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Activity of four entomopathogenic nematode species against young adults of *Sitophilus granarius* (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) under laboratory conditions

Wirkung von vier entomopathogenen Nematodenarten gegenüber *Sitophilus granarius* (Coleoptera: Curculionidae) und *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) unter Laborbedingungen

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Summary

Four entomopathogenic nematode species (*Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, and *Heterorhabditis megidis*) were tested in a laboratory bioassay with the aim of studying their efficacy in control of the adults of two stored grain pests, *Sitophilus granarius* and *Oryzaephilus surinamensis*. Activity of the biological agents studied was determined at three different concentrations (500, 1000, and 2000 infective juveniles [IJs] per adult) and temperatures (15, 20, and 25°C). The granary weevil mortality rate was higher than the mortality rate of the saw-toothed grain beetle. *Heterorhabditis megidis* proved to be the least efficient in control of both pests, while no significant differences were recorded between any of the other three nematode species. The experiment demonstrated that the entomopathogenic nematodes were most efficient in the control of *S. granarius* at 20°C (LC_{50} after 7-day exposure 803-1195 IJs/adult) and 25°C (LC_{50} 505-1175 IJs/adult). A satisfactory level in control of the pest *O. surinamensis* was reached at 20°C (LC_{50} 921-1335 IJs/adult). The concentration of the suspension used in our experiment was shown to be a less important factor affecting the biological activity of nematodes against the adults of both stored grain pests. Though the use of entomopathogenic nematodes for control of the tested pests is not possible at the present time, it may be possible to combine this approach with some other (biotechnical) methods in the future.

Key words: biological control, efficacy, entomopathogenic nematodes, *Oryzaephilus surinamensis*, *Sitophilus granarius*

Zusammenfassung

Vier entmopathogene Nematodenarten (*Steinernema feltiae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* und *Heterorhabditis megidis*) wurden in einem Labor-Biotest hinsichtlich ihrer Wirksamkeit bei der Bekämpfung zweier Vorratsschädlinge des Getreides, *Sitophilus granarius* und *Oryzaephilus surinamensis*, untersucht. Ihre Wirksamkeit wurde in drei verschiedenen Konzentrationen (500, 1000 und 2000 Infektionslarven [IL] pro Adultem) und Temperaturen (15, 20 und 25°C) ermittelt. Die Mortalität des Kornkäfers *S. granarius* übertraf dabei die des Getreideplattkäfers *O. surinamensis*. *Heterorhabditis megidis* war die ineffektivste Nematodenart gegenüber beiden Vorratsschädlingen, während zwischen den drei anderen Nematodenarten keine signifikanten Unterschiede auftraten. Das Experiment zeigt, dass die entomopathogenen Nematoden den Kornkäfer am effektivsten bei Temperaturen von 20°C (LC_{50} nach 7 Tagen 803-1195 IL/Adultem) und 25°C (LC_{50} 505-1175 IL/Adultem) kontrollieren. Ein befriedigender Bekämpfungserfolg gegenüber dem Getreideplattkäfer wurde bei 20°C (LC_{50} 921-1335 IL/Adultem) erreicht. Die Konzentration der verwendeten Nematodensuspension beeinflusste den Bekämpfungserfolg gegenüber beiden Schädlingsarten dagegen weniger. Obwohl die Verwendung entomopathogener Nematoden zur Kontrolle der beiden untersuchten Vorratsschädlinge des Getreides zur Zeit noch nicht praktikabel ist, könte dieser Ansatz zukünftig in Kombination mit anderen (biotechnischen) Verfahren zum Erfolg führen.

Stichwörter: biologische Bekämpfung, Effizienz, entomopathogen Nematoden, *Oryzaephilus surinamensis*, *Sitophilus granarius*

1 Introduction

The granary weevil, *Sitophilus granarius* (L.), and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), are listed among important pests of stored grain, the former being a primary pest (Ungsunantwiwat and Mills 1985; Kucerova et al. 2003) occurring mostly in temperate climates, and the latter being a typical cosmopolitan and a secondary pest (Trematerra et al. 2000; Weston and Rattlingourd 2000). Both species feed on a variety of cereals or cereal products, although wheat and barley are among the most frequent sources of their nutrition (Schwartz and Burkholder 1991; Buchelos and Athanassiou 1999).

