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Efficacy of entomopathogenic nematodes for control of large pine weevil, *Hylobius abietis:* effects of soil type, pest density and spatial distribution.

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Running title: *Biological control of large pine weevil with entomopathogenic nematodes*

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Author Contribution Statement: AK CTG and POT conceived and designed research. AK BM SQ LM CDW conducted experiments. AP provided the entomopathogenic nematodes. AK and CTG analyzed data and wrote the manuscript. All authors read and approved the manuscript.

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Key Message:

- Entomopathogenic nematodes (EPN) were applied to conifer stumps for control of the large pine weevil *Hylobius abietis* LPW.
- Species with different foraging strategies (ambushers *vs* cruisers) provided the same level of control.
- EPN efficacy is predicted to be increased in organic soils. However, EPN efficacy in suppressing LPW populations in peaty (organic rich) and in mineral soils was equal.
- Weevil density and spatial distribution within stumps, which both vary depending on soil type, explain how EPN parasitize and suppress the pests.

Abstract

The large pine weevil *Hylobius abietis* (L.), LPW, is a major pest of trees in replanted coniferous forests in northern Europe. The use of entomopathogenic nematodes (EPN) applied against developing stages for population suppression is increasingly recognised as an effective alternative to plant protection using chemical pesticides. Here we report results from a series of trials we conducted over two years using two species of EPN, *Steinernema carpocapsae* (Weiser) and *Heterorhabitis downesi* (Stock, Griffin, and Burnell) with different foraging strategies. Trials were conducted at lodgepole pine sites in Ireland on both mineral and peat soil type. EPN suspension was applied to the stumps of felled pine trees and EPN efficacy was determined afterwards by directly assessing parasitism rates after debarking one quarter of the stumps and by collecting emerging adult weevils from traps erected over other treated and control stumps. Our results suggest that both species of EPN are equally effective in suppressing LPW populations to below the current, informal thresholds of economic damage. EPN were equally efficient in controlling LPW in peat and in mineral (lithosols/regosols and acid brown earth/brown pozolics) soils. Weevil density and distribution within pine stumps in peat *vs.* mineral sites can explain patterns of LPW parasitism and suppression. Our results also suggest that infestation level (number of weevils per stump) can be an important factor in forecasting EPN application success.

Keywords: forest pest, root feeding insect, entomopathogenic nematodes, foraging strategy, soil type, density-dependent parasitism.

Introduction

The large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) is the most important pest of tree seedlings in replanted coniferous forests in Northern Europe, costing an estimated €140 million in Europe of which €2.75 million in the UK alone (Evans et al. 2015). The weevils are attracted to clear-felled areas by volatile chemicals emitted by the stumps of recently felled trees; they oviposit in the stumps and immature weevils develop under the bark (Leather et al. 1999). Upon emergence, in late summer to autumn, adult weevils feed on young seedlings and can destroy 100% of newly planted trees with an estimated mortality in UK and Ireland of 50% within the first few years in sites untreated with insecticides (Heritage & Moore 2001). A single adult can damage or kill several young plants (Eidmann and Lindelöw 1997; Wainhouse et al. 2007), and thus even a low number of adults emerging from stumps can have a significant impact on sites that have been replanted. In recent years concerns over weevil damage have increased due to climate change and rising temperatures which not only leads to a shorter life cycle and increased flight and dispersal of the LPW (Inward et al. 2012; Tan et al. 2010), but also shifts in the distribution of areas suitable for the large pine weevil (Barredo et al. 2015).

Current practices for managing LPW rely on a variety of chemical, cultural and biological methods. Treatment of the young plants prior to planting with pyrethroids and an additional top-up spray of planted trees is the most popular method, but cypermethrin and alpha-cypermethrin, the most effective pesticides, are only available for use in UK and Ireland for a limited period under derogation from the Forest Stewardship Council (Anonymous 2014). In addition, concerns over environmental impacts lead to withdrawal of many synthetic pesticides based on EU directives (Directive 91/414/EEC, Regulation 1107/2009/EC). Before pesticides are used, biological control measures, together with physical and other non-chemical methods, should have first preference (Directive

2009/128/EC). Delay of restocking sites for at least two years has been reported to be helpful if there are no clear-felled areas nearby (Örlander and Nilsson 1999; Örlander and Nordlander 2003; Leather et al. 1999). Management of felling and restocking dates using decision support systems integrated with GIS to minimize weevil impacts has become standard practice in UK (Wainhouse et al. 2007; Evans et al. 2004). Entomopathogenic nematodes (EPN) applied in an inundative fashion are a promising tool in the management of the pine weevil (Torr et al. 2005; Brixey et al. 2006; Dillon et al. 2006, 2007, 2008; Williams et al. 2013a). In addition they are environmentally safe (Ehlers and Hokkanen 1996) and have little impact on non-target species in the pine weevil habitat (Dillon et al. 2012).

Previous trials in Ireland have shown that the most promising species is the native *Heterorhabditis downesi*, a cruise-foraging nematode (Dillon et al. 2006; Williams et al. 2013a). However, these studies also highlighted that *Steinernema carpocapsae* (Weiser), an ambush-type forager, can also be quite effective against LPW, contrary to the assumption that EPNs with an ambush foraging strategy are not efficient in controlling subterranean pests (Gaugler et al. 1997; Grewal et al. 2005). The former species occurs naturally in Ireland, Britain and in continental Europe (Stock et al. 2002) but it is still not commercially produced, whereas the latter is cultured by many commercial producers of biological control agents and thus it is readily available for use in management of LPW. Furthermore, a meta-analysis study has shown that the efficacy of EPN against LPW is predicted to be greater in peat soils which are characterised by a high level of organic matter than in mineral soils which have lower organic matter (Williams et al. 2013b).

