Nontarget Effect of Entomopathogenic Nematodes on Larvae of Twospotted Lady Beetle (Coleoptera: Coccinellidae) and Green Lacewing (Neuroptera: Chrysopidae) Under Laboratory Conditions

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ABSTRACT The nontarget effect of *Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, and three mixed suspensions of two species of entomopathogenic nematodes on the larvae of the twospotted lady beetle, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), and on the larvae of the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), were studied in a laboratory bioassay. The assay was performed at three temperature (15, 20, and 25°C) and at three different concentrations of the suspension (500, 2,500, and 5,000 infective juveniles [IJs]/ml). The larvae of *A. bipunctata* were more susceptible to nematode attack than the larvae of *C. carnea*. Four days after treatment, significantly the lowest mortality of *A. bipunctata* and *C. carnea* larvae was recorded at 15°C, whereas no significant differences were noted between 20 and 25°C. At 500 IJs/ml, the nematodes had significantly the lowest nontarget effect on the larvae of both aphid predators, whereas no significant differences in this regard were established between 2,500 and 5,000 IJs/ml. We conclude that the entomopathogenic nematodes under investigation exhibit a pronounced nontarget effect on the larvae of both predators mentioned.

KEY WORDS *Chrysoperla carnea*, *Adalia bipunctata*, laboratory bioassay, predators, entomopathogenic nematodes

Entomopathogenic nematodes have been proven effective in controlling some foliar pests (Trdan et al. 2009), but they do have some negative properties, e.g., the wide spectrum of their efficacy includes a negative influence on beneficial organisms (Hazir et al. 2003). Up to now, studies on the nontarget effects of entomopathogenic nematodes have been performed on various species of nontarget organisms, and a large range—from complete harmlessness to pronounced harmful effect—was established (Bathon 1996, Farag 2002, Powell and Webster 2004). The results of some field trials show a moderate influence of entomopathogenic nematodes on nontarget arthropods or even the absence of such an effect (Georgis et al. 1991). Bathon (1996) reported that mortality can be observed among the nontarget organisms, but the influence of these agents should be temporary and local and so only a part of the population is under attack. Georgis et al. (1991) demonstrated a negligible influence of entomopathogenic nematodes on nontarget organisms if they are used only in short term pest control.

Farag (2002) reported a high mortality of the larvae of *Coccinella undecimpunctata* L. caused by *Heterorhabditis taysearae* Shamseldean and *Steinernema carpocapsae* strain S2 in a laboratory assay, so Farag does not recommend the use of entomopathogenic nematodes when these predators are present on the plants in high number. Likewise, *Heterorhabditis bacteriophora* Poinar and *Steinernema carpocapsae* (Weiser) were— under laboratory conditions—very harmful to the following predators: *Coleomegilla maculata* (De Geer), *Olla v-nigrum* (Mulsant), *Harmonia axyridis* (Pallas), and *Coccinella septempunctata* L. In contrast, Shapiro-Ilan and Cottrell (2005) found lady beetles to be substantially less susceptible to nematode infection compared with a known susceptible insect, the black cutworm [*Agrotis ipsilon* (Hüfnagel)].

The twospotted lady beetle, *Adalia bipunctata* (L.), and the lacewing *Chrysoperla carnea* (Stephens) are important beneficials in agriculture. *A. bipunctata* lives on trees higher that 2 m, so it is used in biological control against aphids (Aphididae) in orchards, vineyards, and ornamental plants (Pervez 2005). The larvae of *C. carnea* are predators of some pests on cultivated plants, such as aphids, mites (Acarina), thrips (Thysanoptera), the greenhouse whitefly [*Trialeurodes vaporariorum* (Westwood)], small caterpillars, coleopteran larvae, and some other species (Milevoj

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1999). Because data on the nontarget effect of entomopathogenic nematodes on the mentioned two predators are scarce, we wanted to provide such data with our investigation.

Our investigation was aimed at establishing the degree of the nontarget effect of selected species of entomopathogenic nematodes on the larvae of two of the most important predators of aphids in Europe. The assay was performed under laboratory conditions because the species of entomopathogenic nematodes under investigation—at the time of the experiment—still had the status of exotic organisms. This investigation established the foundations for studying the nontarget effect of entomopathogenic nematodes on beneficial organisms in Europe. This can be upgraded by field assays because of the recent finding of entomopathogenic nematodes species that are new in Slovenia (Laznik et al. 2008a,b). Such studies will yield valuable information on more rational use of entomopathogenic nematodes in growing food, fodder, or ornamental plants.

