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ORIGINAL PAPER

Possibilities of using entomopathogenic nematodes to reduce the number of the large pine weevil *Hylobius abietis* (L.) – the effect of abiotic factors

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ABSTRACT

In searching of biological methods which can be included in the integrated management of the large pine weevil *Hylobius abietis* in the reforestations, the small- and large-scale field trials were carried out in 2014-2016 to evaluate the potential of using different species of entomopathogenic nematodes from the genera *Steinernema* and *Heterorhabditis* to reduce the damage and population of this pest.

The experiments were conducted in 2014-2016 in central Poland on one year clearcuts with *Pinus sylvestris* L. stumps left after harvesting 90-110-year-old Scots pine stands. In the small-scale trials, the soil around the 30 stumps was sprayed with a suspension of *Steinernema carpocapsae* (Weiser), *S. feltiae* (Filipjev), *Heterorhabditis bacteriophora* (Poinar) and *H. downesi* Stock, Griffin and Burnell. In the large-scale trials, the treatments were carried out an the area of 1 ha, and the stumps were sprayed with *S. carpocapsae* and *H. downesi*. In all applications, the nematodes were applied at a dose of 3.5 million infective juveniles per stump. The roots of experimental stumps were collected 4 weeks after treatment. After the roots debarking, the large pine weevil larvae were isolated and the percentage of insects parasitized by nematodes were assessed. The results of small-scale treatments indicated low parasitism of *H. abietis* larvae (5-25%) by the applied nematodes. The highest mortality of *H. abietis* larvae (11-25%) was observed in the roots of *P. sylvestris* stumps treated with *H. downesi*. Large-scale applications of the nematodes *S. carpocapsae* and *H. downesi* resulted in parasitazion of up to 17% of *H. abietis* larvae developing in treated stumps. These applications did not affect the extent of damage caused by *H. abietis* beetles to *P. sylvestris* seedlings in the experimental plots. Presumably, the low level of *H. abietis* parasitism was influenced by the weather conditions unfavourable for nematode development, *i.e.*, lack of precipitation leading to the decreased moisture of forest litter and soil. The results are discussed in the context of abiotic factors that may influence the effectiveness of nematode applications in reforested areas.

KEY WORDS

biological control, Heterorhabditidae, pest insects, reforestations, Steinernematidae

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Introduction

The large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) is one of the most destructive pests in reforestations in many European countries, including Poland (Leather *et al.*, 1999; Grégoire and Evans, 2004; Skrzecz, 2017). It breeds in fresh conifer stumps, and adults feed on the bark of young trees, especially seedlings of Scots pine *Pinus sylvestris* L., (Pinales, Pinaceae), Norway spruce *Picea abies* (L.) H. Karst. (Pinales, Pinaceae), and larch *Larix decidua* Mill. (Pinales, Pinaceae). In case of heavy infestation, the bark of the seedlings can be destroyed by the beetles, and the complete 'ringing' of the trees can lead to their death.

In Poland, protection of forest plantations against *H. abietis* in case of lower occurrence of pine weevils consists mainly of mechanical methods (Skłodowski and Gadziński, 2001; Skrzecz, 2017) with the use of freshly cut and split billets and traps with food attractants. In case of a massive occurrence of the pine weevil and a threat to the stability of reforestations, a chemical method is used as a last method, based mainly on the use of pyrethroids with contact stomach effect (Skrzecz, 2017).

In search of non-chemical methods that could be an element of Integrated Pest Management (IPM), research was conducted in many European countries on the biology of natural enemies of the large pine weevil and the possibilities of using them to reduce the pest population. There are two ways that biological agents might reduce the large pine weevil population. The one of them is the reduction in the quality of breeding material for the large pine weevil development by stump deterioration with the use of wood-colonising fungi (Skrzecz, 2001). The second one is direct reduction of *H. abietis* larvae or beetles by fungi, parasitoids or nematodes. In Austria, attempts have been made to use the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. against the pine weevil (Wegensteiner and Führer, 1988). Despite promising laboratory results (86-100% beetle mortality), there was no development of fungal infections in *H. abietis* beetles feeding on patches of bark treated with fungus in the reforestations. In the 1990s, research was undertaken in Great Britain on the potential uses of the parasitoid *Bracon hylobii* Ratz. (Hymenoptera, Braconidae) in the biological protection of crops against the pine weevil (Henry and Day, 2001). In these studies, it was found that *B. hylobii* can cause mortality of up to 50% of the larvae of this species developing in the stumps of Sitka spruce *Picea sitchensis* (Bong.) Carr. (Pinales, Pinaceae). In Poland, the parasitoid of the large pine weevil is *Peritilus rutilus* Nees (Hymenoptera, Braconidae). The observations made by Korczyński (1984) on the parasitization of weevils by *P. rutilus* revealed an inconsistent and low level of infestation of 2.5% of the studied population.