As part of the BIOCOMES (2013-2017) consortium which promotes the development and use of biopesticides, our purpose in these studies was to directly compare the two species *H. downesi* and *S. carpocapsae* which seem to show the most promising results against LPW. In contrast to previous trials (Dillon et al. 2006; Williams et al. 2013b), where *H. downesi* was produced in wax moth larvae, in the present study both nematode species were produced in bioreactors under commercial conditions (Friedman 1990). Moreover, we explicitly tested the conclusion of the meta-analysis that peat soils favour nematode control of LPW by including both peaty and mineral soils in each of two trial years. We conducted all our trials in pine sites (*Pinus* spp.), as weevils develop in higher numbers than in spruce (*Picea* spp.) (von Sydow and Birgersson 1997; Dillon et al. 2008; Williams et al. 2013b). A direct relationship between number of weevils developing in stumps and subsequent damage by adults on replanted seedlings has not yet been demonstrated; however, current experience and practice in both Ireland and UK (Wainhouse et al. 2007; unpublished note Coillte, Ireland) show that 20 weevils/stump will result in emergence of adult weevils at levels requiring plant protection. Previous studies have compared nematode efficacy in relation to control stumps but in this set of trials we also directly compare numbers of adult weevils emerging from stumps with the target threshold which should be more informative for foresters and pest management decision makers. Lastly we investigate how weevil infestation and spatial distribution within stumps influences EPN parasitism and consequently efficacy in controlling LPW.

Materials and methods

Sites of field studies

Trials were conducted on three field sites in 2014 and on four field sites in 2015 which are summarized in Table 1. All sites were clear-felled lodgepole pine *Pinus contorta* Dougl. var. *latifolia*. Sites were categorized as peat and as mineral (*ca.* 5-10 cm of organic litter layer overlying mineral soil). Mineral soils were further classified to the respective great soil group by reference to the interactive soil maps of the National Biodiversity Data Centre (http://maps.biodiversityireland.ie/). At each site, treatments were arranged in a randomized block design with each block bearing a control stump, a stump treated with *S. carpocapsae* and a stump treated with *H. downesi*. For each treatment there were 20 blocks; 10 of these blocks were selected for assessment of parasitism rates (destructive sampling of 30 stumps) and the other 10 were used for monitoring emergence of weevils (placement of traps over 30 stumps). Stumps were approximately of equal size across all treatments and sites. Application of nematodes took place at the time that weevils were in late larval and/or pupal stage, which was confirmed by destructively sampling a number of stumps one to two weeks before the application.

Application of entomopathogenic nematodes

S. carpocapsae (EN03) and *H. downesi* (K122) used for the trials were provided by e-nema GmbH. Packages with EPN infective juveniles (IJs) were stored for less than a week at 9°C until the day of application. On the day of application aqueous suspensions were prepared and kept in 5 L bottles with aquarium pumps for aeration until they were transferred to the field. At the field, 500 ml of the suspension (~ 3.5×10^6 IJs) was applied around the base of each stump (Torr et al. 2005). In control stumps there was no treatment (application of only water as control does not have any effect based on earlier studies).

Assessment of efficacy

Efficacy of treatments was assessed by destructive sampling (hacking) four weeks after application of EPNs and by trapping adult weevils emerging from stumps, following established methods (e.g., Dillon et al. 2006, 2007, 2008). Destructive sampling was performed by removing the bark of about one quarter of the stump with a chisel to a depth of at least 40 cm under the soil surface by clearing away the soil from the stump and associated roots, and recording the stage (larva, pupa, adult), status (healthy, parasitized by nematode, parasitized by fungi, dead by undetermined reason) and location (depth relative to soil level and distance from bole) of each individual pine weevil. Weevils were removed with clean forceps, placed in 24-well plates and transferred to the laboratory. They were then incubated at ~20°C for another two weeks to check for post-sampling EPN mortality.

Modified emergence traps (Moore 2001) were erected about two weeks after EPN application and were then sampled every 2-4 weeks throughout the season, starting mid July until weevil emergence ceased in November. For the control stumps, we also compared directly the weevil number (all stages) observed during hacking (multiplied by four) with the ones collected in the traps. However, a limited number of control stumps were hacked in 2015 due to the limited time window to complete the volume of work (Table 2).

Statistical analysis

Comparison of 'in-root' weevil distribution - depth under soil surface and distance from bole- between mineral and deep peat sites was achieved with a non-parametric Kolmogorov-Smirnov test. Standard *t*-tests were used to compare weevil catches in traps and weevils found during hacking for control stumps in order to assess weevil emergence, and also to compare in-root depth and distance of weevils between peat and mineral sites. Analysis of factors influencing immature weevil parasitism rates and adult weevil emergence was performed with Generalized Linear Models (GLMs) (Crawley 2007). We assumed quasi-binomial error variance for parasitism (proportional) data and significance of effects was assessed by the change in deviance when a variable was removed from the full model. We also used a mixed effect logistic regression analysis to explore parasitism rates in relation to depth below soil surface and horizontal distance from the bole of the stump. Nematode species (two level factor), weevil number, site, depth and distance were introduced as fixed effects whereas each stump was introduced in the analysis as a random effect. We present the raw means of proportional data because they are biologically more relevant than transformed data along with asymmetrical standard errors. (All analyses were performed using GENSTAT statistical package (Version 14, VSN International, Hemel Hempstead, U.K.).