Materials and Methods

Entomopathogenic Nematodes, Predators, and Aphids. Three species of entomopathogenic nematodes were used in this study: *Steinernema feltiae* (Filipjev), *S. carpocapsae*, and *Heterorhabditis bacteriophora* Poinar; larvae of the lacewing and twospotted lady beetle were treated. All the agents were provided by Koppert B. V. (Berkel en Rodenrijs, The Netherlands) as commercial bioinsecticides Entonem, Capsanem, Larvanem, Chrysopa, and Aphidalia. The following leaf aphids from the following cultivated and wild-growing plants (collected at the Experimental Field of the Biotechnical Faculty in Ljubljana, Slovenia, 46° 04′N, 14° 31′E, 299 m above sea level) were used as the main food source of the twospotted lady beetle and lacewing larvae during laboratory bioassay: *Brevicoryne brassicae* (L.) from *Brassica oleracea* L. variety *gemmifera* DC./Zenk, *Macrosiphoniella millefolii* (De Geer) from *Achillea millefolium* L., and *Aphis craccivora* Koch from *Vicia cracca* L.

Laboratory Bioassay. The assay was carried out in special petri dishes (7 cm in diameter; for details regarding the special petri dishes and the bioassay procedure, see Rojht 2007 and Trdan et al. 2009), which were placed into a rearing chamber at 85% RH and constant darkness. Nematode treatments included single species treatments and mixed species treatments (1:1) (*S. feltiae* × *S. carpocapsae*, *S. feltiae* × *H. bacteriophora*, and *S. carpocapsae* × *H. bacteriophora.*) at concentrations of 500, 2,500, and 5,000 infective juveniles [IJs]/ml, and each treatment × concentration was replicated five times at 15, 20, and 25°C. Five control petri dishes (just water was added with no nematodes) were evaluated at each rearing temperature. Efficacy was evaluated by counting dead larvae 4 d after application. If necessary, food for larvae was added 2 d after treatment.

Statistical Analysis. A multifactor analysis of variance (ANOVA) was conducted to determine differences in mortality rates (percentage) between the larvae of both predators, assayed with different treatments at three different temperature. Before the analysis, each variable was tested for homogeneity of treatment variances. Mortality rate data were corrected for control mortality using Abbott's formula (Abbott 1925) and normalized using the arcsine square-root transformation before analysis. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments (Hoshmand 2006). All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, MD). The data are presented as untransformed means.

Table 1. ANOVA results for corrected mortality of *A. bipunctata* larvae 4 d after treatment

Source	df	*F*	*P*
Temp	2	70.80	<0.0001
Nematode species	5	4.39	0.0008
Dose of nematodes	2	86.69	<0.0001
Temp × nematode species	10	3.43	0.0003
Temp × dose of nematodes	4	1.18	0.3222
Nematode species × dose of nematodes	10	2.30	0.0135

Results and Discussion

All main effects as well as their associated interactions (except temperature × dose of nematodes for *A. bipunctata* and nematode species × dose of nematodes for *C. carnea*) were significant at $P < 0.05$ (Tables 1 and 2). Four days after treatment significantly the lowest mortality of *A. bipunctata* and *C. carnea* larvae was recorded at 15°C, whereas no significant differences were noted between 20 and 25°C. At 500 IJs/ml the nematodes had significantly the lowest nontarget effect on the larvae of both aphid predators, whereas no significant differences in this regard were established between 2,500 and 5,000 IJs/ml. Between the larvae of both studied predators, those of *C. carnea* was generally less susceptible to attack of entomopathogenic nematodes.

The mortality rate for the twospotted lady beetle larvae at 25°C was >93% with the exception of the lowest concentration. At both higher concentration and at 20°C, the lowest mortality rate for the larvae of the same predatory species was 75%. The mortality rate for the lacewing larvae at the both higher temperatures was over 42%. These results confirm that the

Table 2. ANOVA results for corrected mortality of *C. carnea* larvae 4 d after treatment

Source	df	*F*	*P*
Temp	2	80.09	<0.0001
Nematode species	5	15.33	<0.0001
Dose of nematodes	2	26.38	<0.0001
Temp × nematode species	10	5.67	<0.0001
Temp × dose of nematodes	4	3.23	0.0124
Nematode species × dose of nematodes	10	1.51	0.1326

Table 3. Mean ± SE mortality of green lacewing and twospotted lady beetle larvae treated with three different concentrations of three EPN species and three mixed suspension of two EPN species at 15, 20, and 25°C, 4 d after treatment