The number and size of grain storage facilities are increasing with progressive growth of the human population. These are the habitats of storage pests. Lately, growing attention has been focused on preventative and curative measures against storage pests (Fleurat-Lessard 2003), curative measures including more environment-friendly substances (Aslan et al. 2004; Athanassiou et al. 2005) and organisms (Pratissoli et al. 2004). So far, the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Searle and Doberski 1984; Hluchy and Samsinakova 1989) and some parasitoids (Ahmed 1996; Oliveira et al. 2003) have been tested for the control of *S. granarius* and *O. surinamensis*.

Entomopathogenic nematodes (EPNs) as potential biological agents have received increasing attention recently. They are mutually associated with bacteria of the family Enterobacteriaceae. The third-stage infective juveniles (IJs) of these nematodes locate and infect suitable insect hosts. After infection, the symbiotic bacteria are released into the insect hemocoel, causing septicemia and death of the insect (Kaya and Gaugler 1993; Abdel-Razek 2003). In the beginning, EPNs were mostly known as natural enemies of soil pests (Ishibashi

and Choi 1991; Glazer et al. 1992) but now, they are frequently included in studies on the control of foliar pests (Chyzik et al. 1996). Some experts disagree on the pest-control abilities of EPNs and thus on the economics of their use (Grewal et al. 1997; Smith 1995).

It is well known that EPNs act efficiently in a humid environment (Ebssa et al. 2004b) and that lack of water is the most limiting environmental factor influencing the "survival biology" of EPNs (Glazer 2002). Further, it is known that EPNs are more efficient against the larvae and other preimaginal stages of insects because they can enter their body more easily (LeBeck et al. 1993). However, it has also been demonstrated several times already that nematodes in higher concentrations can kill adult insects too (Cabanillas 2003).

Investigations on relationships between EPNs and larvae of stored-grain pests have not been rare in recent years (Shapiro-Ilan et al. 2005), but only limited data are available on the efficacy of some species of these biological agents against adult beetles. So far, *Typhaea stercorea* (L.) (Svendsen and Steenberg 2000), *Cosmopolites sordidus* (Germar) (Schmitt 1993), and *Sitophilus granarius* (Bednarek 1986a, b) have been examined in this regard. The initial aim of our research was to study the activity of four EPNs against adult beetles of two hosts, the granary weevil and the saw-toothed grain beetle, since few data have so far been published on this topic. The nature of this research was primarily fundamental.

2 Materials and methods

2.1 Entomopathogenic nematodes and stock cultures of both stored grain pests

Our investigation was carried out in the Entomological Laboratory of the Chair of Entomology and Phytopathology (University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy) in Ljubljana, Slovenia. The following four species of EPNs were tested: *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser) (both Rhabditida: Steinernematidae), *Heterorhabditis bacteriophora* Poinar and *H. megidis* Poinar (both Rhabditida: Heterorhabditidae) (commercial biopreparations of EPNs from Koppert B.V., Berkel en Rodenrijs, The Netherlands). All the biopreparations, which were sent by air-mail, were used within 2 months of their receipt. The stock cultures of both stored grain pests, *S. granarius* and *O. surinamensis,* were reared at the Chair of Entomology and Phytopathology. The granary weevil and the saw-toothed grain beetle were fed wheat grain and a mixture of wheat and barley grits, respectively. Populations of both pests have been maintained in the laboratory, at room temperature (20 ± 2°C) in the dark, since 1998.

The adults used in the experiment were the offspring of parent couples. The latter were reared separately from the stock cultures under the same conditions. Parent couples were put in rearing vessels (20 vessels for each species), where their food was the same as that of the stock culture.

2.2 Laboratory bioassay

Application of the suspensions of EPNs was performed after the first adults appeared (35–45 days after the males and females were put together), following a preliminary test procedure (Svendsen and Steenberg 2000). Adults of the same age (± 1 day) were used in the experiment. For this purpose, all adult beetles were removed from the rearing chambers one day prior to the application of nematodes. Only those beetles which emerged in the last 24 hours were used. Twenty grains of wheat were placed in 5-cm (diameter) Petri dishes lined with filter paper disks. Ten adults of one species were put in each Petri dish.

