

Differential susceptibility of pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae), larvae and pupae to entomopathogenic nematodes and death of adults infected as pupae

Christopher D. Williams · Aoife B. Dillon ·
Darragh Ennis · Roseanne Hennessy ·
Christine T. Griffin

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Abstract The large pine weevil *Hylobius abietis* is a serious pest of reforestation in northern Europe. Development takes place in the stumps of felled conifer trees and emerging adults feed on and kill newly planted trees. Application of entomopathogenic nematodes around tree stumps has been shown to reduce the emergence of adult weevils. In order to target application at the most susceptible stage, the susceptibility of larvae and pupae to *Heterorhabditis downesi* and *Steinernema carpocapsae* was compared in a close-contact assay on filter paper. An average of 95.8 % of larvae were killed by *H. downesi* and 82.1 % by *S. carpocapsae* while only 16.3 and 15.0 % of pupae were killed by these two species, respectively. However, many of the *H. abietis* that were exposed as pupae died after metamorphosis to callow adult, with mortality of pupae and callow adults combined reaching 62.5 % for *H. downesi* and 69.9 % for *S. carpocapsae*. For both nematode species significantly more insects died as larvae than as either pupae or

pupae/callow adults. When pupae were exposed to infective juveniles (IJs) for 2 days and were then washed while still pupae to remove surface IJs, adults were later found to be infected indicating that IJs can infect pupae, survive metamorphosis and subsequently kill adults.

Keywords Pine weevil · Entomopathogenic nematodes · Differential susceptibility · Forest pests · Biocontrol

Introduction

The large pine weevil, *Hylobius abietis* (L.) (Coleoptera: Curculionidae), is the most serious pest of reforestation in Europe, costing the forestry sector millions of euro per annum e.g. €2.57 million (\$3.36 million) a year in the UK and up to €30 million (\$38.84 million) annually in Sweden (Weslien 1998; Leather et al. 1999; Långström and Day 2004). If no chemical control measures were used against the pine weevil, the most recent estimate for the economic damage that would result across Europe was €140 million (\$181.26 million) per annum (Långström and Day 2004). Adult weevils are attracted to volatile chemical cues which are emitted when coniferous trees are felled. Females oviposit in the stump and larvae and pupae develop in or under the bark (Leather et al. 1999) often below soil level at depths in excess of 20 cm. On emergence,

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C. D. Williams (✉) · A. B. Dillon · D. Ennis ·
R. Hennessy · C. T. Griffin
Department of Biology, Maynooth University, Maynooth,
Co. Kildare, Ireland
e-mail: chris.david.williams@gmail.com

R. Hennessy
Teagasc Kinsealy Research Station, Kinsealy, Co. Dublin,
Ireland

adults feed on the bark of young trees planted on the clearfelled site, which can result in death through ‘ring barking’. Without control measures, weevils can destroy up to 100 % of newly planted trees, with a UK national average estimate of 50 % mortality within the first few years at untreated sites (Heritage and Moore 2001).

Current control measures include the synthetic chemicals alpha cypermethrin or cypermethrin, which are administered in nursery pre-treatment either via electrodyne application or dipping of saplings prior to planting and/or through on-site post-planting spray. However, with concerns over potential environmental impacts, cypermethrin is being phased out across Europe (EC 2012). Also, under Forest Stewardship Council (FSC) guidelines, alpha cypermethrin and cypermethrin are considered “highly hazardous chemicals” applied only under derogation, so there is an obligation on FSC certified companies to find alternatives to chemical control. Furthermore, current pesticides have a repellent effect on the pine weevil and, while this protects young plants, it does little to impact on the local populations of the pest (Torr et al. 2005; Leather et al. 1999).

Entomopathogenic nematodes (EPN) have been used as a sustainable method of controlling pine weevils (Torr et al. 2005; Dillon and Griffin 2008). Nematodes are applied as inundative biological control agents (biopesticides) targeted against pine weevil larvae, pupae and callow adults developing within the stumps. We have previously shown that *Heterorhabditis downesi* (Stock, Griffin and Burnell) was best at controlling this subterranean cryptic pest, but *Steinernema carpocapsae* (Weiser) was also effective (Dillon et al. 2006). At operational level, *S. carpocapsae* is applied by pressure hose, from a tank mixer mounted on a modified forwarder, at an average rate of 3.5 million nematodes per stump (Torr et al. 2005). These operations are conducted by growers in the UK and Ireland, mainly the Forestry Commission and Coillte, respectively.

In field trials the various life stages of *H. abietis* differed in susceptibility to EPN infection. Dillon et al. (2006) found that 45 % of larvae, 32 % of pupae and 30 % of adults in stumps were infected by EPN when assessed 4 weeks after nematode application. However, uninfected larvae and pupae may have gone on to develop into pupae and adults, respectively. Brixey et al. (2006) adjusted for this and estimated that 14 %

of larvae, 13 % of pupae and 44 % of callow adults were infected by *S. carpocapsae* after application to stumps. In laboratory trials on a cotton substrate Pye and Burman (1978) found that larvae were more susceptible—between 80 and 95 % were killed by *S. carpocapsae* with no pupae and only 5 % of adults killed. In contrast, Brixey (2000) reported that pupae were more susceptible than late instar (4–5th instar) larvae, and Torr et al. (2005) recommended targeting pupae.

