo	30.3	30.3	0.00	1.0000	27.3	54.5	5.08	0.0243
72	ZWO 23	25.8	33.3	0.42	0.5193	46.3	56.7	0.74	0.3900
	ZWO4	16.1	46.7	6.63	0.0100	77.4	73.5	0.13	0.7161
	ZAG15	56.9	79.3	6.81	0.0090	87.5	100	4.01	0.0453
	K13	29.0	53.3	3.72	0.0537	68.8	84.8	2.37	0.1236
	Wipsowo	36.4	36.4	0.00	1.0000	33.3	57.6	3.91	0.0480
96	ZWO 23	29.0	33.3	0.13	0.7169	53.7	80.0	5.27	0.0217
	ZWO4	32.3	63.3	5.90	0.0151	77.4	76.5	0.01	0.9277
	ZAG15	59.4	82.8	4.00	0.0455	87.5	100	4.01	0.0453
	K13	32.3	60.0	4.73	0.0297	68.8	84.8	2.37	0.1236
	Wipsowo	42.4	42.4	0.00	1.0000	33.3	57.6	3.91	0.0480
120	ZWO 23	32.3	40.0	0.07	0.7884	56.1	80.0	4.42	0.0355
	ZWO4	35.5	80.0	7.28	0.0070	77.4	76.5	0.01	0.9277
	ZAG15	59.4	86.2	5.45	0.0196	87.5	100	4.01	0.0453
	K13	32.3	63.3	5.90	0.0151	68.8	84.8	2.37	0.1236
	Wipsowo	42.4	42.4	0.00	1.0000	33.3	57.6	3.91	0.0480

* Significant differences at $P \leq 0.05$.

At a dose of 50 IJs/larva, temperature had no effect on the mortality of larvae 48 h after application irrespective of the EPN isolate. At a dose of 100 IJs/larva, the higher temperature resulted in higher mortality of larvae only with the *S. feltiae* ZAG 15 and K13 isolates after 48 h (Table 4). No effect of dose was observed with larval mortality at both temperatures tested. The sole exception was the mortality of larvae following exposure to the *S. feltiae* Wipsowo isolate at 15°C at 48 h (Table 5).

No adult or larval mortality was observed in the control treatments regardless of temperature or EPN dose.

Table 4. The effect of temperature on the mortality (%) of cereal leaf beetle larvae by entomopathogenic nematodes (EPNs) applied at two doses. IJs = infective juveniles.

Hours after EPN Application	Isolate	Temperature and Doses of EPNs							
		50 IJs/Larva				100 IJs/Larva			
		15°C	25°C	χ^2	*P**	15°C	25°C	χ^2	*P**
48	ZWO 23	90.3	89.3	0.02	0.8953	96.6	92.6	0.43	0.5109
	ZWO4	86.7	96.4	0.43	0.5109	89.7	96.6	1.07	0.3000
	ZAG15	79.3	89.3	0.76	0.3845	81.8	100	6.60	0.0102
	K13	93.8	93.3	0.00	0.9468	94.6	96.4	0.12	0.7271
	Wipsowo	34.4	83.3	15.24	0.0001	76.7	89.3	1.62	0.2036
72	ZWO 23	100	100	0.00	1.0000	100	100	0.00	1.0000
	ZWO4	100	100	0.00	1.0000	100	100	0.00	1.0000
	ZAG15	100	100	0.00	1.0000	100	100	0.00	1.0000
	K13	100	100	0.00	1.0000	100	100	0.00	1.0000
	Wipsowo	93.8	100	2.86	0.0906	96.7	100	0.95	0.3298

* Significant differences at $P \leq 0.05$.

Discussion

Our previous studies of the susceptibility of *Pieris* spp., *Agrotis exlamationis* (L.), and *Hylobius abietis* (L.) to native EPN isolates demonstrated that hosts differ in their susceptibility to isolates (Mazurkiewicz et al. 2016, 2017; Tumialis et al. 2013). Thus, we recognize the need to assay selected EPNs against each target pest. In the study reported herein, the most effective EPN isolate tested against *O. melanopus* was *S. feltiae* ZAG 15, while the *H. megidis* Wipsowo isolate was the least effective, causing mortality levels that did not exceed 58%.

