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ORIGINAL ARTICLE

Evaluation of biological activity of biopreparations containing nematodes from the genera *Steinernema* and *Heterorhabditis* used for reducing large pine weevil *Hylobius abietis* L. population in pine *Pinus sylvestris* L. stumps

Iwona Skrzecz[1] ✉, *Dorota Tumialis*[2], *Elżbieta Pezowicz*[2], *Alicja Sowińska*[1]

[1] Forest Research Institute, Department of Forest Protection, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland, phone: 48 22 7150541, fax: 48 22 7150557, e-mail: i.skrzecz@ibles.waw.pl

[2] Warsaw University of Life Sciences, Animal Sciences Faculty, Department of Zoology, Ciszewskiego 8, 02-786 Warsaw, Poland

Abstract

The use of entomopathogenic nematodes (EPNs), such as *Steinernema* and *Heterorhabditis* spp., against the large pine weevil *Hylobius abietis* has been mainly studied in the United Kingdom and Ireland. The results of these studies show a great potential of nematodes for *H. abietis* control. Similar research conducted in Poland aimed at evaluating effectiveness of commercially produced biopreparations that contained entomopathogenic nematodes and were applied against the large pine weevil.

The treatments consisted of spraying soil around *Pinus sylvestris* stumps with an aqueous suspension of *Steinernema carpocapsae, S. feltiae* and *Heterorhabditis bacteriophora*, *H. downesi*, *H. megidis* at a dose 3.5 million IJs/1000 ml of water per stump. The roots of experimental stumps were analyzed 4 weeks subsequent to the treatments. After roots debarking, *H. abietis* larvae were isolated and the percentage of insects parasitized by EPNs was assessed. All tested nematodes showed ability to parasitize *H. abietis* larvae overwintering in *P. silvestris* stumps. The highest extensivity of parasitizm was observed in *H. abietis* larvae parasitized by *S. carpocapsae* and *H. downesi*, and the lowest – in the case of larvae collected from the roots of the stumps sprayed with *H. megidis*. There were no nematodes found in *H. abietis* larvae collected from untreated – control stumps.

Key words

biological control, *Steinernema*, *Heterorhabditis*, *Hylobius abietis*

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Introduction

Among insect species causing economic damage in the youngest coniferous stands, those from the genus Hylobius (Coleoptera: Curculionidae) are of particular importance, at the same time as the large pine weevil *Hylobius abietis* (L.) is a particularly harmful pest. The species is widespread in Asia (Siberia and Japan) and Europe. In Poland it is year on year observed all through the area of entire country. The area of 20–30 thousand ha of Poland's forests have been threatened by *H. abietis* for more than 10 years, thus all reforested areas are systematically observed for the assessment of pest population numbers (Kolk et al. 2004). The large pine weevil can damage just about all coniferous tree species, and it also attacks deciduous trees. Large pine weevil beetles feed on stems and shoots of tree seedlings, which results in patchy damage of tree bark followed by seedling deformation and wilt. Opposite to imagines, large pine weevil larvae do not cause economic damages as they develop in the roots of left behind pine (*Pinus* spp.) and spruce (*Picea* spp.) stumps.

The protection of newly established coniferous stands against large pine weevil beetles has become one of key issues of forest conservation, and as a consequence in many European countries there has been carried out continuous research on natural enemies of *H. abietis* and their potential to reduce pest population numbers. Recent investigations have been focused for the most part on possibilities of using entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis* for decreasing population numbers of large pine weevil larvae developing in tree stumps. First experiments with the use of EPNs were conducted in Sweden, where more than 50% mortality of large pine weevil larvae isolated from the stumps was observed after treatments with the suspension of *Steinernema carpocapsae* Weiser (Pye 1979; Pye and Burman 1985). Several field studies on nematode applications were carried out in the United Kingdom (UK) by Brixey et al. (2006) and Torr et al. (2007). These studies concerned invasiveness of nematode species from the genera Steinernema (*S. carpocapsae, S. feltiae* Filipjev) and Heterorhabditis (*H. bacteriophora* Poinar, *H. downesi* Stock, Griffin & Burnell). The results obtained showed that June-July treatments of litter around the stumps attacked by the large pine weevil caused more than 60% reduction of its population numbers. Many studies of this kind were also conducted in Ireland, the results of which confirmed high effectiveness of nematodes in *H. abietis* control, and especially species from the genera *Steinernema* and *Heterorhabditis* (Dillon et al. 2006).