The efficacy of EPNs was tested in three concentrations: 500, 1000, and 2000 IJs per adult; or 5000, 10000, and 20000 IJs in 1 ml of water per Petri dish. The suspensions of nematodes were prepared in glass jars, and in each Petri dish was given 1 ml of suspension. The Petri dishes were then closed and sealed with parafilm to prevent the beetles from escaping. Suspensions were administered with a pipette, and tips were changed after every treatment. The fifth treatment was for control purposes: instead of nematode suspension, distilled water was added to the Petri dishes.

Petri dishes were put in a rearing chamber (type: RK-900 CH, producer: Kambič Laboratory equipment, Semič, Slovenia), every treatment in 10 replications. Efficacy was tested in the dark at three different temperatures (15, 20, and 25°C) and a relative humidity of 95%. After seven days, the number of dead beetles of both species was determined. By that time, the next generation of nematodes from the genus *Steinernema* was found in the dead beetles. The mortality rate in beetles exposed to nematodes from the genus *Heterorhabditis* was determined after seven days, and the emergence of a new generation of nematodes was determined after 14 days. The cause of death was thus confirmed.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between adults of *S. granarius* and *O. surinamensis* reared in 36 different treatments (four species of EPNs − each species in three different concentrations and at three different temperatures). Before the analysis, the mean mortality was tested for homogeneity of treatment variances. Mortality rate data were corrected for control mortality, using Abbott's formula (Abbott 1925). The arcsine square-root was transformed before the analysis here. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. Values of LC_{50} and LC_{90} (numbers of IJs/adult causing 50% and 90% mortality) were determined, and efficacy of the tested nematodes was estimated on their basis. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA) and figures were created with MS Office Excel 2003. The data are presented as untransformed means ± SE.

3 Results

3.1 Sitophilus granarius

Group analysis indicated that the percentage of mortality of granary weevil adults was statistically significantly influenced by the nematode concentration (F = 8.38; df = 2, 140; $P = 0.0004$), temperature (F = 138.55; df = 2, 140; $P < 0.0001$), EPN species (F = 21.61; df = 3, 140; $P < 0.0001$), and interaction between temperature and EPN species (F = 4.21; df = 6, 140; $P = 0.0006$), while the influence of interaction between nematode concentration and temperature (F = 0.58; df = 4, 140; $P = 0.6780$), influence of interaction between nematode concentration and EPN species (F = 0.87; df = 6, 140; $P = 0.5182$), and that of interaction between nematode concentration, temperature, and EPN species (F = 1.23; df = 12, 140; $P = 0.2685$) were not significant. In all treatments total mortality was significantly different from the control treatment. Corrected mortality was therefore calculated.

Significantly the lowest percentage of pest mortality in all four EPN species was determined at 15°C (35.31 ± 5.49 with *S. feltiae*, 7.07 ± 2.43 with *S. carpocapsae*, 27.21 ± 4.96 with *H. bacteriophora*, and 9.93 ± 3.45 with *H. megidis*). No significant differences of this parameter were determined between EPN species at 20°C (80.56 ± 5.15 with *S. feltiae*, 83.33 ± 4.41 with *S. carpocapsae*, 76.39 ± 4.35 with *H. bacteriophora*, and 43.06 ± 4.86 with *H. megidis*) or 25°C (74.15 ± 5.24 with