Laznik et al. (2010a, b) also reported that the native *Steinernema carpocapsae* C101 isolate caused 100% mortality of cereal leaf beetle adults within 48 h of exposure to a dose of 500 IJs/adult. The *Heterorhabditis bacteriophora* D54 isolate was the least effective at temperatures of 15°C and 20°C. Laznik et al. (2010a, b) also found similar patterns of mortality response to EPN dose and duration of exposure, as we noted in our study.

Laznik et al. (2010c) and Trdan et al. (2009) indicate that 25°C is the optimum temperature for *S. feltiae* strains used to manage the rice weevil, *Sitophilus oryzae* (L.). Similarly, Trdan et al. (2008) found that both steinernematids and heterorhabditids were more effective at 25°C than at 15°C or 20°C, especially when applied at higher doses, against *Phyllotreta* spp. adults. Our results corroborate those findings as we also found that the EPN isolates we tested were more effective at the higher temperatures tested.

Table 5. The effect of entomopathogenic nematode (EPN) doses applied at two temperatures on the mortality (%) of cereal leaf beetle larvae. IJs = infective juveniles.

Hours after EPN Application	Isolate	Temperature and Doses of EPNs							
		15°C				25°C			
		50 IJs/Larva	100 IJs/Larva	χ^2	*P**	50 IJs/Larva	100 IJs/Larva	χ^2	*P**
48	ZWO 23	90.3	96.6	0.93	0.3337	89.3	92.6	0.18	0.6698
	ZWO4	86.7	89.7	0.13	0.7227	96.4	96.6	0.00	0.9798
	ZAG15	79.3	81.8	0.06	0.8031	89.3	100	3.72	0.0538
	K13	93.8	94.6	0.02	0.8810	93.3	96.4	0.28	0.5948
	Wipsowo	34.4	76.7	11.18	0.0008	83.3	89.3	0.43	0.5112
72	ZWO 23	100	100	0.00	1.0000	100	100	0.00	1.0000
	ZWO4	100	100	0.00	1.0000	100	100	0.00	1.0000
	ZAG15	100	100	0.00	1.0000	100	100	0.00	1.0000
	K13	100	100	0.00	1.0000	100	100	0.00	1.0000
	Wipsowo	93.8	96.7	0.88	0.3493	100	100	0.00	1.0000

* Significant differences at $P \leq 0.05$.

Our results and those of Laznik et al. (2010b) demonstrate the potential for using EPNs for management of the cereal leaf beetle. Other studies with other beetle species support this possibility. Campos-Herrera and Gutiérrez (2009) and Wright et al. (1987) obtained less than desirable levels of mortality (30–40%) of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larvae treated with *S. feltiae*. Tumialis et al. (2013), however, reported larval mortality exceeding 80% after challenging large pine weevils, *H. abietis,* with isolates of *S. feltiae* and *Steinernema kraussei* Steiner. Mortality ranging from 78 to 94% were observed by Santos et al. (2011) following exposure of *Diabrotica speciosa* Germar larvae to various isolates of *Heterorhabditis* sp. and *Steinernema glaseri* Steiner. And, Yadav and Lalramliana (2012) reported 100% mortality of *Aplosonyx chalybaeus* (Hope) larvae treated with isolates of *Heterorhabditis indica* Poinar, Karunakar and David, *Steinernema thermophilum* Ganguly and Singh, and *S. glaseri*.

Beetle larvae, in general, appear to be more susceptible to EPNs than adults (Toepfer et al. 2005, Trdan et al. 2009). Determination of appropriate EPN dose might be affected by the presence of coat of fecal wastes on larvae (Kher et al. 2011), which might be a barrier to penetration of EPN IJs.

In our opinion, an important objective of laboratory bioassays as reported herein is the selection of EPN isolates as potential biological control agents. The study by Santos et al. (2011) demonstrated that mortality obtained in laboratory bioassays may translate to effective (e.g., 70% mortality) levels of biological control in crops. Similarly, Laznik et al. (2010d) proved that an *S. feltiae* isolate identified in

laboratory studies was effective in field trials against Colorado potato beetle larvae. Those results also showed efficacy of *S. feltiae* against the foliage-feeding pest (Laznik et al. 2010d). Therefore, our laboratory assays indicate that native isolates of EPNs have potential as biological control agents of *O. melanopus* in crops.