Entomopathogenic nematodes (EPNs) are another group of organisms that potentially exhibit many of the characteristics of an excellent biological control agent (Ehlers, 1998, 2001; Lacey and Georgis, 2012; Labaude and Griffin, 2018). They occur naturally in the soil environment, are harmless to mammals, can be used on a large scale, and their activity is not affected by the concurrent use of pesticides. A number of studies have been conducted in Ireland and the United Kingdom to evaluate the feasibility of using nematodes of the order Rhabditida, genera *Steinernema*: *S. carpocapsae* (Weiser), *S. feltiae* (Filipjev) and *Heterorhabditis*: *H. bacteriophora* (Poinar), *H. downesi* (Stock, Griffin and Burnell) to reduce pine weevil larvae developing in the stumps of conifers (Brixey *et al.*, 2006; Dillon *et al.*, 2006, 2007; Torr *et al.*, 2007; Williams *et al.*, 2013). It was found that spraying tree stumps and surrounding litter with nematode suspensions resulted in over 60% reduction of *H. abietis* population. The results of these studies led us to consider the use of EPNs as a possible control agent against *H. abietis*.

The objective of this study was to evaluate the possibility of using commercial biopreparations containing nematodes of the genera *Steinernema* and *Heterorhabditis* to reduce the population of large pine weevil larvae developing in stumps of *P. sylvestris* left on cleared areas. The second objective was to determine if damage to seedlings resulting from larval parasitization and beetle migration from hatching sites through nematode-infested soil to feeding sites could be reduced.

Material and methods

NEMATODES. The following nematode species were tested in the trials: *S. carpocapsae*, *S. feltiae*, *H. bacteriophora* and *H. downesi*. The nematodes were provided by E-NEMA GmbH in the form of biopreparations containing invasive juveniles of EPN with clay mineral powder. These products were recommended for use in agriculture and forestry against pests living or developing in the soil: Nemastar® (*S. carpocapsae*), Nemaplus® (*S. feltiae*), Hd-Nematodes® (*H. downesi*), Nematop® (*H. bacteriophora*). The preparations were delivered up to two weeks before the start of the experiment and stored at 10°C during this time until the day of application.

LOCALIZATION. The experiments were conducted in 2014-2016 in central Poland on one-year clearcuts with *P. sylvestris* stumps left after harvesting 90-110-year-old Scots pine stands on the site of fresh coniferous forests (Table 1). Clearcuts were established in October-November of the years preceding the experiments and replanted with 2-year-old *P. sylvestris* seedlings in March-April of the following year. In a given year, the distance between the observed clearcuts (=plots) was 0.5-1.0 km. In each experimental plot, 30 stumps were randomly selected and their height and diameter at the top (surface of the cut) were measured.

TREATMENTS. The nematode applications were carried out in the third decade of September and early October when the large pine weevil larvae of overwintering generation were found in the stumps.

Small-scale treatments in 2014-2015

Following nematode species were tested in the experiments:

– in 2014: *S. carpocapsae*, *H. downesi*, *H. bacteriophora;*

– in 2015: *S. carpocapsae*, *S. feltiae*, *H. downesi*.

In each plot, the experiment consisted of 4 variants in which 10 stumps were sprayed with individual nematode species at a dose of 3.5 million IJs (infected juveniles of nematodes)/stump suspended in 10 l of water per stump, and with the same amount of water – comparison control.

Table 1.
Localization and characteristic of experimental plots

Localization (Forest District and coordinates)	Date of treatment	Plot no.	Area [ha]	Stump dimensions (mean ±SD cm)	
				diameter	height
Celestynów 52.0757N; 21.2857E	20.09.2014	1	2.7	46 ±13	22 ±4
		2	2.3	53 ±10	19 ±8
Garwolin 51.8624N; 21.4316E	22.09.2015	3	3.2	59 ±9	16 ±11
		4	4.1	57 ±8	27 ±6
	04.10.2016	5	3.8	60 ±11	23 ±8
		6	3.5	61 ±8	25 ±7

The nematodes were applied to the stumps and the soil around them with a backpack sprayer. A total of 160 stumps (2 years × 2 locations × 4 variants × 10 stumps) were treated.

Large-scale treatments in 2016

On each clearcut, the experiment consisted of three following variants (experimental plots) with 300 tree stumps and their surrounding soil (approximately 1 ha) sprayed with a suspension of *S. carpocapsae* (variant 1) and *H. downesi* (variant 2), both at a concentration of 3.5 million IJs/stump, which corresponded to a dose of 1.05×10^9 EPNs in 300 l water/variant. The stumps sprayed with 300 l of water represented a comparison variant. Aquatic nematode suspensions were prepared immediately prior to treatment. The nematodes were applied using a tractor-mounted sprayer consisting of a 300-litre tank with an agitator ensuring circulation of the suspension and two booms with 10 nozzles each (Fig. 1). Buffer zones of about 10 m were left between the variants.