For emergence data (cumulative trap collections over the season) which followed a normal distribution based on Anderson-darling test, we used a two way-ANOVA with nematode species and site introduced as factors; the controls from this analysis were excluded as the purpose was to compare the two EPN species at different locations. Analysis was performed separately for each year. In addition we performed one way ANOVAs followed by a Kramer–Tukey test, to detect differences among means across all site and treatment combinations, with the controls included. Within locations we compared different treatments with a Fisher's least significant difference (LSD) procedure which is a more liberal post-hoc test, while preserving the experiment wise type I error rate at the nominal level of significance, if the number of treatment groups is three (Meier 2006). A complementary one-tailed *t*-test comparing trap catches with a mean of 20 which is the number of weevils per stump that are indicated as a threshold for chemical treatment as recommended by Coillte (Ireland’s national forestry company), was also performed.

Results

Population structure and distribution of weevils in stumps.

Based on hacking control stumps four weeks post application, weevils seemed to be earlier in their development in 2015 than 2014 (Table 2). Weevil distribution in stump roots was different between peat and mineral sites (Figure 2, Smirnov-Kolmogorov test for comparing distributions between two samples, depth: $D = 0.064$, $P < 0.001$; distance: $D = 0.099$, $P < 0.05$). The average depth of weevils was greater in peat vs. mineral sites (14.89±0.236 cm *vs* 12.51±0.387 cm; $t_{2690} = 4.904$, $P < 0.001$). Similarly the average distance of weevils from bole was greater in peat vs. mineral sites (13.57±0.351 cm *vs* 11.23±0.561 cm; $t_{2690} = 3.264$, $P = 0.001$). Thus, weevils were more likely to be found in the roots deepest and farthest from the bole in stumps on peat than in stumps on mineral soils (Fig. 1). The site in Clonoghil (peat) had a much higher percentage of weevils at depths > 20cm compared to the other sites – 55% versus 9-31% for the other six sites (Table 2).

There was also a positive relationship between the number of weevils per stump and their average distance from the bole of the stump (GLM model with weevils: $F_{1,115} = 22.46$, $P < 0.001$; soil type: $F_{1,115} = 3.83$, $P = 0.053$) but there was not a significant relationship between weevil number and average depth (GLM model with weevils: $F_{1,115} = 0.13$, $P = 0.720$; soil type: $F_{1,115} = 3.83$, $P = 0.053$).

Parasitism rates: differences among sites and nematode species

Parasitism rates (after a two week post sampling incubation period) were the same for both nematode species in both years (GLM analysis, 2014: $F_{1,56} = 2.18$, $P = 0.116$; 2015; $F_{1,68} = 0.61$, $P = 0.437$, Fig. 2). Parasitism rates did not differ across the three sites in 2014 ($F_{2,56} = 2.27$, $P = 0.114$), but they were significantly different across sites in 2015 ($F_{3,68} = 14.37$, $P < 0.001$). However, no clear trend existed in comparing parasitism rates between peat and mineral sites (Fig. 3). The interactions between site and nematode species were insignificant for both years and are not shown.

Effects of pine weevil infestation on parasitism rates

For the year 2014 we found no effects of weevil number per stump on parasitism rates ($F_{1,56} = 0.3$, $P = 0.584$), but in 2015 parasitism rates were inversely correlated with weevil number per stump ($F_{1,68} = 6.48$, $P = 0.014$, Fig. 3). Despite a strong negative trend, the effect of number of weevils on parasitism rates was not significant when data from both years were combined ($F_{1,125} = 3.27$, $P = 0.074$) but was significant when instead of site, soil type (peat vs mineral) was introduced in the model ($F_{1,125} = 12.83$, $P < 0.001$).

Parasitism rates in relation to root depth and distance from the stump

Logistic analysis showed that LPW parasitism rates were significantly lower at greater depths in soil ($F_{1,2684} = 70.85$, $P < 0.001$, Fig. 4a) and at greater distance from the bole of the stumps

($F_{1,2684} = 239.76$, $P < 0.001$, Fig. 4b). Parasitism rates in relation to depth and distance did not differ between the two nematode species ($F_{1,2684} = 0.13$, $P = 0.719$), but they did differ significantly among sites ($F_{6,2684} = 68.1$, $P < 0.001$, Fig.4). Furthermore, the interactions between site, depth and distance were also significant (site*depth: $F_{6,2684} = 2.54$, $P = 0.019$, site*distance: $F_{6,2684} = 5.00$, $P < 0.001$). However, trends of parasitism rates in relation to depth and distance among sites of different soil type (peat versus mineral) were not clear; Parasitism rates of LPW at deeper levels seemed to be greater for two of the three mineral sites (Killurney and Tigroney, Fig. 4a), at both of which the soil was classified as lithosol/regosol (Table 1). Parasitism at greater distance from the bole was greater at a peat site (Knockaville, Fig. 4b)

Emergence of pine weevils

Numbers of adult LPW emerging from stumps treated with *H. downesi* or with *S. carpocapsae* did not differ in either year (Table 3, Fig. 5 & 6). Numbers of emerging adult LPW from stumps treated with both species also did not differ amongst the three sites in 2014, but they significantly differed amongst sites in 2015, due to the high infestation in the Clonoghil site (Table 2, Fig. 5 & 6).

In 2014 the number of LPW emerging from stumps treated with *H. downesi* was significantly lower compared with the controls across all three sites, whereas *S. carpocapsae* was effective in two sites (both of peat) (Fig. 5). In 2015, applications of both nematode species led to significant suppression of LPW adult emergence in three out of four sites (Fig. 6). The site at which application did not lead to significant suppression was a peat site (Clonoghil).