Temp (°C)	Nematode species	Nematode concn (IJs/ml)					
		A. bipunctata			*C. carnea*		
		500	2,500	5,000	500	2,500	5,000
15	*S. feltiae*	73.79 ± 8.93c	80.19 ± 9.52b	75.47 ± 7.24ab	18.36 ± 6.71b	54.38 ± 14.75c	85.24 ± 7.84d
	S. carpocapsae	60.64 ± 10.01c	73.14 ± 13.53ab	70.35 ± 2.70a	6.0 ± 4.0a	13.98 ± 2.32b	50.99 ± 10.69bc
	H. bacteriophora	38.96 ± 3.65b	82.56 ± 7.48b	74.51 ± 10.90ab	1.82 ± 1.82a	4.86 ± 3.05a	4.0 ± 4.0a
	SF × SC	8.14 ± 5.86a	67.68 ± 7.04ab	87.44 ± 5.13b	41.0 ± 15.36c	54.48 ± 8.46c	58.91 ± 5.73c
	SF × HB	34.76 ± 5.85b	70.82 ± 5.50ab	78.37 ± 7.33ab	4.86 ± 3.05a	46.60 ± 7.38c	48.02 ± 10.88bc
	SC × HB	30.93 ± 9.70b	60.23 ± 7.91a	84.13 ± 5.81b	30.89 ± 10.73bc	40.67 ± 7.57c	38.0 ± 11.58b
20	*S. feltiae*	95.92 ± 2.50c	100 ± 0.00b	100.0 ± 0.00b	21.86 ± 2.65a	81.67 ± 13.02ab	50.71 ± 20.71a
	S. carpocapsae	48.07 ± 10.32a	84.81 ± 5.71a	100.0 ± 0.00b	68.44 ± 11.88c	84.50 ± 7.68b	94.67 ± 3.43c
	H. bacteriophora	68.48 ± 9.42b	91.38 ± 4.19a	75.80 ± 7.58a	41.08 ± 9.08b	54.67 ± 15.08a	67.50 ± 19.20ab
	SF × SC	76.53 ± 10.41b	92.91 ± 4.62a	100 ± 0.00b	65.91 ± 4.41c	73.45 ± 5.06ab	89.78 ± 5.48bc
	SF ×HB	75.06 ± 9.43b	83.96 ± 6.43a	86.62 ± 11.01ab	38.50 ± 9.22b	70.64 ± 11.62ab	77.33 ± 7.24ab
	SC × HB	79.37 ± 7.83b	100 ± 0.00b	97.73 ± 2.27ab	64.67 ± 8.57c	86.29 ± 5.81b	83.14 ± 6.65b
25	*S. feltiae*	83.96 ± 5.72c	93.21 ± 2.78a	96.99 ± 3.01a	42.12 ± 14.36a	78.59 ± 2.95a	65.75 ± 20.61ab
	S. carpocapsae	79.53 ± 11.49bc	97.89 ± 2.11ab	100 ± 0.00a	89.9 ± 10.03b	100 ± 0.0c	93.63 ± 3.95b
	H. bacteriophora	43.58 ± 4.57a	95.26 ± 2.93ab	97.89 ± 2.11a	36.43 ± 14.77a	65.79 ± 16.63a	42.10 ± 11.79a
	SF ×SC	88.41 ± 5.37c	100 ± 0.00b	100 ± 0.00a	76.81 ± 13.86b	89.55 ± 6.61b	89.97 ± 6.14b
	SF × HB	60.20 ± 11.36b	97.37 ± 2.63ab	100 ± 0.00a	47.71 ± 11.09a	68.81 ± 10.81a	59.89 ± 11.10a
	SC × HB	61.54 ± 11.66b	96.99 ± 3.01ab	100 ± 0.00a	81.11 ± 9.94b	100 ± 0.0c	89.26 ± 10.74b

For each dose, within each temperature, means followed by the same letters are not significantly different ($P \leq 0.05$; Duncan's multiple range test).

SF, *S. feltiae;* SC, *S. carpocapsae;* HB, *H. bacteriophora.*

activity of all infective juveniles increases with increasing temperature. They are most effective and act most quickly from 20 to 30°C (Koppenhöfer 2000). Usually, *H. bacteriophora* and a mixed suspension of *S. feltiae* and *H. bacteriophora* were the least nontarget-efficient agents in our study (Table 3), whereas the other four treatments had approximately the same influence to the larvae of both predators.

Mixed suspensions of two nematode species also were used in the assay to prove a possible synergistic effect of two species of entomopathogenic nematodes and consequently a higher nontarget effect. The synergism between various species (Ansari et al. 2006) as well as the synergism between the organism and the pesticide (Koppenhöfer and Kaya 1998) are studied for the intent of introducing different sustainable strategies of pest control. The results of our study do not support a synergism between various entomopathogenic nematode species in most of the experiments presented.

Despite the results of this study, as well as those of the laboratory assay performed, a high susceptibility of the larvae (Farag 2002) and cocoons (Powell and Webster 2004) of the predators to the entomopathogenic nematodes was confirmed, and a field assay is needed to prove or disprove these findings. We note that optimal conditions for the development of entomopathogenic nematodes were established in the laboratory, which is rarely the case in nature.

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