The aim of the present study is to assess the susceptibility of various life stages of *H. abietis* to EPN infection; larvae and pupae of *H. abietis* were indefinitely exposed to various concentrations of *S. carpocapsae* and *H. downesi* in a close contact assay on filter paper. A second aim of the present paper is to determine whether infective juveniles (IJs) may infect pupae, survive metamorphosis and subsequently kill newly eclosed adults.

Materials and methods

Source of weevils and nematodes; culturing of nematodes

Pine weevil larvae and pupae were collected from clear-felled forest sites by removing the bark of lodgepole pine (*Pinus contorta* Douglas) with a chisel and collecting immature weevils from their galleries. The following nematode isolates were used: *S. carpocapsae* (US-S-25 from Koppert), *S. carpocapsae* (All strain) and *H. downesi* (K122).

Stocks of nematodes were cultured in the laboratory using *Galleria mellonella* (L.) larvae which were placed on White traps (White 1927) and harvested IJs were washed by sedimentation in tap water (Kaya and Stock 1997). Nematodes were stored at 9 °C and were used within 2 weeks.

Susceptibility of *Hylobius abietis* larvae and pupae to infection by *S. carpocapsae* and *H. downesi* on filter paper

Late instar larvae and pupae of *H. abietis* were placed in 1.5 cm diameter wells of 24-well tissue culture plates that had been lined with one 1 cm diameter disc of filter paper. Each insect was then treated with either *S. carpocapsae* (US-S-25 Koppert strain) or *H.*

downesi (K122 strain) applied to the filter paper in 50 µl of water. Control treatments for both larvae and pupae had 50 µl of water applied to the filter paper. The insects were then incubated at 20 °C and, at intervals checked for mortality and the life stage at time of death was noted. There were four experiments:

- In experiment 1, the concentrations used were 25, 50, 100, or 200 IJs for larvae and for pupae. There were 24 insects in each treatment. Mortality and life stage were checked daily for 2 weeks.
- In experiment 2, there were just two concentrations of each nematode species, 50 and 250 IJs for both larvae and pupae. Each treatment was replicated three times with 24 insects in each replicate (total 72 insects). Mortality and life stage was recorded on days 5, 8, 13 and 19.
- Experiment 3 tested pupae only, and included higher nematode concentrations: 250, 1,000 and 2,000 IJs. There were 24 insects per treatment. Mortality and life stage were recorded daily for 18 days. The aim was to test whether a higher concentration of nematodes would result in higher mortality of pupae as pupae rather than as callow adults.
- For experiment 4, a small number of insects (12 per treatment) that had pupated within the previous 24 h were exposed to a single concentration of 2,000 IJs.

Washing experiment to determine whether IJs infecting pupae can cause death following metamorphosis

As we observed that some of the insects exposed to nematodes as pupae died as adults we tested the hypothesis that IJs might enter weevil larvae in the pupal stage, survive metamorphosis and then kill the weevil in the adult stage. To do this we performed a washing experiment. Weevil pupae were exposed to IJs of either *H. downesi* (K122 strain) or *S. carpocapsae* (All strain) (250 IJs) on filter paper in multi-well plates, as above. After 2 days' exposure, weevils were either washed clean of IJs by dipping them in tap water, or were left in contact with the nematode-contaminated filter paper. Washed insects were examined under a dissecting microscope to confirm the absence of visible IJs, and each was then placed in a clean multiwell with moist filter paper. The experiment was repeated four

times, with 10–12 insects per treatment in each experiment. Weevil mortality and stage were recorded daily. Weevils were observed for 1 month following washing. Dead weevils were dissected to confirm infection by nematodes.

Statistical analysis

T tests, Mann–Whitney U tests, χ^2 tests and Probit analysis were all performed on the statistical package Minitab version 16.

Results

Susceptibility of *Hylobius larvae* and pupae to *S. carpocapsae* and *H. downesi* on filter paper

Larvae

The lowest mortality of pine weevil larvae was 62.5 % (50 *S. carpocapsae* IJS per insect in experiment 2); all other treatments 25–250 *S. carpocapsae* or *H. downesi* IJS per insect killed at least 80 % of the exposed larvae (Table 1). Thus, the LC₅₀ for each species is <25 IJS per insect. Overall, more larvae were killed by *H. downesi* than by *S. carpocapsae* (Paired *t* test, *t* = 2.71, *df* = 5, *P* = 0.042) (Table 1).