In addition, two hundred Scots pine seedlings (4 replicates × 50 trees) were selected in June 2017 for all variants to assess damage caused by beetle feeding. The seedlings in each replicate were selected along the transect from the boundary between the clearcut and the surrounding old forest (>50 years old) to the centre of the afforestation and classified into five classes according to the type of damage described by EPPO standard PP 1/127(2):

0 – undamaged seedlings,
1 – up to 5 individual feedings,
2 – from 5 to 15 individual feedings,
3 – more than 15 individual feedings, stem damaged by 50 and more percent,
4 – seedlings killed by weevils.

Fig. 1.
Application of nematodes in 2016 – in the foreground visible a *Pinus sylvestris* stump sprayed with nematode *Heterorhabditis downesi* (Hd)

Assessment of efficacy. Approximately 4 weeks after nematode applications, 10 stumps from each variant in small-scale (total 120 stumps, including 10 stumps × 3 variants × 2 localizations × 2 years) and large-scale treatments (total 60 stumps, including 10 stumps × 3 variants × 2 localizations) were excavated, and 5 root sections 1 m long were collected from each stump to evaluate treatment efficacy. Root samples were debarked in a laboratory and the number of *H. abietis* larvae was compared with nematode treatments. Subsequently, the collected larvae were dissected and analyzed for percentage of parasitism.

Mean daily air temperatures and daily precipitation for the four treatment weeks were obtained from the State Forest meteorological stations in Celestynów Forest District: up to 10 km from experimental plots No. 1, 2 and 30 km from No. 3-6 (Table 1).

The numbers of parasitized larvae in all variants of experiment were analysed using Kruskal-Wallis test with adopted significance level of $P \leq 0.05$, followed by *post-hoc* test (multiple comparisons of mean ranks for all groups) to find treatment effects that are significantly different from each other. The Statistica 10 (StatSoft, 2011) package was used in the analysis.

Results

Weather conditions during experiments. The years 2014-2015 were classified as extremely warm with a lack of precipitations throughout the country. From spring to autumn, there were extremely high air temperatures, accompanied by drought leading to a weakening of stands. Autumn period was assessed as very warm with precipitation deficits in August-through November. During the four week period following the treatments (in both years), conditions were unfavourable for nematode development, as forest litter moisture not exceed 20% (Figs 2a, b). Average daily temperatures varied between 11°C and 20°C, and very little precipitation fell (up to 10 mm/day).

The year 2016 was classified as very warm and normal in terms of moisture conditions. Autumn can generally be described as slightly warm. September was classified as very dry, while October was classified as wet in most parts of the country, including central Poland. The heaviest precipitation, exceeding 40 mm daily, occurred between October 3 and 6 (Fig. 2c). November 2016 was described as average, with mean monthly air temperatures of 4.0°C ±1°C. Precipitation in central Poland was within the normal range, with the greatest precipitation occurring in the first decade of the month.

Assessment of treatment efficacy.
Small-scale treatments. The number of *H. abietis* larvae found in the samples of roots from the stumps in all variants were similar: 11-20 and 12-23 larvae/roots/stump in 2014 and 2015 respectively (Table 2). The highest mortality due to parasitism was noted in the group of larvae collected from the roots of stumps treated with the *H. downesi* (2014 – plot 1: 17,5 ±8 (mean % ±SD), plot 2: 14 ±7; 2015 – plot 1: 13 ±7, plot 2: 12 ±6) (Fig. 3). Considerably lower level of parasitism was observed in the larvae developing in the stumps treated with *S. carpocapsae* (2014 – plot 1: 9.4 ±8.7, plot 2: 7.2 ±6.3; 2015 – plot 1: 8.1 ±5.9, plot 2: 5,5 ±4.1), *H. bacteriophora* (2014 – plot 1: 7.8 ±5.9, plot 2: 3.6 ±2.1) and *S. feltiae* (2014 – plot 1: 3,7 ±2.2, plot 2: 2.1 ±1.5). The large pine weevil larvae isolated from untreated stumps were not parasitized by nematodes. Statistical analysis confirmed the differences between the numbers of larvae parasitized by *H. downesi* and other species of nematodes (2014: localization 1 – H=20.987 p=0.0001, localization 2 – H=24.239 p=0.0001; 2015: localization 1 – H=21.866 p=0.0001, localization 2 – H=22.147 p=0.0001) (Fig. 3).