Analogous research was undertaken in Poland in the year 2008. First field trials concerned early autumn treatments of litter around pest infested *P. silvestris* stumps with bioprepartions containing *S. carpocapsae* and *H. downesi* nematodes. The analysis of treatment effectiveness showed more than 80% mortality of large pine weevil larvae which were overwintering in the stumps. These results justified undertaking further research with the aim to evaluate possibilities of using commercial biopreparations that contain EPNs from the genera *Steinernema* and *Heterorhabditis* to reduce population numbers of large pine weevil larvae in *P. silvestris* stumps.

Material and methods

The experiments were carried out within the area of the Regional Directorate of State Forests in Warsaw, in the Forest District Piotrków (51°22'37'' N; 19°49'01'' E) and Celestynów (55°03'43'' N; 21°23'16'' E). Two clearcut areas (of absolute area more than 3 ha) were selected for experimental treatments. On each observation area there were one-year-old *P. silvestris* stumps with the diameter 32–50 cm (on average 38 ± 5.4 cm) – left behind after harvesting 90–100 years old pine stands.

Nematode applications were carried out in September 2010 and 2011, in the season when large pine weevil larvae of overwintering generation were found in stumps. The following biopreparations were tested in the experiment:

- biopreparation containing *Heterorhabditis downesi* nematodes (Koppert B.V., The Netherlands),
- Capsanem containing *Steinernema carpocapsae* nematodes (Koppert B.V.,The Netherlands),
- Nemasys H containing *Heterorhabditis bacteriophora* nematodes (Becker Underwood Ltd, UK),
- Nemasys G containing *Heterorhabditis megidis* Poinar, Jackson & Klein nematodes (Becker Underwood Ltd, UK),
- Nemasys F containing *Steinernema feltiae* nematodes (Becker Underwood Ltd, UK).

All biopreparations applied included 50 million/package invasive (L_3) EPN larvae. Right before the treatment aquatic suspensions of nematode biopreparations were prepared and litter was removed from around the stumps within the radius of about 1m. The suspension of invasive nematode larvae at a dose 2l/stump (3.5 million nematodes/stump) was applied onto soil around the stumps with the use of a knapsack applicator, and afterwards treated soil was covered back with litter. The stumps sprayed with water were treated as control ones. Each of tested biopreparations as well as water were applied to 30 stumps (3 replications of 10 stumps); in total 210 stumps were observed in both years.

Nematode treatments were carried out in mid-September 2010 and 2011, at air temperature 15–17°C and fully clouded sky. No precipitation was observed during nematode application. For the period of 4 weeks after the treatment (in both years), diurnal temperature measured 10 cm above the soil level fluctuated from 11° to 20°C, whereas soil diurnal temperature measured at the depth of 20 cm was 10 to 14°C. The air humidity measured 10 cm above the soil level oscillated between 68–94%. Weather data were obtained from the meteorological stations of the State Forest, located near the experimental areas.

In mid-October, approximately 4 weeks after the treatment, all the stumps observed were dug out (0.5 m down), and from each one there were collected 1 m long root sections (5–10/stump) for evaluation of treatment effectiveness (Skrzecz 2004). The root samples were debarked and the counts of larvae occurring within 20 cm long sections of samples were compared with reference to nematode biopreparation treatments.

The results obtained were statistically analyzed with the use of Statistica software (StatSoft®, Inc.). The differences in infestation intensity of pine stumps treated with EPNs and not-treated (control sprayed with water) were tested with ANOVA using transformed data according to the equation $y = \sqrt[2]{x + 1}$. The Tukey range test was used for distinction of uniform groups.

H. abietis larvae collected from root samples were sectioned 48 hours after their collection from stump roots and examined under a binocular microscope (enlargement × 5) for determination of:

- larvae mortality,
- extensivity of infection (% of nematode infected larvae in the sample observed),
- intensity of infection (the number of nematodes per one infected larva).

Results

Assessment of a level of stump infestation by *H. abietis*

In the roots of *P. silvestris* stumps there were observed exclusively larva instars of the large pine weevil. No significant differences were found in intensity of large pine weevil infestation in nematode treated and control stumps ($F = 0.007221$; $p = 0.932578$) (Tab. 1).