As regards the speed of kill, in experiment 1, larvae exposed to *H. downesi* showed high mortality by day 5, but larvae exposed to *S. carpocapsae* continued to die up to day 13. For the highest concentration (200 IJS per insect) the LT₅₀ (with fiducial limits) for *H. downesi* was 3.4 (3.0–3.6) days and for *S. carpocapsae* was 4.4 (3.6–5.0) days (data not shown); a marginally significant difference (based on the fact that the fiducial limits just overlap), with *H. downesi* killing more quickly than *S. carpocapsae*. Similarly in experiment 2, larvae continued to die up to day 13 in all treatments, with death in the lower concentrations of those larvae treated with *S. carpocapsae* continuing for longer—up to day 19 when the experiment was terminated (Fig. 1a).

Pupae

In experiment 1, mortality for insects exposed as pupae ranged from 45.8 to 100 % but there was no consistent concentration response over the range of

Table 1 Mortality of *H. abietis* exposed to a range of concentrations of *S. carpocapsae* and *H. downesi* on filter paper for 14 days (Expt 1) or 19 days (Expt 2)

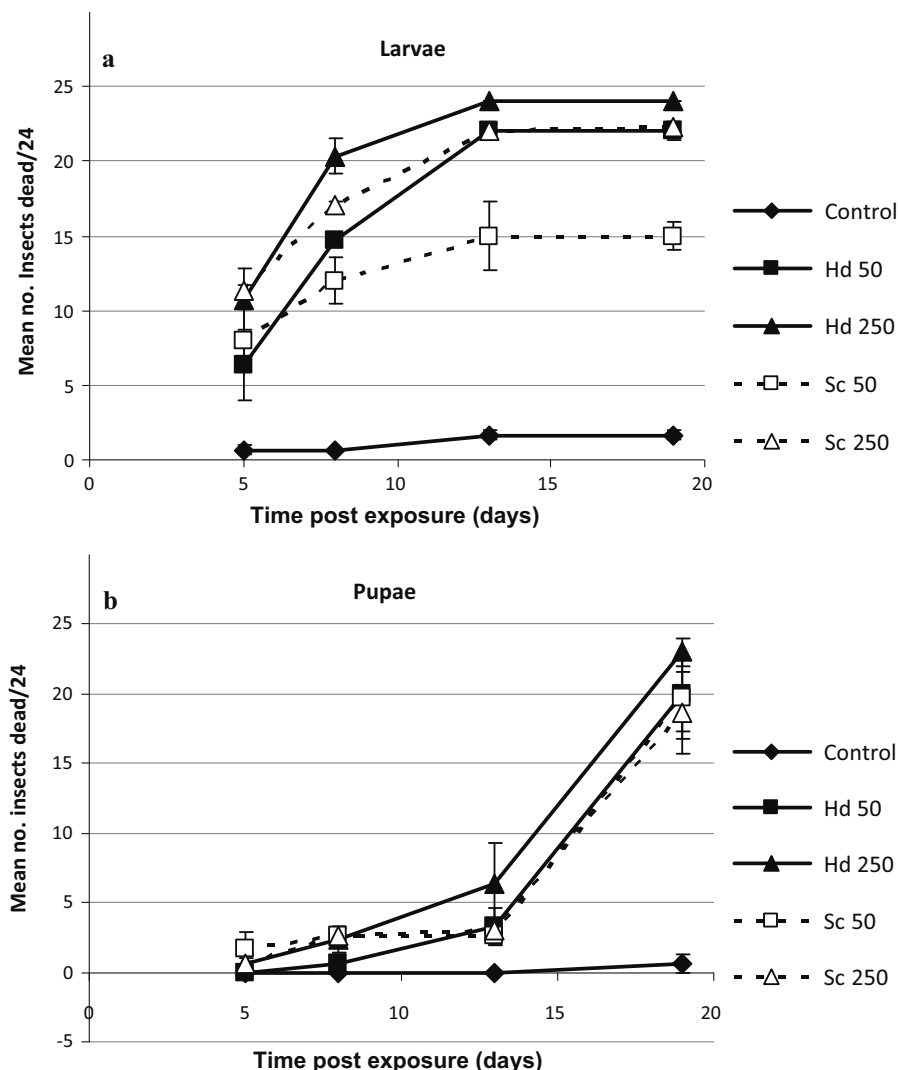
Concentration IJS per insect	n	<i>H. downesi</i> versus		<i>S. carpocapsae</i> versus		<i>S. carpocapsae</i> versus pupae	
		<i>H. downesi</i> pupae		larvae		As pupae and adult	
		As larvae	As pupae and adult	As larvae	As pupae/adult	As pupae and adult	As pupae/adult
Expt 1	25	87.5 (21)	50.0 (12)***	12.5 (3)**/37.5 (9)	83.3 (20)	66.7 (16) ns	20.8 (5)***/45.8 (11)
	50	95.8 (23)	91.7 (22) ns	12.5 (1)***/87.5 (21)	87.5 (21)	54.2 (13)*	29.2 (7)***/25.0 (6)
	100	100 (24)	50.0 (12)***	16.7 (4)***/33.3 (8)	95.8 (23)	62.5 (15)**	33.3 (8)***/29.2 (7)
	200	100 (24)	45.8 (11)***	25.0 (6)***/20.8 (5)	91.7 (22)	100 (24) ns	41.7 (10)***/58.3 (14)
Expt 2	50	91.7 (66)	69.4 (50)***	15.3 (11)***/54.2 (39)	62.5 (45)	70.8 (51) ns	1.4 (1)***/69.4 (50)
	250	100 (72)	68.1 (49)***	19.4 (14)***/48.6 (35)	91.7 (66)	65.3 (47)***	4.2 (3)***/61.1 (44)
Total	240	95.8 (230)	65.0 (156)***	16.3 (39)***/48.8 (117)	82.1 (197)	69.2(166)***	15.0 (36)***/54.2 (130)