Regardless the variant of the experiment, in the roots of stumps there were similar numbers of *H. abietis* larvae: 17-29 specimens (plot 1: H=3.6142, p=0.1641; plot 2: H=8.1377, p=0.1710)

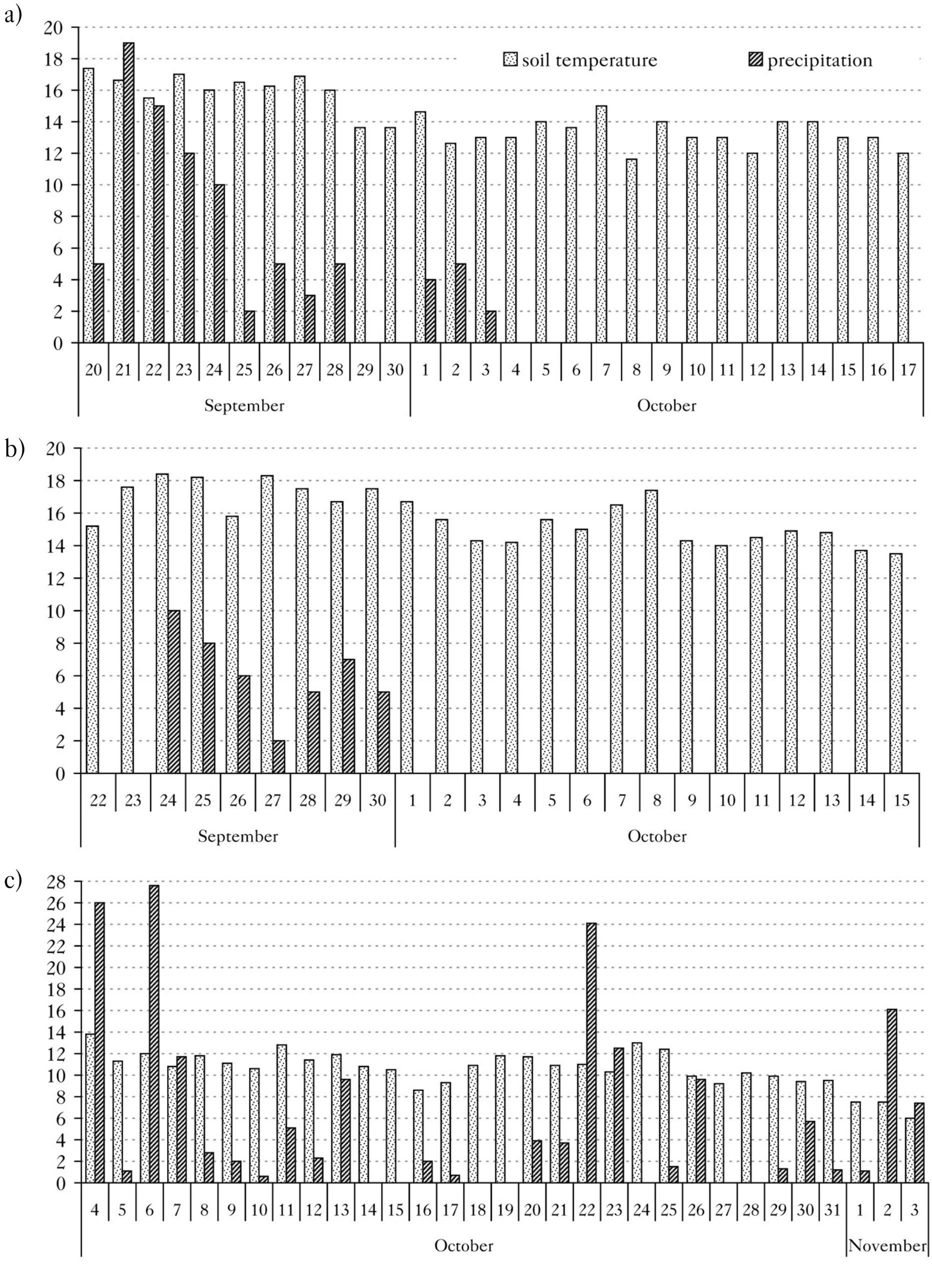

Fig. 2.

Mean daily temperature of the soil at the depth of 20 cm [°C] and daily precipitation [mm] during experiments in 2014 (a), 2015 (b) and in 2016 (c)

(Table 3). Also, there was a similar level of parasitism in the stumps in both locations. Statistical analysis did not confirm the differences between the percentages of larvae parasitized by tested EPNs (localization 1: H=15.3196, p=0.8539; localization 2: H=15.6025, p=0.6012). No nematodes were found in *H. abietis* larvae collected from the roots of stumps treated with water in both years.

Efficacy of treatment based on the numbers of seedlings damaged by *H. abietis* beetles. In the observed reforestations, healthy trees predominated (80-95%), showing no signs of disease caused by *Lophodermium* fungi (no etiological symptoms typical for the pathogen) or changes caused by drought (crown rusting). In addition, there was no damage to the seedlings by other insect or animal species that could affect the general condition of the trees.

Assessment of damage to seedlings showed that 42-60% of seedlings exhibited symptoms of *H. abietis* feeding and 2-4% of trees were killed (Table 4). Similar results were obtained in untreated plots: 50-70% of damaged and 4-6% of seedlings killed by beetles. In localization 2, on plots sprayed with EPNs, the level of damage was lower in all variants, reaching 8-22% of seedlings, while 2-8% of trees were killed by *H. abietis* beetles. The same results were obtained on untreated plots: 14-22% damaged and 2-8% dead seedlings.