Additional research is required to further develop these EPN isolates for these uses.

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References Cited

Adams, B.J. and K.B. Nguyen. 2002. Taxonomy and systematics, Pp. 1–33. *In* Gaugler, R. (ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, U.K.

Arthurs, S., K.M. Heinz and J.R. Prasifka. 2004. An analysis of using entomopathogenic nematodes against above-ground pests. Bull. Entomol. Res. 94: 297–306.

Bedding, R.A. and R.J. Akhurst. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21: 109–110.

Campos-Herrera, R. and C. Gutiérrez. 2009. A laboratory study on the activity of *Steinernema feltiae* (Rhabditida: Steinernematidae) Rioja strain against horticultural insect pests. J. Pest Sci. 82: 305–309.

Dimitrijević, B., M. Jelić and S. Lomović. 1999. The effect of mineral nutrition on the damage degree of spring wheat by *Lema melanopus* L. (Coleoptera: Chrysomelidae). Acta Entomol. Serbica 4: 49–55.

Evans, E.W., J.B. Karren and C.E. Israelsen. 2006. Interactions over time between cereal leaf beetle (Coleoptera: Chrysomelidae) and larval parasitoid *Tetrastichus julis* (Hymenoptera: Eulophidae) in Utah. J. Econ. Entomol. 99: 1967–1973.

Ishibashi, N. and D.R. Choi. 1991. Biological control of soil pests by mixed application of entomopathogenic and fungivorous nematodes. J. Nematol. 23: 175–181.

Karić, N. 2003. Effects of temperature on the development of *Oulema melanopus* L. (Coleoptera: Chrysomelidae). Works Fac. Agric. Univ. Sarajevo 48: 57–68.

Kaya, H.K. and S.P. Stock. 1997. Techniques in insect nematology, Pp. 281–324. *In* Lacey, L.A. (ed.), Manual of Techniques in Insect Pathology. Academic Press, London.

Kher, S.V., L.M. Dosdall and H.A. Carcamo. 2011. The cereal leaf beetle: Biology, distribution and prospects for control. Insects Dis. 4: 32–41.

Laznik, Ž., M. Štrukelj and S. Trdan. 2010a. Activity of entomopathogenic nematodes (Rhabditida) against cereal leaf beetle (*Oulema melanopus* (L.), Coleoptera, Chrysomelidae) adults under laboratory conditions. Acta Agr. Sloven. 95: 141–148.

Laznik, Ž., T. Tóth, T. Lakatos, M. Vidrih and S. Trdan. 2010b. *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) adults are susceptible to entomopathogenic nematodes (Rhabditida) attack: Results from a laboratory study. J. Plant Dis. Protect. 117: 30–32.

Laznik, Ž., T. Tóth, T. Lakatos, M. Vidrih and S. Trdan. 2010c. The activity of three new strains of *Steinernema feltiae* against adults of *Sitophilus oryzae* under laboratory conditions. J. Food Agric. Environ. 8: 150–154.

Laznik, Ž., T. Tóth, T. Lakatos, M. Vidrih and S. Trdan. 2010d. Control of the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) on potato under field conditions: A comparison of the efficacy of foliar application of two strains of *Steinernema feltiae* (Filipjev) and spraying with thiametoxam. J. Plant Dis. Protect. 117: 129–135.

Laznik, Ž., M. Vidrih, F. Vučajnk and S. Trdan. 2012. Is foliar application of entomopathogenic nematodes (Rhabditida) an effective alternative to thiametoxam in controlling cereal leaf beetle (*Oulema melanopus* L.) on winter wheat? J. Food Agric. Environ. 10: 716–719.

LeSage, L., E.J. Dobesberger and C.G. Majka. 2007. Introduced leaf beetles of the Maritime Provinces, 2: The cereal leaf beetle *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae). Proc. Entomol. Soc. Wash. 109: 286–294.

Malschi, D. 2009. Integrated pest management in relation to environmental al sustainability. Part I: Ecological management of wheat pests. Course notes and practical applications. Bioflux Publishing House Cluj-Napoca. 04 June 2018 (http://www.editura.bioflux.com.ro/docs/malschi.I.pdf).