Insects that were exposed as larvae died as larvae. Insects exposed as pupae died either as pupae or as callow adults. χ^2 comparisons show differences between number dead as larvae and both number dead as pupae and number dead as pupae and adult

ns χ^2 is not significant

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fig. 1 Experiment 2. Mortality (number of insects dead/24, mean of three replicates) of *Hylobius abietis* exposed to EPN as larvae (a) and as pupae (b). Hd = *Heterorhabditis downesi*, Sc = *Steinernema carpocapsae*, numbers in legend represent number of infective juveniles applied. Error bars represent \pm SE



concentrations tested (25–200 IJs per insect) (Table 1). Most of the insects exposed as pupae died as callow adults (Table 1). Insects exposed as pupae continued to die until the experiment was terminated on day 13. LT₅₀s were not calculated for pupae, since most of the deaths occurred as adults.

Experiment 2 showed broadly similar results with most of the insects that died following exposure of pupae to nematodes dying as callow adults (Table 1). The death of these newly eclosed adults explains the steep increase in mortality in Fig. 1b. The total mortality (pupae and callow adults) was similar for both nematode species (Table 1).

In experiment 3, the maximum mortality of pupae was 75 % (exposed to 2,000 *H. downesi* per insect)

(Table 2). In experiment 4, when newly developed pupae (within 24 h of pupation) were exposed to 2,000 nematodes, more than 80 % were killed (*H. downesi* 10/12 = 83.3 %; *S. carpocapsae*, 11/12 = 91.7 %), a significant difference to the older pupae of experiment 3 exposed to the same concentration of *S. carpocapsae* ($\chi^2 = 6.02$, df = 1, $P = 0.014$), but not *H. downesi* ($\chi^2 = 0.321$, df = 1, $P = 0.571$).

Larvae versus pupae

Considerably more larvae than pupae were killed at all concentrations of both nematode species in each of the two experiments, and the difference was highly significant (e.g. $\chi^2 = 14.187$ df = 1, $P < 0.001$) in

Table 2 Mortality of *H. abietis* exposed to a range of concentrations of *S. carpocapsae* and *H. downesi* on filter paper

Expt	Concentration IJS per insect	n	% (and no.) dead					
			<i>H. downesi</i>			<i>S. carpocapsae</i>		
			As pupae and adult	As pupae	As adult	As pupae and adult	As pupae	As adult
3	250	24	87.5 (21)	29.2 (7)	58.3 (14)	100 (24)	50.0 (12)	50.0 (12)
	1,000	24	100 (24)	12.5 (3)	87.5 (21)	79.2 (19)	45.8 (11)	33.3 (8)
	2,000	24	100 (24)	75.0 (18)	25.0 (6)	95.8 (23)	50.0 (12)	45.8 (11)
4	2,000	12	100 (12)	83.3 (10)	16.7 (2)	100 (12)	91.7 (11)	8.3 (1)
Total		84	96.4 (81)	45.2 (38)	51.2 (43)	92.9 (78)	54.8 (46)	38.1 (32)

Pupae in experiment 4 had been prepupae at most 24 h while those in experiment 3 were older

each case (Table 1). On average, 16.9 and 21.8 % of pupae died as pupae when exposed to *H. downesi* and *S. carpocapsae*, respectively, compared to 95.8 and 82.1 % of larvae. However, additional insects died following metamorphosis to callow adult, with mortality reaching 62.5 and 69.9 % of insects exposed as pupae/callow adults to *H. downesi* and *S. carpocapsae*, respectively. There was no significant difference between those infected with *H. downesi* and those infected with *S. carpocapsae* (paired *t* test, $t = -0.61$, $df = 5$, $P = 0.570$). The difference between mortality of larvae and that of pupae plus callow adults combined was still significant in most cases (Table 1).