There were no statistical differences between the number of seedlings in each class of damage growing on the plots treated with tested nematodes und untreated (Table 5).

Table 2.
Mean number (±SD) of *Hylobius abietis* larvae found in the roots of stumps treated with EPNs in 2014-2015

Year	Plot no.	No. of larvae/roots/stump in the variants				
		S. carpocapsae	*S. feltiae*	*H. bacteriophora*	*H. downesi*	untreated stumps
2014	1	15.7 ±1.9	–	17.4 ±2.4	16.6 ±2.9	11.6 ±2.2
	2	14.6 ±2.8	–	15.2 ±1.8	18.3 ±2.6	13.7 ±1.9
2015	3	16.4 ±3.4	13.9 ±2.8	–	15.0 ±2.5	13.5 ±2.9
	4	14.8 ±2.2	13.4 ±2.3	–	15.7 ±3.3	12.9 ±3.0

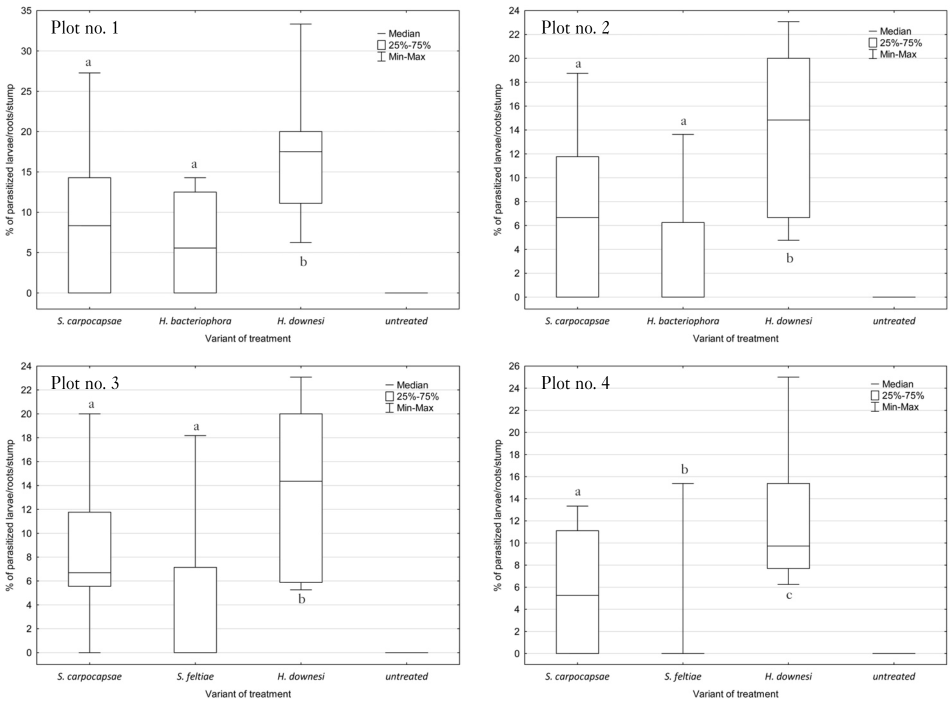

Fig. 3.
Number of *Hylobius abietis* larvae found in the roots of stumps treated with EPNs in 2014-2015

Table 3.

Mean number (±SD) of *Hylobius abietis* larvae found in the roots of stumps treated with EPNs in 2016

Plot no.	Variant of treatment	No. of larvae/roots	No. of parasitized larvae/roots/stump	% of parasitized larvae/roots/stump
5	*S. carpocapsae*	24.6 ±4.4	2.0 ±1.3	8.3 ±5.2
	H. downesi	23.0 ±3.4	2.0 ±1.1	8.9 ±5.1
	untreated	26.4 ±3,3	0	0
6	*S. carpocapsae*	22.7 ±3.4	2.0 ±1.2	8.8 ±6.2
	H. downesi	24.1 ±2.8	2.9 ±1.7	10.5 ±6.9
	untreated	21.0 ±3.9	0	0

Table 4.

Mean number ±SD of *Pinus sylvestris* seedlings damaged by *Hylobius abietis* beetles on the plots treated with EPNs in 2016

Plot no.	Variant of treatment	% of seedlings		
		undamaged (scale 0)	damaged (scale 1-3)	dead (scale 4)
5	*S. carpocapsae*	46.5 ±9.6	51.0 ±8.9	2.5 ±1.0
	H. downesi	48.5 ±8.1	48.0 ±8.5	3.0 ±1.1
	untreated stumps	38.8 ±9.7	57.5 ±8.7	4.5 ±1.0
6	*S. carpocapsae*	86.0 ±6.3	11.5 ±5.9	2.5 ±1.0
	H. downesi	79.5 ±8.1	15.0 ±7.0	5.5 ±1.9
	untreated stumps	78.5 ±3.4	17.5 ±3.4	4.0 ±2.8

Table 5.