Tab. 1. Intensity of *H. abietis* infestation in *P. sylvestris* stumps treated with EPNs and not-treated (control)

Stump treatment	% of root sections infested by *H. abietis*	Total number of larvae	Mean number of larvae/stump (± SD)
S. carpocapsae	62	157	1.6 ± 0.5
S. feltiae	60	167	1.9 ± 0.7
H. bacteriophora	65	148	1.3 ± 0.4
H. downesi	68	139	1.5 ± 0.6
H. megidis	61	172	1.7 ± 0.5
H. downesi	64	133	1.8 ± 0.6
Control	69	150	1.7 ± 0.4

The pest was found in 60–68% of root sections taken from EPN treated stumps and in 69% of those collected from not-treated ones. Mean numbers of large pine weevil larvae depended on the treatment and ranged from 1.3 (roots of stumps treated with *H. bacteriophora*) to 1.9 (roots of stumps treated with *S. feltiae*).

Evaluation of large pine weevil infection with EPNs

On nematode treated stumps there was observed mortality of large pine weevil larvae at a level from 20% to about 86% (Tab. 2). All infected *H. abietis* larvae found in the stumps were parasitized by tested nematode species. The results of microscope analyses indicated differentiated levels of large pine weevil infection level. The highest extensivity of nematode parasitism (> 40%) was noted in the larvae collected from the stumps sprayed with the suspension of invasive nematodes *S. carpo-*

capsae and *H. downesi*. Considerably lower extensivity of parasitism (< 15%) was observed in the larvae from the stumps treated with *S. feltiae*, *H. bacteriophora* and *H. megidis.* The highest intensity of parasitism was found in the larvae parasitized by *H. bacteriophora* (8.4), whereas for other EPNs there was observed low intensity of larvae infection (2–4). The cause of death of large pine weevil larvae which were not parasitized with nematode was not explainable.

Tab. 2. Level of EPN infection in *H. abietis* larvae

Stump treatment	Mortality of larvae (%)	Extensivity of parasitism (%)	Intensity of parasitism
S. carpocapsae	81.0	42.5	3.0
S. feltiae	20.0	10.9	2.0
H. bacteriophora	23.3	14.9	8.4
H. downesi	86.0	44.2	4.2
H. megidis	23.5	5.9	2.0
Control	0.0	0.0	0.0

No nematode were observed in the larvae collected from the roots of control stumps.

Discussion

The large pine weevil and its natural enemies have been the subject of many research undertaken in Europe. In Austria, there were carried out trials on application of fungus *Beauveria bassiana* (Balls.) Vuill against this weevil, and 80% mortality of adults was obtained, but only under laboratory conditions. Furthermore, in the UK there were conducted investigations on possibilities of using a parasitic wasp *Bracon hylobii* Ratz. (Hymenoptera: Braconidae) in biological control of the large pine weevil in reforested areas (Henry and Day 2000). In the nineties of last century, studies on biological methods of decreasing large pine weevil population numbers based on the use of saprophytic fungus *Phlebiopsis gigantea* (Fr.) Jülich were carried out in Poland. The fungus causes decaying of pine stump wood which constitutes pine weevil breeding environment. The results obtained showed that stump treatment with *Ph. gigantea* reduced their infestation with pine weevils, and that fungus mycelium growing in the stumps impeded development of pest larvae which were hatching from sparsely laid eggs (Skrzecz and Moore 1997; Skrzecz 2001). Nonetheless, the method has not been implemented in the countries of northern Europe due to the lack of native *Ph. gigantea* strains. In that case, in the UK and Ireland there was carried out research on using nematodes *Steinernema* sp. and *Heterorhabditis* sp. for control of the large pine weevil at larva stage (Dillon et al. 2008).

The promising results obtained in nematode experiments in the abovementioned countries encouraged similar research in Poland. Nematodes were applied according to the procedures used in the studies carried out in the UK and Ireland, which then were based on spraying litter with an aquatic suspension of invasive EPN larvae ((Brixey et al. 2006; Dillon et al. 2006; Torr et al. 2007). Another way of nematode application was put into use in Sweden (Pye and Burman 1985), where apart from litter spraying with a solution containing invasive *S. carpocapsae* forms there were carried out injections of nematodes directly under stump bark. Nevertheless, there were not found any significant differences in mortality of *H. abietis* larvae being a result of applying aforesaid methods.