In one site (Gurtnapisha, mineral) the average adult weevil number/ control stump was lower than the suggested threshold of 20 weevils/ stump and from a management perspective there was no need for treating this site (Fig. 6). However, the higher number of weevils in control stumps at destructive sampling than the number of adult weevils collected in traps indicates that weevil emergence during the late summer-autumn was incomplete (Table 2). In the remaining six sites, treatment with EPN led to suppression of weevil emergence below the suggested threshold of 20 weevils/stump in five out of six sites, but for each location one species only provided the level of control sought; however, there was no relationship between soil type and which species was most effective (Figures 5 & 6).

Assessment of weevil emergence

In three sites (one in 2014 and two in 2015) weevil emergence was determined to be incomplete based on comparisons between weevils found in stumps during hacking and adult weevils collected in traps, in control stumps. Two of these sites were mineral (Killurney 2014 and Gurtnapisha 2015, Table 2).

Discussion

Our study confirms previous studies showing that the use of EPN can be efficient in controlling LPW (Dillon et al. 2006, 2007). However, it adds new information that is highly pertinent to controlling LPW by application of EPN and also suggests that the importance of factors such as soil type and infestation load (i.e., number of weevils developing in the stump, Williams et al. 2013b) should be at least considered in the future. Differences in parasitism rates were strikingly different among the two years of our trials. Ambient and soil temperatures were higher in 2014 than in 2015 especially in June and July, the months immediately following application (supplementary material). In addition to the direct effect of temperature on nematode efficacy (Grewal et al. 1994; Wilson et al. 2016), the higher temperatures of 2014 may have influenced nematode efficacy indirectly through an effect on weevil development. In 2014 weevils were more advanced in their development at the time of application. While LPW pupae are in general less susceptible to EPN than are larvae, there is evidence that both newly pupated insects and callow adults are susceptible (Williams et al.

2015). Application at a time when many of weevils are transitioning from larva to pupa or from pupa to callow adult may favour EPN.

Overall, our trials suggest that both *H. downesi* and *S. carpocapsae* are equally efficient in parasitizing the LPW developing in stumps and subsequently suppressing adult numbers coming out of the stumps as shown by our emergence trap data. This is perhaps not surprising; although previous studies showed that *H. downesi* is superior to *S. carpocapsae*, it was suggested that the latter should not be underrated as a biological control agent (Dillon et al. 2006, 2007). In our trials, *S. carpocapsae* not only provided considerable suppression relative to controls in many cases, but also suppressed the numbers of emerging adult weevils below the targeted threshold of 20/stump, as many times as *H. downesi* did. It is also noteworthy that in the current study parasitism rates in relation to depth and distance from the base of the stump were equal for both species. These results are also intriguing given the ambushing foraging behaviour of this species; because S. *carpocapsae* can find and infect relatively immobile insects at considerable distances even deep within soil, the current classification of EPN based on their foraging behaviour (ambushers vs cruisers) is under question (Wilson et al. 2012; Griffin 2015). On the other hand, it might be possible that nematodes are carried passively along the roots either by the suspension water or later through rainfall which was adequate in summer of both years (supplementary material). Other studies have similarly confirmed the effectiveness of *S. carpocapsae* in parasitizing and controlling other root feeding insects (Jansson et al. 1993; de Altube et al. 2008). The *H. downesi* used in the present experiments was the same strain as used in our previous trials, but was produced in bioreactors, formulated and shipped from Germany to Ireland, instead of being produced in the laboratory in wax moth larvae as previously (Dillon et al. 2006, 2007, 2008; Williams et al. 2013a). Production methods may impact on quality of EPN (Grewal and Peters 2005), but there was no evidence that bioreactor-produced *H. downesi* were of lower quality than the insect-produced nematodes used in previous trials (see analysis in supplementary material).

Many studies have addressed the effects of soil texture on EPN efficacy, with the emphasis on the mineral component of the soil (e.g. Choo and Kaya, 1991; Koppenhöfer and Fuzy 2006), but much of the coniferous forestry in northern temperate regions is planted in peat soils. For example, 45% of Irish forests have a peat depth of over 30 cm (Anonymous, 2007). Peat soils are characterised by very high organic matter, derived from the accumulation of dead plant material under water-logged, anaerobic conditions. Several recent studies suggest that media with high organic content including peat are favourable for EPN (Kruitbos et al. 2010; Ansari and Butt 2011; Nielsen and Lewis 2011; Wilson et al. 2012). Our results show that both EPN species were as efficacious in peat as in soils classified as mineral. The suitability of this medium for nematodes may be in part due to the high moisture content of peaty soils (Paavilainen and Päivänen 1995; Grant and Villani 2003; Preisser and Strong 2004), movement of nematodes though rootways that might be more accessible in peaty soils (Ennis et al. 2010), and carriage of cues needed for host location at longer distances (Hitpold and Turlings 2008; Turlings et al. 2012). Our trials do not support the previous meta-analysis of studies on using EPN for controlling the LPW suggesting that efficacy was greater in peat than in mineral soils (Williams et al. 2013b). “Mineral” is a broad category, encompassing many different sub-types used for forestry, ranging from acid brown earths (well drained productive soils with good physical properties) to gleys (poorly drained soils with poor soil physical properties (Kennedy 2002). In addition, peat soils also vary based on formation type and subsequent peat extraction practices (Renou and Farrell 2005). For example, deeper layers of cutover blanket bog have poor hydraulic conductivity (hence poor drainage) (Renou and Farrell 2005). Thus, a more refined soil classification would aid in predicting EPN efficacy against LPW. Nonetheless, our study suggests at least that the use of

EPN for controlling LPW should not only be determined by soil type, but other factors might also be important (see below).