Washing experiment

In the washing experiment 11.3–27.5 % of the weevils died as pupae, but also an additional 15.0–32.5 % died as adults (Table 3). Some weevils that died as adults were callow adults and others were fully sclerotized. In cases where adults died following washing (Table 3), this indicates that IJs can survive metamorphosis within weevil pupae and can subsequently kill adults. This may take place anytime between 4 and 13 days from exposure of weevils to nematodes. There was no significant difference in percentage infectivity between “washed” and “unwashed” treatments for pupae or adults of either species ($P > 0.05$ for all four Mann–Whitney U tests—see Table 3). Furthermore, washing had no effect on the proportion of infected insects that died as adults for either species (*H. downesi*: $\chi^2 = 0.776$, $df = 1$, $P = 0.379$; *S. carpocapsae*: $\chi^2 = 1.802$, $df = 1$, $P = 0.179$). Overall, adults accounted for 70.0 % of the “washed” insects killed and infected by *H. downesi* and 35.3 % of the

insects killed and infected by *S. carpocapsae*. All dissected adults infected with nematodes had second generation adult nematodes present. For those infected with *S. carpocapsae* this means at least two IJs entered each pupa. Since *H. downesi* are hermaphrodite in the first generation only a single IJ had to enter each pupa.

For those insects that died as adults, the time from washing (2 days after initial exposure) to death of adults ranged from 2 to 11 days. This is divided into two periods—the time from washing to eclosion, which gives a measure of the age of the pupae, and the time from eclosion to death, which gives an indication of the speed at which nematodes killed adults, assuming that the process began at eclosion. The time between washing and eclosion of adults had a median (range given in parentheses) of 1 (1–4) days and 4 (1–9) days for *H. downesi* and *S. carpocapsae* infected individuals, respectively and the time between eclosion of adults and death had a median of 2 (1–5) days and 4 (2–5) days for *H. downesi* and *S. carpocapsae* infected individuals, respectively. The time between eclosion and death of adults was significantly longer in those insects infected with *S. carpocapsae* compared to those infected with *H. downesi* (Mann–Whitney U test: $W = 78$, $P = 0.034$).

Discussion

The susceptibility of insects, including beetles, to EPN often changes with life stage. The susceptibility of Chrysomelidae is sometimes higher in the larval stage (Saleh et al. 2009), sometimes in the pupal stage (Xu et al. 2010) and which stage is more susceptible is sometimes dependent on temperature (Yang et al.

Table 3 Percentage (mean \pm SE) of *Hylobius abietis* dead and infected by entomopathogenic nematodes as either pupae or adults

Species	Treatment	As pupae	As adults
<i>Heterorhabditis downesi</i>	Unwashed	15.8 \pm 7.11	19.2 \pm 10.83
	Washed	11.3 \pm 6.57	26.3 \pm 4.73
<i>Steinernema carpocapsae</i>	Unwashed	27.5 \pm 11.81	32.5 \pm 16.52
	Washed	27.5 \pm 13.77	15.0 \pm 5.00

Pupae were exposed to 250 nematode infective juveniles for two days and were then either washed free of surface nematodes or were left unwashed. Infection was confirmed by dissection. There were no significant differences between washed and unwashed treatments (Mann–Whitney U test $P > 0.05$ for all four tests). N = 4 experiments

2003). For the Scarabaeidae both Lacey et al. (2001) and Khatri-Chhetri et al. (2011) found that pupae were more susceptible than larvae to EPN. Ramos-Rodríguez et al. (2006) found EPN were less efficacious against pupae and adults than larvae of *Tribolium castaneum*.

Among the weevils (Curculionidae), however, Loya and Hower (2003), Jansson et al. (1990), Abbas et al. (2001) and Mannion and Jansson (1992) demonstrated a higher susceptibility of larvae compared to pupae. Thus, whereas it may be difficult to draw general conclusions as to the susceptibility of different beetle stages to nematodes, the Curculionidae, at least, all appear to have more resistant pupal than larval stages. Our data show that pine weevil larvae are more susceptible than pupae to both *S. carpocapsae* and *H. downesi*. Furthermore, for *H. downesi*, we show that most deaths that occur following exposure of pupae do so following metamorphosis. As they were continually exposed in the initial set of experiments we do not know whether they were infected as pupae, as callow adults or both. Most studies, however, do not report whether deaths of insects exposed in the pupal stage occur before or after metamorphosis, and it may be that such a phenomenon is common among the Curculionidae. We conclude that applying nematodes to stumps earlier after felling to target *Hylobius* larvae, in contrast to Brixey et al. (2006) and Torr et al. (2005) recommendations, may be more efficacious in controlling pine weevil. Brixey (2000) found that pupae were about twice as susceptible as late instar larvae. It is unclear why Brixey produced different results to us.