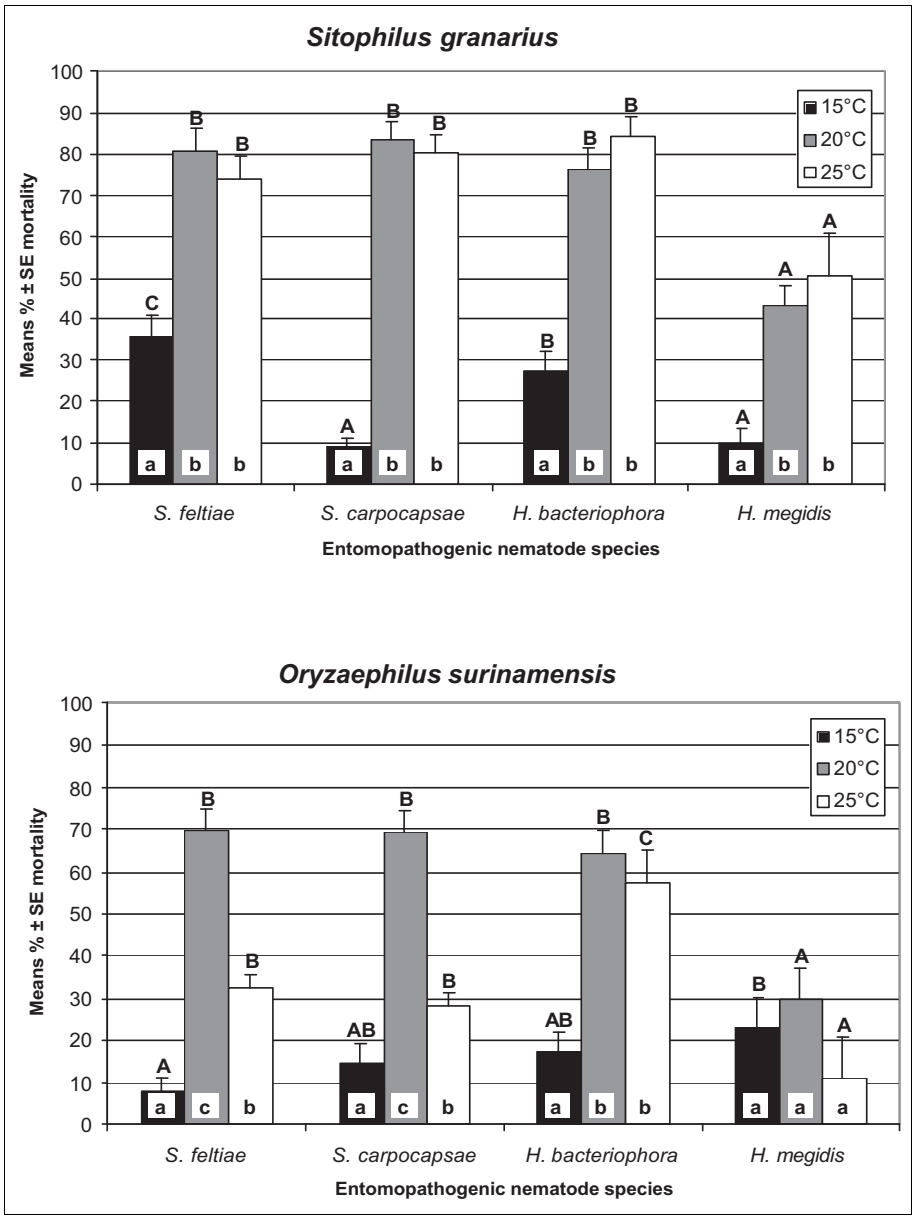

Fig. 1: Mean adult mortality of *Sitophilus granarius* and *Oryzaephilus surinamensis* treated with four different species of entomopathogenic nematodes depending on rearing temperature. Data shown are corrected for control mortality and analyzed by multifactor ANOVA. Capital and lower-case letters correspond to the grouping of means by Duncan's multiple range test ($P \leq 0.05$) for EPN species and temperature, respectively. The same letters do not differ significantly.

S. feltiae, 80.27 ± 4.12 with *S. carpocapsae*, 84.35 ± 4.46 with *H. bacteriophora*, and 50.48 ± 9.80 with *H. megidis*) (Fig. 1). *H. megidis* was significantly the least efficient at 20°C and 25°C, while no significant differences of efficacy were ascertained between any of the other EPN species.

The highest efficacy at 15°C, in both the lowest and highest concentrations, was found with *S. feltiae*, while at 1000 IJs/adult *H. bacteriophora* was the most effective (Table 1). Efficacy of *H. megidis* was the lowest at a concentration of 500 IJs/adult. This species and *S. carpocapsae* were the least efficient at a concentration of 1000 IJs/adult. *Steinernema carpocapsae* was also the least efficient with the highest concentration at 15°C, but on the contrary it was significantly the most efficient at 20°C, with the lowest and the highest concentration. At the same temperature and concentration 1000 IJs/adult *S. feltiae* was the most efficient. *Heterorhabditis megidis* was the least efficient at 20 and 25°C in all three concentrations. At the highest temperature, similar mortality values were found between the other three EPN species at 2000 IJs/adult, while at 500 and 1000 IJs/adult *H. bacteriophora* showed the best efficacy against the pest.