Comparison of the tree damage caused by *Hylobius abietis* beetles to *Pinus sylvestris* seedlings in 2016 – results of Kruskal-Wallis test

Plot no.	Variant of treatment	Seedlings		
		undamaged (scale 0)	damaged (scale 1-3)	dead (scale 4)
5	*S. carpocapsae* *H. downesi* untreated stumps	H=0.2096 *P*=0.3313	H=1.8431 *P*=0.3979	H=5.0833 *P*=0.0787
6	*S. carpocapsae* *H. downesi* untreated stumps	H=2.8277 *P*=0.2432	H=1.9406 *P*=0.3790	H=4.3461 *P*=0.1138

Discussion

Contemporary forest protection is based on the integration of various methods that lead to the reduction of the extent of damage caused by insects. The principles of integrated pest management emphasize the significant part of biological methods using biopreparations based on organisms pathogenic to insects. Due to the diversity of biocenotic compounds in the forest environment, the development of methods that use natural enemies (predators, parasites, and pathogenic microorganisms) to reduce insect numbers is extremely difficult. The frequently observed lack of transfer of results obtained under laboratory conditions to field conditions has resulted that few biopreparations have found practical application in the integrated protection of the forest against insects. This group of preparations includes biological insecticides based on the bacterium *Bacillus thuringiensis* Berliner.

In Central European countries, biopreparations containing EPNs have not yet found practical application in forestry because they are very sensitive to adverse weather conditions, especially high temperatures and droughts, which affect their viability or cause them to disappear. However, they have been used to protect forest crops against the large pine weevil in the United Kingdom and Ireland, where climatic conditions are more favourable (higher humidity and lower temperatures in summer) and ensure better survival of nematodes in the soil (Brixey *et al*., 2006; Dillon *et al*., 2006, 2007; Torr *et al*., 2007; Williams *et al*., 2013, 2015; Kapranas *et al*., 2017a,b). The promising results of the nematode trials in the above countries encouraged similar research in Poland.

In the present study, the efficacy of EPNs formulated as commercial biopreparations was evaluated in the control of the great large pine weevil. Nematode species that caused greater than 50% mortality of the large pine weevil in field trials conducted by other authors were selected for testing (Pye and Burman, 1978; Dillon *et al*., 2006, 2007; Skrzecz *et al*., 2011). The group of these species included mainly the nematodes *S. carpocapsae* and *H. downesi*, which showed high activity in studies conducted in northern European countries (Pye and Burman, 1978; Dillon *et al*., 2006; Williams *et al*., 2013). Also in Poland, Skrzecz *et al*. (2011, 2012) observed more than 80% mortality of *H. abietis* larvae developing in *P. sylvestris* stumps treated with *S. carpocapsae* and *H downesi*.

Nematodes were applied following the procedures of studies conducted in the United Kingdom and Ireland based on spraying litter with an aqueous suspension of IJs at a dose of 3.5 million/stump (Brixey *et al*., 2006; Dillon *et al*., 2006; Torr *et al*., 2007) to reduce the number of pest larvae developing in the stumps. Larvae are the stage most susceptible to nematode infection. Ansari *et al*. (2012) and Williams *et al*. (2015) presented the analysis of the extent of EPN infection in different developmental stages of the pests. They found that *H. abietis* larvae were the most infected with nematodes (almost 50%) compared to pupae and imagines.

To minimize the effects of summer weather conditions, which are generally unfavorable for EPNs in central European countries, we used EPN in early autumn against second-generation larvae of *H. abietis* overwintering in the stumps. The change in treatment timing compared to applications described by other authors was influenced by the results of our previous observations when stump treatments were applied in the first half of June. *Hylobius abietis* mortality of a few percent was observed in treated stumps, which was not different from natural pest mortality in untreated stumps (Skrzecz *et al*., 2011). These results were influenced by weather conditions unfavorable for nematode development during the study (high air and soil temperatures, lack of precipitation), which may have led to increased mortality of nematodes deployed at that time. On the other hand, results of initial trials of EPN application in early autumn, when weather conditions were much more favorable for nematode development, showed that 40-80% of the larvae of the great pine weevil that overwintered in treated stumps were parasitized by nematodes (Skrzecz *et al*., 2012).

In the present studies, the parasitism of *H. abietis* larvae was low in all trials (≤25%). The species *H. downesi* parasitized most larvae of the pest (up to 25%), while other nematode species (*S. carpocapsae*, *S. feltiae* and *H. bacteriophora*) caused infection of up to 15% of *H. abietis* larvae. It is well known that environmental parameters, especially temperature extremes, UV radiation, low humidity, and soil properties, can affect the survival and virulence of EPNs. Certainly, these results were not affected by the temperature of the soil and air, which was 15-20°C on the days of application. These temperatures are optimal for the development of most species of insecticidal nematodes EPNs (Griffin and Downes, 1991; Chojnacki, 2010; Dzięgielewska and Skwiercz, 2018; E-nema, 2023). Both low and excessive (above 30°C) air and soil temperature severely limit the ability of EPNs and the pathogenicity of symbiotic bacteria (Griffin and Downes, 1991;

Shapiro-Ilan *et al.*, 2014). Steinernematidae can survive at temperature range of 4 to 30°C, while Heterorhabditidae can survive in an optimal range of 15 to 28°C (Odendaal *et al.*, 2016). In addition, EPNs were applied in the morning and in early autumn, which certainly led to minimizing the harmful effect of UV radiation on the survival of nematodes (van Dijk *et al.*, 2009; Shapiro-Ilan *et al.*, 2015).