The results of our washing experiment indicate that IJs may infect pupae and then survive metamorphosis and subsequently kill adults (either callow adults or fully sclerotized adults) following eclosion from the pupa. The fact that washing had no effect on the

proportion of infected insects that died as adults, for either species, is exactly the result one would predict if nematodes were invading pupae and surviving metamorphosis within the weevil. Other parasites have been known to survive metamorphosis in amphibians such as the intestinal nematode *Oswaldocruzia filiformis* in *Rana temporaria temporaria* (Griffin unpublished), but this is, to our knowledge, the first report of such a phenomenon in entomopathogenic nematodes and insects. It is possible that IJs entering pupae are first encapsulated, but during metamorphosis in which tissues are extensively destroyed and remodelled (Richards and Davies 1977) they are subsequently freed from encapsulation and are at liberty to kill adult weevils. Encapsulation is a common immune response among insects, and encapsulation of EPN has been reported in *H. abietis* larvae and adults (Pye and Burman 1977; Girling et al. 2010). However, nematodes may escape encapsulation (Li et al. (2007). Girling et al. (2010) found that live adult *H. abietis* harboured encapsulated or dead nematodes and these weevils may have successfully defended themselves against the nematodes and might have survived had they not been sacrificed. Other live weevils sacrificed at the time (5 days post exposure) harboured live nematodes instead (Girling et al. (2010), which may either have escaped from encapsulation or evaded it in the first place but had not yet killed the insect. Another possible explanation for the results of our washing experiment is that IJs wait in the intestine or tracheal system of pupae free from encapsulation. Further research is required to decide between these two hypotheses. Either of these scenarios (surviving for days as IJ without killing the host) may reflect the evolutionary origins of EPN as necromenics (where dauer juveniles wait for a host to die naturally before commencing feeding) (Sudhaus 2008).

In our experiments the time between washing and eclosion of adults was generally low (median values of 1 and 4 days) showing that most weevils were fairly well advanced as pupae prior to infection with nematodes. However, the range of values was quite high (up to 9 days) showing that pupae can be infected when they are much younger, and can be subsequently killed as adults. The pupal stage of *H. abietis* should last for 17.2 days at 20 °C, based on the thermal constants provided by Inward et al. (2012). The fact that the time to death after eclosion was significantly longer for *S. carpocapsae* compared to *H. downesi* reflects the situation in the larval experiments, where *H. downesi* also killed somewhat faster than *S. carpocapsae*.

Girling et al. (2010) showed that mortality of adult pine weevil at relatively high concentrations of IJs (500 and 4,000) of *S. carpocapsae* and *H. downesi* was lower than the mortality of larvae and pupae caused by the same EPN species reported here, even at lower concentrations. As we show that the larvae are the most susceptible stage, field application of nematodes earlier in the season when a high proportion of pine weevil are in this stage is likely to be more efficacious than later in the season when the proportion of pupae and callow adults is higher. It should be noted that IJs applied to target larvae may persist or may recycle through hosts and also kill pupae and callow adults even when they are applied earlier in the season.

With other EPN species and/or hosts there may be an EPN-host stage interaction as Ramos-Rodriguez et al. (2006) found for *T. castaneum*. However, when testing nematode efficacy against pine weevil under field conditions, Dillon et al. (2006) found no such interaction of weevil stage \times nematode species, and this is supported by the laboratory experiments reported here as both *H. downesi* and *S. carpocapsae* were more effective against larvae than against later stages. There are many possible reasons for the different susceptibilities between life stages. Activity and attraction of nematode IJs to active, feeding larvae (Lewis et al. 1992) may be higher than to the inactive pupae, there may be greater possibilities of IJ entry into larvae compared to pupae, or the stages may differ in their immune response.

LT₅₀ values and times to death have been widely reported for *S. carpocapsae*. Grewal et al. (1993) report the species killing the wax moth (*G. mellonella*) within 24 h and Feng et al. (2006) report slightly

longer LT₅₀s of between 26 and 27.25 h depending on long-term storage conditions. Saleh et al. (2009) reported LT₅₀s of around 37 h for larvae of the sugar beet beetle (*Cassida vittata*) exposed to *S. carpocapsae* and Schroer et al. (2005) reported an LT₅₀ of <25 h for the diamondback moth (*Plutella xylostella*) when exposed to *S. carpocapsae* and adjuvants. Other insects are more resistant, with cockroaches taking a particularly long time to kill (Appel et al. 1993; Koehler et al. 1992). Our LT₅₀s for late larval instars of *H. abietis* (~4 days) are close to the upper range of those reported in the literature.

Our close-contact bioassay results show a similar pathogenicity for both *H. downesi* and *S. carpocapsae*. However, Dillon et al. (2006, 2007) and Williams et al. (2013a, b) showed conclusively that *H. downesi* is more efficacious than *S. carpocapsae* against pine weevil in the field as assessed by both emergence of adult weevils and by infection data gleaned from destructive sampling of stumps. We suggest that the difference between our close-contact bioassay results and the previously reported field results are due to the different foraging strategies, which are thought to be employed by the two species (Lewis et al. 1992; Grewal et al. 1994). *Heterorhabditis downesi* is described as a “cruise” forager whereas *S. carpocapsae* is described as a typical “ambush” forager, though there is some evidence that *S. carpocapsae* can cruise forage in organic substrates (Kruitbos et al. 2010). Such differences in foraging strategies are probably not important in close-contact bioassays, but would be important under field conditions where weevil hosts must first be located prior to infection.