Table 2 summarizes LC_{50} and LC_{90} values calculated from the bioassay. Results of determining the percentage of mortality in beetles reared at 15°C were not taken into account, since all four nematode species showed the worst activity at this temperature. At 20°C, the lowest LC_{50} value was obtained with *S. feltiae* (803 IJs/adult) and the highest with *H. megidis* (1195 IJs/adult). At 25°C, *S. carpocapsae* showed the lowest LC_{50} value (505 IJs/adult), while *H. megidis* exhibited the highest value (1175 IJs/adult).

3.2 Oryzaephilus surinamensis

Group analysis indicated a statistically significant effect of temperature (F = 29.71; df = 2, 140; $P < 0.0001$), EPN species (F = 8.09; df = 3, 140; $P < 0.0001$), nematode concentration (F = 5.24; df = 2, 140; $P = 0.0066$), and interaction between temperature and EPN species (F = 2.95; df = 6, 140; $P = 0.0102$) on the percentage of mortality in adults. The influence of interaction between nematode concentration and EPN species (F = 1.85; df = 6, 140; $P = 0.0960$), influence of interaction between nematode concentration and temperature (F = 1.40; df = 6, 140; $P = 0.2397$), and that of interaction between nematode concentration, temperature, and EPN species (F = 1.61; df = 12, 140; $P = 0.0980$) were not significant. In all treatments total mortality was significantly different from the control treatment. Corrected mortality was therefore calculated.

S. feltiae (8.11 ± 2.70), *S. carpocapsae* (14.59 ± 4.64), and *H. bacteriophora* (17.12 ± 5.91) caused significantly the lowest

percentage of mortality of the beetles at 15°C. Significantly the highest values of this parameter were observed at 20°C with the first two species (68.38 ± 6.12 with *S. feltiae* and 68.60 ± 5.57 with *S. carpocapsae*); and at 20°C (63.25 ± 6.53) and 25°C (57.20 ± 8.81) with the third species (Fig. 1). Temperature did not influence the efficacy of *H. megidis*, and mortality of the beetles ranged between 11.94 ± 9.27 at 25°C and 29.72 ± 8.92 at 20°C.

Similar mortality values were observed between EPN species in the highest concentration at 15°C. *S. feltiae* was the least efficient at the lowest concentration, but its activity against hosts did not differ much from that of *S. carpocapsae* and *H. bacteriophora* (Table 1). The latter two species were also the most efficient at a concentration of 1000 IJs/adult. At 20°C, *H. megidis* was the least efficient at all three concentrations, but its activity in a concentration of 1000 IJs/adult did not differ much from that of *S. feltiae*. *S. carpocapsae* and *H. bacteriophora* were the most efficient at a the concentration of 1000 IJs/adult. At 25°C also, *H. bacteriophora* caused the highest mortality of the beetles at concentrations of 500 and 1000 IJs/adult, but surprisingly, this species was the least efficient ath the highest concentration. *H. megidis* was the least efficient at the highest temperature, while its activity in the lowest concentration did not differ substantially from that of either species from the genus *Steinernema* or from that of *H. bacteriophora* at the highest concentration.

Table 2 summarizes LC_{50} and LC_{90} values calculated from the bioassay. Results of determining the percentage of mortality in beetles reared at 15°C were not taken taken into account, since three of the biological agents (with the exception of *H. megidis*, which showed no statistical differences in activity at any of the three temperatures) showed the lowest activity at this temperature. At 20°C, the lowest LC_{50} value was obtained with *S. carpocapsae* (921 IJs/adult) and the highest with *H. megidis* (1335 IJs/adult). At 25°C, *S. feltiae* showed the lowest LC_{50} value (896 IJs/adult), while *S. carpocapsae* exhibited the highest value (1695 IJs/adult).