Analysis of soil properties showed that even the pH of forest soils, which was 5.0-5.5, did not affect the effectiveness of EPN treatments (Hara *et al.*, 1991; Griffin *et al.*, 1994; Stock, 1995; Dzięgielewska and Skwiercz, 2018). Nyasani *et al.* (2008) found that the soil pH range of 4.5 to 5.7 was best tolerated by EPNs. In our studies, EPNs were applied to podzolic soils, which are characterised by low competitive pressure from understory vegetation and provide optimal conditions for Scots pine regeneration. Kapranas *et al.* (2017b) showed that the spread of EPNs (IJs) is influenced by soil parameters such as sand content and compaction. They found that both compaction and increasing peat content generally reduced the pathogenicity of *S. carpocapsae*, *S. feltiae*, and *H. downesi*. Therefore, it can be assumed that the structure of podzolic soils with a grain size of loose sand, and rarely loamy sand, favours the spread of EPNs.

Soil moisture is probably the most important factor affecting EPN efficacy in our studies. The year 2014, the year in which our studies began, initiated a trend in Poland that continues to this day, in which prolonged droughts combined with extremely high air temperatures in the growing season have become the norm, with all the consequences for the natural environment. In 2014-2016, the average moisture in *P. sylvestris* litter in August-September did not exceed 20%, several percent below long-term averages. Water scarcity in soils, which has been worsening for years, is certainly not conducive to EPN activity, especially in forests where rainfall is the only way to replenish water.

Nematode application did not significantly affect the extent of damage to seedlings caused by *H. abietis* beetles. However, it could be expected that large-scale application of nematodes to the afforested area (on the stumps and soil) could result in a reduction in the number of *H. abietis* beetles hatching from the stumps, thus reducing damage to seedlings. However, there are different opinions about the relationship between pest abundance and the extent of damage. Szmidt and Korczyński (1982) considered that even in periods of high beetle abundance, damage to seedlings may be low, whereas in low beetle abundance, severe damage to seedlings can be recorded. On the other hand, Swedish observations indicate some correlation between the number of weevils and the number of damages to reforestations established on 1-2 year old clearcuts (Nordenhem, 1989; Örlander *et al.*, 1997). It should also be considered that increases in pest numbers on fresh clearcuts are also influenced by pest migration from surrounding stands (Skrzecz *et al.*, 2021). In addition, Girling *et al.* (2010) found that beetle infection or exposure to EPNs did not affect their feeding.

Conclusions

In summary of the presented research results, the high sensitivity of EPN to extreme environmental factors is the main obstacle to exploiting its full potential as a biological agent for controlling insect pests in forest crops. Our results indicate low parasitism of *H. abietis* larvae by the nematodes *S. carpocapsae*, *S. feltiae*, *H. bacteriophora*, and *H. downesi* applied to the stumps of *P. sylvestris* and the soil surrounding them. Despite the fact that the treatments were carried out in autumn, a significant water deficit in the soil was not conducive to the development of nematodes in the years studied, resulting in low parasitism of the pest, *i.e.* up to 25% of larvae developed in the stumps treated with nematodes. The highest mortality of *H. abietis* larvae was observed in roots of *P. sylvestris* stumps treated with *H. downesi*. These applications did not affect the extent of damage caused by *H. abietis* beetles to *P. sylvestris* seedlings in the experimental plots. The low level of *H. abietis* parasitism was influenced by weather conditions unfavourable for nematode development, *i.e.*, lack of precipitation resulting in lower forest litter and soil moisture. In Poland in 2014-2016, prolonged drought and high air temperatures, referred to as 'physiological drought,' led to weakening of *P. sylvestris* stands throughout the country. In this case, the application of EPN requires a lot of water and special equipment, which makes the use of nematodes very troublesome and expensive. However, if temperature, humidity, and UV radiation are optimal for EPN development and pathogenicity, their use in soil application can provide satisfactory results. Certainly, more research is needed to increase the survival rate of EPNs in soils in open areas under warming climate conditions. Exploring the possibility of using surfactants, UV protectants, or more technically advanced technologies such as nematode-filled capsules offers hope (Labaude and Griffin, 2018).

Authors' contributions

I.S. – experimental design, field trials, data collection and analysis, manuscript writing; D.T. – data analysis and interpretation, participation in manuscript writing ; T.J. – field trials, statistical analysis, manuscript improving; A.M. – microscopic analysis, manuscript improving.