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References

- Abbas MST, Saleh MME, Akil AM (2001) Laboratory and field evaluation of the pathogenicity of entomopathogenic nematodes to the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) (Col.: Curculionidae). J Pest Sci 74(6):167–168
- Appel AG, Benson EP, Ellenberger JM, Manweiler SA (1993) Laboratory and field evaluations of an entomogenous

- nematode (Nematoda: Steinernematidae) for German cockroach (Dictyoptera: Blattellidae) control. *J Econ Entomol* 86:777–784
- Brixey JM (2000) The use of entomopathogenic nematodes to control the immature stages of the large pine weevil, *Hylobius abietis* L. (Unpublished doctoral dissertation), The University of Reading, Reading
- Brixey JM, Moore R, Milner ADJ (2006) Effect of entomopathogenic nematode (*Steinernema carpocapsae* Weiser) application technique on the efficacy and distribution of infection of the large pine weevil (*Hylobius abietis* L.) in stumps of Sitka spruce (*Picea sitchensis* Carr.) created at different times. *For Ecol Manage* 226:161–172
- Dillon A, Griffin C (2008) Controlling the large pine weevil, *Hylobius abietis*, using natural enemies. *Silviculture/Management No. 15*. Coford Connects, Dublin
- Dillon A, Ward D, Downes MJ, Griffin CT (2006) Suppression of the large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes with different foraging strategies. *Biol Control* 38:217–226
- Dillon AB, Downes MJ, Ward D, Griffin CT (2007) Optimizing application of entomopathogenic nematodes to manage large pine weevil *Hylobius abietis* L. (Coleoptera: Curculionidae) populations developing in pine stumps, *Pinus sylvestris*. *Biol Control* 40:253–263
- EC (2012) Proposal for a revised directive of the European parliament and of the council on priority substances in the field of water quality. *Memo 12/59*
- Feng S-P, Han R-C, Qui X-H, Cao L, Chen J-H, Wang G-H (2006) Storage of osmotically treated entomopathogenic nematode *Steinernema carpocapsae*. *Insect Sci* 13:263–269
- Girling RD, Ennis D, Dillon AB, Griffin CT (2010) The lethal and sub-lethal consequences of entomopathogenic nematode infestation and exposure for adult pine weevils, *Hylobius abietis* (Coleoptera: Curculionidae). *J Invertebr Pathol* 104:195–202
- Grewal PS, Gaugler R, Kaya HK, Wusaty M (1993) Infectivity of the entomopathogenic nematode *Steinernema scapterisci* (Nematoda: Steinernematidae). *J Invertebr Pathol* 62:22–28
- Grewal PS, Lewis EE, Gaugler R, Campbell JF (1994) Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. *Parasitology* 108:207–215
- Heritage S, Moore R (2001) The assessment of site characteristics as part of a management strategy to reduce damage by *Hylobius*. *Forestry Commission Information Note 38*. HMSO, UK
- Inward DJG, Wainhouse D, Peace A (2012) The effect of temperature on the development and life cycle regulation of the pine weevil *Hylobius abietis* and the potential impacts of climate change. *Agric For Entomol* 14:348–357
- Jansson RK, Lecrone SH, Gaugler RR, Smart GC Jr (1990) Potential of entomopathogenic nematodes as biological control agents of sweet potato weevil (Coleoptera: Curculionidae). *J Econ Entomol* 83:1818–1826
- Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey LA (ed) *Manual of techniques in insect pathology*. Academic Press, New York, pp 281–324
- Khatri-Chhetri HB, Timsina GP, Manhandhar HK, Moens M (2011) Potential of Nepalese entomopathogenic nematodes as biocontrol agents against *Holotrichia longipennis* Blanch. (Coleoptera: Scarabaeidae). *J Pest Sci* 84:457–469
- Koehler PG, Patterson RS, Randy MW (1992) Susceptibility of cockroaches (Diuictyoptera: Blattellidae, Blattidae) to infection by *Steinernema carpocapsae*. *J Econ Entomol* 85:1184–1187
- Kruitbos LM, Heritage S, Hapca S, Wilson MJ (2010) The influence of habitat quality on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis*. *Parasitology* 137:303–309
- Lacey LA, Rosa JS, Simoes NO, Amaral JJ, Kaya HK (2001) Comparative dispersal and larvicidal activity of exotic and Azorean isolates of entomopathogenic nematodes against *Popillia japonica* (Coleoptera: Scarabaeidae). *Eur J Entomol* 98:439–444
- Långström B, Day KR (2004) Damage, control and management of weevil pests, especially *Hylobius abietis*. In: Lieutier F, Day KD, Battisti A, Grégoire J-C, Evans HF (eds) *Bark and wood boring insects in living trees in Europe, a synthesis*. Springer, Dordrecht, pp 415–444
- Leather SR, Day KR, Salisbury ANJ (1999) The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bull Entomol Res* 89:3–16
- Lewis EE, Gaugler R, Harrison R (1992) Entomopathogenic nematode host finding: response to host contact cues by cruise and ambush foragers. *Parasitology* 105:309–315
- Li XY, Cowles RS, Cowles EA, Gaugler R, Cox-Foster DL (2007) Relationship between the successful infection by entomopathogenic nematodes and the host immune response. *Int J Parasitol* 37:365–374
- Loya LJ, Hower AA (2003) Infectivity and reproductive potential of the Oswego strain of *Heterorhabditis bacteriophora* associated with life stages of the clover root curculio, *Sitona hispidulus*. *J Invertebr Pathol* 83:63–72
- Mannion CM, Jansson RK (1992) Comparison of ten entomopathogenic nematodes for control of sweet-potato weevil (Coleoptera, Apionidae). *J Econ Entomol* 85:1642–1650
- Pye AE, Burman M (1977) Pathogenicity of the nematode *Neoaplectana carpocapsae* (Rhabditida, Steinernematidae) and certain microorganisms towards the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). *Ann Entomol Fenn* 43:115–119
- Pye AE, Burman M (1978) *Neoaplectana carpocapsae*: infection and reproduction in large pine weevil larvae, *Hylobius abietis*. *Exp Parasitol* 46:1–11
- Ramos-Rodríguez O, Campbell JF, Ramaswamy SB (2006) Pathogenicity of three species of entomopathogenic nematodes to some major stored-product insect pests. *J Stored Prod Res* 42:241–252
- Richards OW, Davies RG (eds) (1977) *Imms' general textbook of entomology: Tenth edition*. Volume 1: structure, physiology and development. Chapman and Hall, London
- Saleh MME, Draz KAA, Mansour MA, Hussein MA, Zawrah MFM (2009) Controlling the sugar beet beetle *Cassida vittata* with entomopathogenic nematodes. *J Pest Sci* 82:289–294