Mazurkiewicz, A., M. Jakubowska, D. Tumialis, E. Pezowicz and I. Skrzecz. 2016. Sensitivity of *Agrotis exclamationis* L. (Lepidoptera: Noctuidae) larvae to native strains of entomopathogenic nematodes. Acta Sci. Pol. Hortorum Cultus 15: 121–127.

Mazurkiewicz, A., D. Tumialis, E. Pezowicz, I. Skrzecz and G. Błażejczyk. 2017. Sensitivity of *Pieris brassicae*, *P. napi* and *P. rapae* (Lepidoptera: Pieridae) larvae to native strains of *Steinernema feltiae* (Filipjev, 1934). .J. Plant Dis. Protect. 124: 521–524.

Meindl, P., B. Kromp, B. Bartl and E. Ioannidou. 2001. Arthropod natural enemies of the cereal leaf beetle (*Oulema melanopus* L.) in organic winter wheat fields in Vienna, Eastern Austria. IOBC Bull. 24: 79–86.

Olfert, O., R.M. Weiss, S. Woods, H. Philip and L. Dosdall. 2004. Potential distribution and relative abundance of an invasive cereal crop pest, *Oulema melanopus* L. (Coleoptera: Chrysomelidae). Can. Entomol. 36: 277–287.

Poinar, G.O. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae, Pp. 23–58. *In* Gaugler, R. and H.K. Kaya (eds.), Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL.

Santos, V., A. Moino, Jr., V. Andaló, C.C. Moreira and R.A. Olinda. 2011. Virulence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) for the control of *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae). Cienc. Agrotecnol. 35: 1149–1156.

Tanasković, S., M. Madić, D. Đurović, D. Knežević and F. Vukajlović. 2012. Susceptibility of cereal leaf beetle (*Oulema melanopa* L.) in winter wheat to various foliar insecticides in western Serbia region. Rom. Agric. Res. 29: 361–366.

Toepfer, S., C. Gueldenzoph, R.U. Ehlers and U. Kuhlmann. 2005. Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe. Bull. Entomol. Res. 95: 473–482.

Tóth, T. 2006. Collection of entomopathogenic nematodes for the biological control of insect pests. J. Fruit Ornam. Plant Res. 14: 225–230.

Trdan, S., M. Vidrih, L. Andjus and Ž. Laznik. 2009. Activity of four entomopathogenic nematode species against different developmental stages of Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). Helminthologia 46: 14–20.

Trdan, S., M. Vidrih, N. Valič and Ž. Laznik. 2008. Impact of entomopathogenic nematodes on adults of *Phyllotreta* spp. (Coleoptera: Chrysomelidae) under laboratory conditions. Acta Agric. Scand. Sect. B Soil Plant Sci. 58: 169–175.

Trzmiel, K., B. Wielkopolan and M. Lewandowska. 2015. Using RT-PCR assay for the detection of *Brome mosaic virus* (BMV) in cereal leaf beetle (*Oulema melanopus* L.). Zastosowanie techniki RT-PCR do wykrywania wirusa mozaiki stokłosy (*Brome mosaic virus*, BMV) w skrzypionce zbożowej (*Oulema melanopus* L.). Progr. Plant Protect. 55: 494–497.

Tumialis, D., L. Skrzecz, A. Mazurkiewicz, E. Pezowicz and K. Góral. 2013. Sensitivity of *Hylobius abietis* (L.) larvae on native species and isolates of entomopathogenic nematodes. Sylwan 157: 769–774.

Ulrich, W., A. Czarnecki and T. Kruszyński. 2004. Occurrence of pest species of the genus *Oulema* (Coleoptera: Chrysomelidae) in cereal fields in Northern Poland. Electron. J. Pol. Agric. Univ. 7: 1–6.

Wenda-Piesik, A. and D. Piesik. 1998. The spring cereals food preferences of *Oulema* ssp. in pure and mixed crops. Electron. J. Pol. Agric. Univ. 1: 1–7.

White, G.F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66: 302–303.

Wright, R.J., F. Agudelo-Silva and R. Georgis. 1987. Soil applications of steinernematid and heterorhabditid nematodes for control of Colorado potato beetles, *Leptinotarsa decemlineata* (Say). J. Nematol. 19: 201–206.

Yadav, K.A. and Lalramliana. **2012.** Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope). J. Parasit. Dis. 36: 149–154.