4 Discussion

The investigation demonstrated that entomopathogenic nematodes control granary weevil adults more efficiently than they control adults of the saw-toothed grain beetle. The application of *S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* at 20 and 25°C resulted in mortality rates of over 57% in granary weevils. Satisfactory results in control of the saw-toothed grain beetle were achieved only at 20°C, with the percentage

Table 1: Mean adult mortality (± SE) of *Sitophilus granarius* and *Oryzaephilus surinamensis* treated with three different concentrations of four entomopathogenic nematode species at 15, 20, and 25°C. Data shown are corrected for control mortality

Temperature (°C)	Nematode species	*S. granarius*			*O. surinamensis*		
		Nematode concentration (IJs/adult)					
		500	1000	2000	500	1000	2000
15	*S. feltiae*	42.86 ± 9.46	18.78 ± 6.93	44.90 ± 8.90	6.15 ± 3.15	6.15 ± 3.15	13.54 ± 6.89
	S. carpocapsae	8.98 ± 5.23	5.31 ± 3.63	6.94 ± 3.39	8.0 ± 5.27	23.08 ± 11.58	8.31 ± 5.16
	H. bacteriophora	7.14 ± 7.71	33.69 ± 8.90	30.61 ± 8.78	8.92 ± 4.92	30.77 ± 13.98	18.46 ± 9.36
	H. megidis	0.00 ± 0.00	8.98 ± 4.11	20.82 ± 6.43	27.69 ± 17.93	20.92 ± 13.93	5.85 ± 3.28
20	*S. feltiae*	64.58 ± 12.59	89.58 ± 4.66	87.50 ± 7.65	51.06 ± 18.61	49.47 ± 2.66	65.43 ± 2.66
	S. carpocapsae	85.42 ± 7.06	72.92 ± 8.46	91.67 ± 5.10	44.15 ± 6.69	81.38 ± 5.09	70.74 ± 5.09
	H. bacteriophora	68.75 ± 5.71	79.17 ± 10.93	81.25 ± 8.96	52.13 ± 13.39	65.43 ± 11.79	60.11 ± 10.97
	H. megidis	41.67 ± 11.69	37.50 ± 5.71	50.00 ± 8.96	5.85 ± 3.18	36.17 ± 10.64	33.51 ± 2.66
25	*S. feltiae*	73.47 ± 4.08	57.14 ± 10.41	91.84 ± 3.82	9.21 ± 5.84	28.95 ± 2.63	23.68 ± 2.63
	S. carpocapsae	67.35 ± 8.78	81.63 ± 6.77	91.84 ± 3.82	15.79 ± 3.50	21.05 ± 3.04	42.11 ± 10.96
	H. bacteriophora	81.63 ± 9.89	83.67 ± 10.00	87.76 ± 3.82	28.95 ± 10.85	61.40 ± 14.04	2.63 ± 1.52
	H. megidis	35.10 ± 18.51	48.98 ± 16.77	67.34 ± 14.21	18.42 ± 6.62	5.26 ± 3.72	2.63 ± 1.52

Table 2: Dose effect of four different entomopathogenic nematode species on young adults of *Sitophilus granarius* and *Oryzaephilus surinamensis* at two different temperatures

Nematode species	Prey species	LC_{50}[z] (95% CL[y])		LC_{90}[z] (95% CL[y])	
		20°C	25°C	20°C	25°C
S. feltiae	*Sitophilus granarius*	803 (166 – 1440)	776 (227 – 1305)	1267 (891 – 1644)	1410 (994 – 1826)
S. carpocapsae		1005 (211 – 1799)	505 (0 – 1151)	1354 (999 – 1709)	1368 (1016 – 1720)
H. bacteriophora		1141 (519 – 1763)	973 (121 – 1825)	1388 (960 – 1816)	1197 (809 – 1584)
H. megidis		1195 (821 – 1569)	1175 (828 – 1522)	1477 (520 – 2435)	1430 (908 – 1952)
S. feltiae	*Oryzaephilus surinamensis*	993 (525 – 1461)	896 (406 – 1386)	1436 (882 – 1990)	–
S. carpocapsae		921 (371 – 1471)	1695 (1352 – 2038)	1505 (850 – 2160)	2578 (1882 – 3273)
H. bacteriophora		1034 (509 – 1560)	1074 (578 – 1572)	1386 (717 – 2056)	1418 (783 – 2052)
H. megidis		1335 (850 – 1821)	927 (304 – 1550)	1800 (703 – 2897)	–