A different time of treatment was applied in the present study. Nematodes were not applied in summer season as in the aforementioned countries, but in early autumn. The choice of treatment timing was based on the conclusions from earlier experiments, when litter around pine stumps was treated with the suspension of *S. carpocapsae* in mid-June. Then the results obtained showed only 5% mortality of *H. abietis* in treated stumps which did not differ from pest natural mortality in not-treated stumps (unpublished data, Skrzecz and Bednarek 1998). Most probably this end result was influenced by disadvantageous for nematode development weather conditions during the study (high air and soil temperatures, lack of precipitation) that might have caused raised mortality of nematodes applied at that time. On the other hand, the results of initial trials on application of *S. carpocapsae* and *H. downesi* conducted in early autumn 2008 – when weather conditions were considerably more beneficial for nematode development – indicated nematode parasitism in 80% of large pine weevil larvae overwintering in treated stumps (Skrzecz et al. 2011).

In the present study, the effectiveness of commercial nematode biopreparations was evaluated. For testing there were chosen the preparations containing those nematode species, which when applied under field conditions caused more than 50% mortality of the large pine weevil (Pye and Burman 1985; Dillon et al. 2006, 2007, 2008; Skrzecz et al. 2011). More to the point, in the nineties of the last century, there were carried out laboratory trials by Skrzecz (1998) on infection of pine weevil larvae developing on *P. sylvestris* shoots covered with soil. At that time, in soil there were placed greater wax moth *Galleria mellonella* (L.) larvae infected with nematodes *S. feltiae* and *H. bacteriophora* isolated from soils of reforested areas under mass attack of the large pine weevil. The results of these experiments indicated 100% mortality of *H. abietis* due to nematode parasitism.

In this study, litter and soil around *P. silvestris* stumps were sprayed with invasive nematode larvae at a dose of about 3.5 million/stump, following application doses tested in the UK and Ireland. (Dillon at al 2006; Torr et al. 2007). Considerably lower doses (up to 800 thousand EPNs/stump) were applied and tested in Sweden (Pye 1979; Pye and Burman 1985).

The highest effectivness was shown by invasive nematode larvae *S. carpocapsae* and *H. downesi*. In the stumps treated with these species, there were found more than 80% dead *H. abietis* larvae, and this more than 40% was caused by applied pathogens. Higher level of parasitism of *H. abietis* larvae was obtained in the Ireland where nematodes *S. carpocapsae* and *H downesi* were the cause of death of up to 60% of large pine weevil larvae in *Picea sitchensis* Carr. stumps (Dillon et al. 2006). Also in Sweden, an application of *S. carpocapsae* nematodes at 4-fold lower dose caused death of 50–60% larva found in the stumps (Pye and Burman 1985). Different results were obtained in UK, were invasive nematode larvae *H. downesi*, infected less than 20% *H. abietis* specimens (Brixey et al. 2006).

Other nematode species observed (*H. bacteriophora* and *S. feltiae*) caused infection of up to 15% of *H. abietis* larvae, and the least parasitism level (approximately 6%) was observed in large pine weevil larvae found in the stumps treated *H. megidis* suspension. Low insecticidal activity of *H. megidis* and *S. feltiae* could have been caused by soil temperature during the experiment which was never higher than 14°C. As shown by laboratory research conducted by Dzięgielewska and Kieras-Kokot (2009), substrate temperature ≤ 15°C slows down/inhibits development of *S. feltiae* and *H. downesi* nematodes. Different results were obtained in Ireland, where the smallest number of infected *H. abietis* larvae was noted in the stumps treated with *S. feltiae* (< 40%), and *H. megidis* nematodes infected up to 50% of large pine weevil found in *Pinus* sp. stumps.

Evaluation of treatment effectiveness was carried out in October, when in *P. sylvestris* stumps there were observed only large pine weevil larvae, and these instars are most susceptible to nematode infections. Dillon et. al. (2006) presented the analysis of EPN infection extent in different pest development stages. The authors stated that *H. abietis* larvae were infected by nematodes to the highest extent (48%) when compared to pupae (32%) and imagines (30%).

Conclusions

There was demonstrated insecticidal effectiveness of biopreparations containing nematodes of the genera *Heterorhabditis* (*H. bacteriophora, H. downesi, H megidis*) and *Steinernema* (*S. carpocapsae, S. feltiae*) against *H. abietis* larvae colonizing *P. silvestris* stumps.

Sprays with the use of tested biopreparations at a dose 3.5 million invasive nematode larvae/stump applied to soil and litter around the stumps resulted in increased mortality of large pine weevil larvae.

The highest extensivity of parasitism was shown by *H. downesi* and *S. carpocapsae* nematode species.

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