Our trials also provide some evidence that level of infestation can have important effects on LPW parasitism rates. This can be further confirmed by looking at weevil trap catches; the only site on which EPN did not provide any significant suppression over the control stumps, nor achieved the target number of 20 weevils/stump, was the site with the highest weevil infestation (Clonoghil, adult weevils emerging max = 468, median = 102, fig. 5). Mechanistically, density dependence can be explained by the reduced capacity of nematodes to reach weevils which are located in deeper roots and horizontally farthest from the bole. In stumps bearing a high numbers of weevils, more of the weevils are located at more distant parts of the roots and thus a higher percentage of weevils escape parasitism by nematodes. Density dependent parasitism can explain patterns of weevil suppression observed in our trials, and also bears important consequences for the use of EPN as biocontrol agents for LPW. For instance, more inoculum might be needed in cases of high infestations (Shapiro-Ilan et al. 2012). However, it should be noted that in our trials the EPN dose applied as determined by other studies (Torr et al. 2005; Dillon et al. 2006, 2007) provided satisfactory control in moderate to high infestation levels, except in one case where infestation levels were so high; in this case the efficacy of any other alternative control measure is questionable.

Passive movement of EPN either by suspension or by later rainfall might be more favoured in peaty soils (Wheeler 1995) but average weevil depth in mineral sites tends to be lower than in deep peat sites. This trend was even more extreme in Clonoghil, a peat site where a relatively small fraction of weevils were found closer to the surface in comparison with other sites of either soil type. In addition, when infestations are high, weevils are found further from the bole, both for mineral and peat sites. More distantly located weevils along the roots are parasitized at lower rates as we demonstrate here and in other studies (Dillon et al. 2006, 2007). These properties of weevil distribution in stumps could explain the relatively equal efficacy of EPN in mineral and peat soils. In other words, EPN movement and survival might be more constrained in mineral sites, but in these sites target weevils are closer to the application point making it easier to be reached by EPN. Moreover, if LPW infestations in peat sites are moderately high then it is likely that EPN will provide at least an adequate to good level of control. In our study we also observed that weevils are more abundant in peat sites than in mineral ones. Thus EPN efficacy in mineral sites can at least be explained by lower weevil infestation rates.

Lastly, we should point out that our trials were in pine stumps which sustain a higher number of weevils than in spruce stumps (von Sydow and Birgersson 1997; Dillon et al. 2008; Williams et al. 2013b), and thus the use of EPN in spruce sites might provide even better control of LPW. Other topics of investigation would be on optimizing application method of the suspension (e.g. Brixey et al. 2006) and also assessing how soil compaction due to timber harvesting machinery can influence EPN efficacy. In conclusion the results of our trials not only confirm previous studies suggesting that EPN are efficient inundative biological control agents of LPW, but also show that two species with different foraging strategies are equally efficient in suppressing LPW populations at the target level sought. In addition, EPN application should not only be determined by soil type but also on other factors such as infestation levels, which is even more encouraging in widening their use in more cases where LPW control is sought.

This article does not contain any studies with animals performed by any of the authors.

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Table 1. Location and characteristics of field sites.

Site name	Location	Altitude	Soil type	Felling month/year	Application date
Cloondara	Co. Longford 53°44'16.7"N 7°54'15.7"W	41m	Peat[1]	04-05/2013	12/06/2014
Knockaville	Co. Westmeath 53°29'25.9"N 7°13'46.0"W	95m	Peat[2]	07-08/2013	10/06/2014
Killurney	Co. Tipperary 52°25'01.5"N 7°36'13.0"W	371m	Mineral[3]	03-04/2013	13/06/2014
Clonoghil	Co. Laois 52°58'45.8"N 7°37'35.5"W	127m	Peat[2]	04-06/2013	27/05/2015
Doon	Co. Offaly 53°19'53.6"N 7°51'42.3"W	57m	Peat[2]	03-03/2014	03/06/2015
Tigroney	Co. Wicklow 52°53'04.5"N 6°12'11.6"W	207m	Mineral[3]	06-08/2013	17/06/2015
Gurtnapisha	Co. Tipperary 52°26'33.3"N 7°33'32.9"W	466m	Mineral[4]	01-02/2014	09/06/2015

[1]Fen peat [2]Raised bog/cutaway [3]Lithosol/regosol [4]Acid brown earths/brown podsols

Table 2. Population structure and abundance of *Hylobius abietis* in control stumps four weeks post application and comparison with number of weevils caught in emergence traps over the remainder of the season.

Site name (no. stumps)	% larvae	% pupae	% adults	totals	% weevils within 20cm depth	% weevils within 50cm distance from bole	Hacking* average ± SE	Difference** from emergence
Cloondara (10)	38.02	33.33	28.64	192	77.73%	97.8%	76.8 ± 11.2	t = 0.74 P = 0.465
Knockaville (10)	53.03	33.03	13.95	215	71.06%	96.5%	86.0 ± 22.9	t =1.33 P = 0.19
Killurney (10)	18.18	68.18	13.63	132	90.9%	98.3%	52.8 ± 9.4	t =3.50 P < 0.05
Clonoghil (8)	89.47	10.53	0.00	304	45.02%	71.8%	152 ± 26.9	t =0.30 P = 0.076
Doon (6)	50.25	49.25	0.50	197	70.28%	75.6%	131.3 ± 24.9	t = 3.27 P < 0.05
Tigroney (4)	4.54	88.64	6.82	44	68.98%	96.3%	44 ± 12.1	t = 0.12 P = 0.902
Gurtnapisha (6)	76.00	24.00	0.00	75	85.62%	85.6%	50 ± 12.5	t =3.90 P < 0.05

*Estimated per stump after multiplying by 4

** difference between number of weevils found per stump at hacking (ca. four weeks post application) and number of adult weevils collected in traps erected over control stumps (compare with control graphs of figures 5 & 6).