Conflict of interest statement

The authors declare no conflict of interest.

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STRESZCZENIE

Możliwości wykorzystania entomopatogenicznych nicieni do redukcji liczebności szeliniaka sosnowca *Hylobius abietis* L. – wpływ czynników abiotycznych

Zabezpieczanie upraw drzew iglastych przed szeliniakiem sosnowcem *Hylobius abietis* (L.) jest ważnym problemem ochrony lasu i dlatego w wielu krajach europejskich prowadzone są ciągłe badania nad biologią wrogów naturalnych tego szkodnika i możliwościami ich użycia do redukcji jego populacji. Prace te koncentrują się przede wszystkim na wykorzystaniu entomopatogenicznych nicieni (EPNs) z rodzajów *Steinernema* i *Heterorhabditis* do ograniczania liczebności szeliniaka sosnowca rozwijającego się w pniakach. Pierwsze doświadczenia z wykorzystaniem EPNs wykonano w Szwecji, gdzie w wyniku opryskiwania pniaków zawiesiną nicienia *Steinernema carpocapsae* (Weiser) uzyskano ponad 50% śmiertelności larw szkodnika rozwijających się w traktowanych pniakach. W Wielkiej Brytanii oraz w Irlandii wykonano liczne badania terenowe z użyciem nicieni, w których testowano inwazyjność kilku gatunków nicieni z rodzaju *Steinernema* (*S. carpocapsae*, *S. feltiae* (Filipjev)) i *Heterorhabditis* (*H. bacteriophora* (Poinar), *H. downesi* Stock, Griffin & Burnell). Stwierdzono, że wykonane w okresie maj-czerwiec zabiegi opryskiwania pniaków *Pinus* spp. i *Picea* spp. zasiedlonych przez szeliniaka sosnowca spowodowały redukcję jego liczebności o ponad 60%. Badania te stały się inspiracją do przeprowadzenia podobnych prób w kraju.

W latach 2014-2016 wykonano doświadczenia terenowe oceniające możliwość wykorzystania różnych gatunków owadobójczych nicieni z rodzajów *Steinernema* i *Heterorhabditis* do ograniczenia populacji szeliniaka sosnowca oraz wyrządzanych przez niego szkód w uprawach leśnych.

Badania zlokalizowano w nadleśnictwach Celestynów i Garwolin (RDLP w Warszawie) (tab. 1). Każdego roku do zabiegów aplikacji nicieni wybierano po 2 zręby zupełne o areałach powyżej 3 ha. Na każdym ze zrębów znajdowały się roczne pniaki *P. sylvestris* powstałe po usunięciu 90--110-letnich drzewostanów.

W latach 2014-2015 wykonano doświadczenia „w małej skali", w których na każdej z powierzchni doświadczalnych opryskano zawiesiną nicieni *S. carpocapsae*, *S. feltiae*, *H. bacteriophora* i *H. downesi* po 30 pniaków wraz z glebą wokół nich w promieniu około 0,5 m. W doświadczeniach „w dużej skali" zastosowano nicienie *S. carpocapsae* i *H. downesi*, którymi opryskiwano pniaki i glebę na powierzchni 1 ha (ryc. 1). We wszystkich próbach aplikacje nicieni w dawce 3,5 miliona larw inwazyjnych nicieni (IJs) na pniak wykonywano na przełomie września i października, kiedy w korzeniach pniaków znajdowały się larwy szeliniaka sosnowca pokolenia zimującego. Po około 4 tygodniach od zabiegów dokonano oceny skuteczności polegającej na wykopaniu i pobraniu z wszystkich pniaków doświadczalnych po 5 odcinków korzeni o długości około 1 m. Następnie korzenie okorowano i porównano liczby larw szeliniaka sosnowca oraz oceniono odsetek owadów zarażonych przez nicienie.

Wyniki zabiegów „na małą skalę" wykazały, że w analizowanych korzeniach pniaków znajdowało się do 23 larw/korzenie/pniak (tab. 2). Jednocześnie stwierdzono niskie spasożytowanie larw szeliniaka sosnowca (5-25%) przez zastosowane nicienie (ryc. 3). Największą śmiertelność szkodnika (11-25%) stwierdzono w korzeniach pniaków traktowanych *H. downesi*. Również w zabiegach „na dużą skalę" stwierdzono niską skuteczność zabiegów, ponieważ nicienie *S. carpocapsae* i *H. downesi* spowodowały spasożytowanie do 17% larw rozwijających się w traktowanych pniakach (tab. 3). Aplikacje nicieni nie miały wpływu na poziom uszkodzenia upraw sosnowych założonych na powierzchniach doświadczalnych (tab. 4 i 5). Z pewnością przyczyną tak niskiego stopnia spasożytowania szeliniaka sosnowca były niekorzystne dla rozwoju nicieni warunki atmosferyczne panujące w okresie badań, zwłaszcza niska wilgotność gleby wynikająca z braku opadów atmosferycznych (ryc. 2).