[z] LC_{50} and LC_{90} expressed as number of IJs per adult.
[y] Confidence limits, CL, are given in parentheses.

of mortality of the beetles ranging between 44 and 81%. These outcomes partly agree with the results of some related works, where an optimal biological activity of *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* was determined in the temperature range from 22 to 24°C (Choo et al. 2002), from 22 to 26°C (Kaya et al. 1993; Doucet et al. 1996), and at 25°C (Belair et al. 2003; Yang et al. 2003), respectively.

An older study on the efficacy of different insecticides in controlling both pests indicated the highest mortality at 25°C, while only an insignificant effect on the mortality of *S.granarius* was recorded at 15°C (Tyler and Binns 1982). This corresponds with low efficacy of the biological agents studied in our experiment at 15°C. However, in the control of *O. surinamensis*, a low efficacy of the nematodes was determined at 25°C. This confirms our assumption that pest susceptibility to nematodes is a complex process and depends not only on current viability of the pest but also on aggressiveness of the "invader". Both conditions are mostly dependent on abiotic factors (Jian et al. 2002). Nevertheless, the role of biotic factors is not to be dismissed, as it is known that the host's excrements consist of compounds which make it easier for nematode invasive larvae to find the host and have a stimulating effect on nematodes (Alikhan et al. 1985).

Due to the very low efficacy of nematodes in concentrations under 250 IJs/adult in our preliminary studies, nematode concentrations in the present study were higher than what is usually recommended for pest control (Ishibashi and Choi 1991; Ebssa et al. 2004a). All four species showed a certain level of efficacy against adults. The least efficient was *H. megidis*, which manifested similar activity against adults of the beetle *Typhaea stercorea*, but in the case of the latter at even higher concentrations. However, in this study (Svendsen and Steenberg 2000) the efficacy of *H. megidis* did not differ significantly compared to *S. feltiae* and *H. bacteriophora*. This leads to the conclusion that all of the biological agents in our experiment exhibited higher efficacy against both storage pests.

In higher concentrations (500 IJs/adult and more), entomopathogenic nematodes can be considered efficient biological agents in efforts to control the adults of *S. granarius* and *O. surinamensis*. Temperature was an important factor for activity of the nematodes in addition to which it may affect the pest as well, especially its viability (Thurston and Kaya 1994). Here the concentration of nematodes was shown to be a less important factor acting on their efficacy. A recent study supports this finding, although it should be emphasized that the distinction is species-specific (Arthurs et al. 2004). Where concentration of the nematode suspension plays a minor role, this is possibly attributable to the fact that the number of invasive larvae of EPNs penetrating an insect is regulated by the number of invasive larvae which earlier managed to penetrate it (Bednarek and Nowicki 1986). The general impression is that use of the highest nematode concentrations (2000 IJs/adult) is not economically justified. *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* can be recommended for storage pest control, since they were the most effective in controlling both storage pests. This finding is corroborated by results of research, indicating that larvae of *S. granarius* in the weight variant were more intensively infected with EPNs than were larvae of *Pieris brassicae* and *Mamestra brassicae* (Pezowicz 1992), which are otherwise susceptible to attack by the mentioned biological agents (Nadasy et al. 1999).

We are aware of the fact that young adults – compared to older beetles – could be more susceptible to attack by entomopathogenic nematodes. However, such sensitivity of some other beetles (Jaworska and Ropek 1996) cannot be simply transferred to *S. granarius* and *O. surinamensis*, especially since it has been determined that older beetles of the former species are more susceptible to gamma radiation than are younger beetles (Ismail et al. 1990). Further studies in this area are needed in order to discern whether the results of our research are applicable to beetle adults of different ages. As a possibility to enhance the applicability of EPNs against storage pests we recommend a combination with pest attractants. For this purpose pheromone traps (Plarre 1996; Mabbett, 2003) or food volatiles (Pierce et al. 1990) are already used.

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