Table 3. The effect of nematode species and site on the emergence of adult *Hylobius abietis*. (control stumps are excluded).

Source	2014 d.f	2014 F	2014 P	2015 d.f	2015 F	2015 P
Species	1	1.07	0.306	1	0.01	0.930
Site	2	0.77	0.468	3	27.87	< 0.001
Species *x* Site	2	1.37	0.262	3	0.25	0.861
Error	54			72		
Total	59			79		

FIGURE LEGENDS

Figure 1. *Hylobius abietis* distribution (depth from soil surface and distance from bole in cm) in pine stumps.

Figure 2. Percentage parasitism of *Hylobius abietis* by *Heterorhabditis downesi* and *Steinernema carpocapsae* in stumps at different sites in 2014 (a) and 2015 (b). Bars show average values with asymmetrical, quasi-binomially distributed standard errors. Abbreviations: Peat. = peaty soil type, min. = mineral soil type.

Figure 3. Influence of *Hylobius abietis* infestation (number of weevils/stumps) on parasitism rates by entomopathogenic nematodes across different sites. Regression lines are added for the sites in year 2015 wherein there was a significant relationship.

Figure 4. The influence of depth below soil level (a) and distance from the bole of the stump (b) on parasitism rates of *Hylobius abietis* by entomopathogenic nematodes. Data are presented across different sites (logistic analysis of co-variance) and are slightly displaced vertically for clarity. Abbreviations provided regarding and the soil type (peat. and min. for peaty and mineral soils, respectively) and year (2014 and 2015).

Figure 5. Numbers of adult *Hylobius abietis* (average ± s.e.) emerging from control stumps and stumps treated with entomopathogenic nematodes (*Heterorhabditis downesi* and *Steinernema carpocapsae*) across three sites in the year 2014. Capital letter above bars show significantly different treatments across all sites (Tukey-Kramer test), asterisks show Fisher's (LSD) post-hoc tests within each site separately. Checkmarks denote treatments wherein weevil numbers are less than 20/stump (denoted by the horizontal dashed line).

Figure 6. Numbers of adult *Hylobius abietis* (average ± s.e.) emerging from control stumps and stumps treated with entomopathogenic nematodes (*Heterorhabditis downesi* and *Steinernema carpocapsae*) across three sites in the year 2015. Capital letter above bars show significantly different treatments across all sites (Tukey-Kramer test), asterisks show Fisher's

(LSD) post-hoc tests within each site separately. Checkmarks denote treatments wherein weevil numbers are less than 20/stump (denoted by the horizontal dashed line).

Figure 1

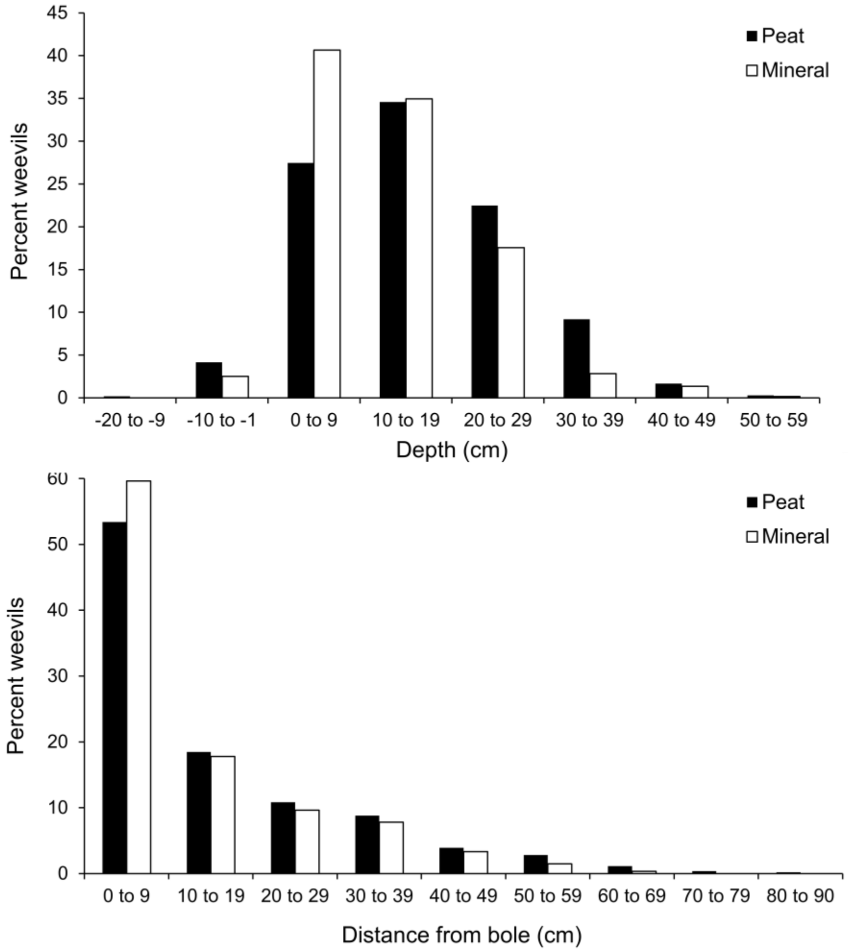

Percent weevils
45
40
35
30
25
20
15
10
5
0
■ Peat
□ Mineral
-20 to -9
-10 to -1
0 to 9
10 to 19
20 to 29
30 to 39
40 to 49
50 to 59
Depth (cm)
Percent weevils
60
50
40
30
20
10
0
■ Peat
□ Mineral
0 to 9
10 to 19
20 to 29
30 to 39
40 to 49
50 to 59
60 to 69
70 to 79
80 to 90
Distance from bole (cm)

Figure 2

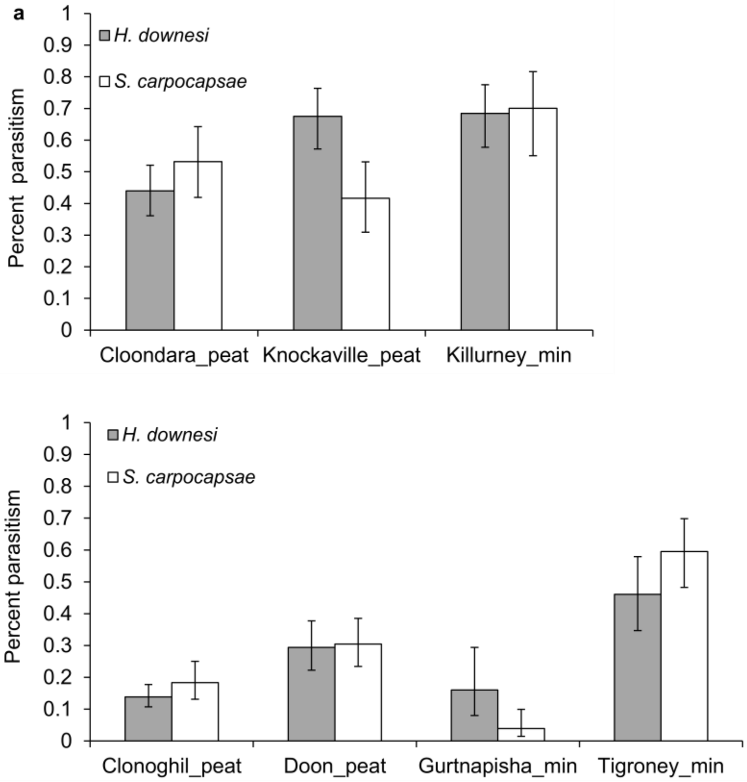


Figure 3

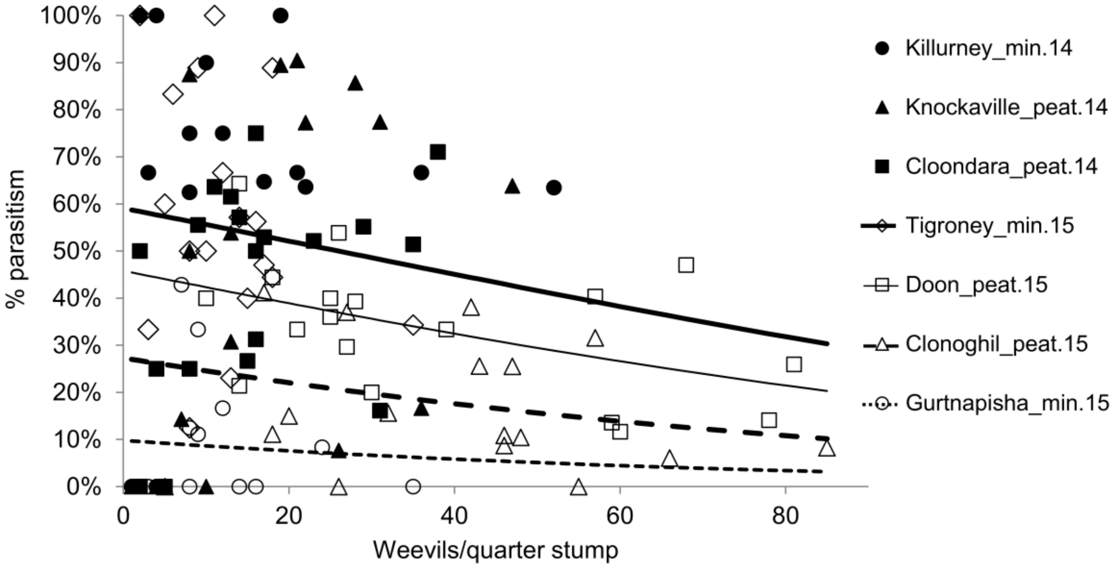


Figure 4

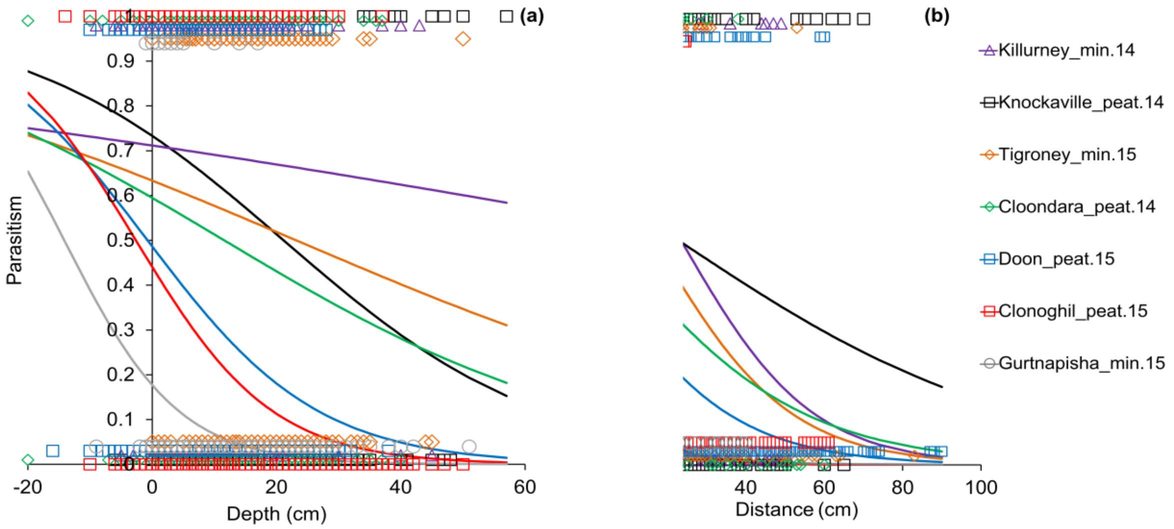


Figure 4

Figure 5

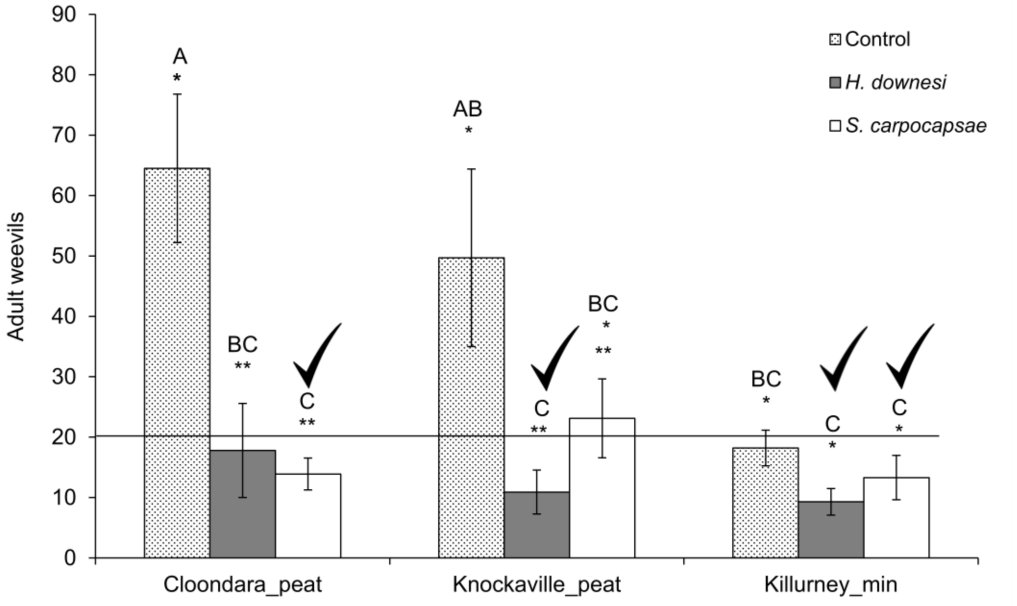
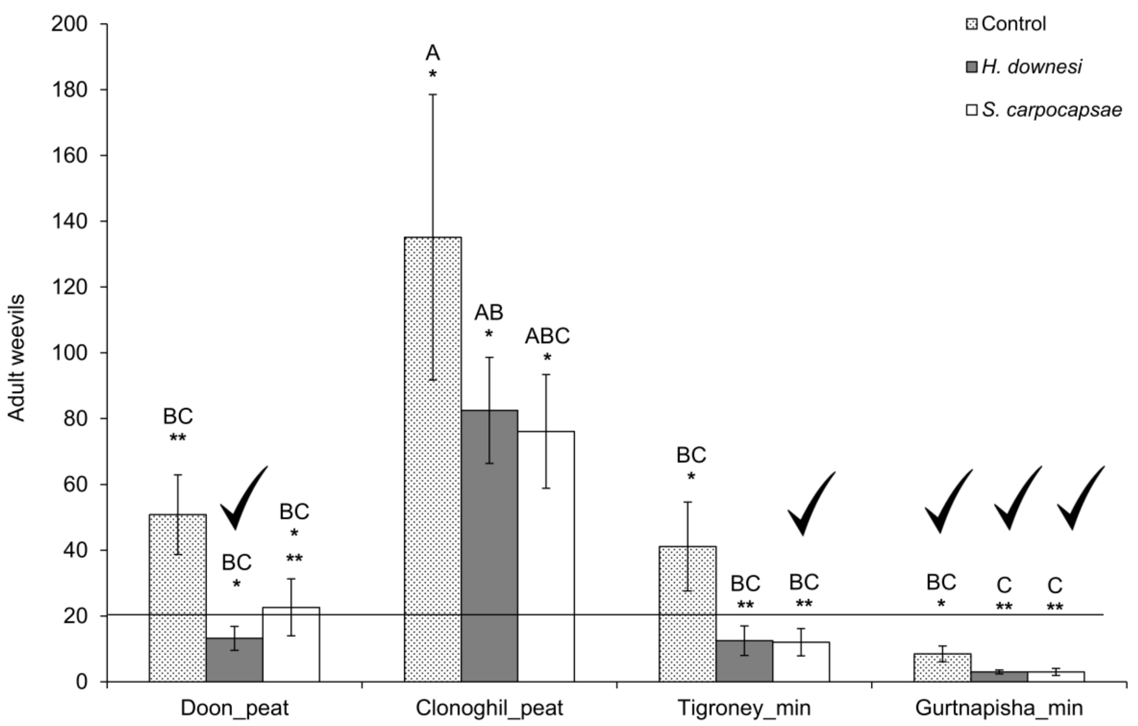


Figure 6



Adult weevils
200
180
160
140
120
100
80
60
40
20
0
Control
H. downesi
S. carpocapsae
A
*
AB
*
ABC
*
BC
**
BC
*
BC
*
**
BC
*
BC
**
BC
**
BC
*
C
**
C
**
Doon_peat
Clonoghil_peat
Tigroney_min
Gurtnapisha_min

652