- Schroer S, Yi X, Ehlers R-U (2005) Evaluation of adjuvants for foliar application of *Steinernema carpocapsae* against larvae of the diamondback moth (*Plutella xylostella*). *Nematology* 7:37–44
- Sudhaus W (2008) Evolution of insect parasitism in rhabditid and diplogastrid nematodes. Papers dedicated to Prof. Dr Bozidar Curcic. In: Makarov SE, Dimitrijević RN (eds) *Advances in arachnology and developmental biology*. Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, pp 143–161
- Torr PS, Wilson MJ, Heritage S (2005) Forestry applications. In: Grewal PS, Ehlers R-U, Shapiro-Ilan DI (eds) *Nematodes as biocontrol agents*. CABI Publishing, Oxfordshire, pp 281–293
- Weslien J (1998) Vad kostar snytbaggeskadorna? (How much does the pine weevil damage cost?) *Kunglige Skogs-och Lantbruksakademins Tidskrift* 137: 19–22
- White GF (1927) A method for obtaining infective nematode larvae from cultures. *Science* 66:302–303
- Williams CD, Dillon AB, Girling R, Griffin CT (2013a) Organic soils promote the efficacy of entomopathogenic nematodes, with different foraging strategies, in the control of a major forest pest: a meta-analysis of studies to date. *Biol Control* 65:357–364
- Williams CD, Dillon AB, Hennessy R, Mc Namara L, Harvey CD, Griffin CT (2013b) Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic nematodes and fungi using eradicator and prophylactic strategies. *For Ecol Manage* 305:212–222
- Xu CX, De Clercq P, Moens M, Chen SL, Han RC (2010) Efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) against the striped flea beetle, *Phyllotreta striolata*. *BioControl* 55:789–797
- Yang X, Jian H, Liu Z, Yang H, Yuan J, Quanli Z, Shuangyue L (2003) Evaluation of entomopathogenic nematodes for control of the beetle, *Luperomorpha suturalis* Chen (Col., Chrysomelidae). *Zeitschrift für Angewandte Entomologie* 127:377–382

Christopher D. Williams is an applied entomologist with interests in insect conservation and biological control. He has recently been working on the biological control of pine weevil using entomopathogenic nematodes and fungi.

Aoife B. Dillon is the bioinsecticides specialist at BASF and was involved in part of this project when working as the IPM coordinator at the Irish forestry company Coillte.

Darragh Ennis is an entomologist who worked on this project as a PhD student, investigating behavioural interactions between pine weevils and entomopathogenic nematodes.

Roseanne Hennessy was an MSc student investigating biological control of the vine weevil and pine weevil using combined applications of entomopathogenic nematodes and entomopathogenic fungi.

Christine T. Griffin is a lecturer at Maynooth University with research interest in entomopathogenic nematodes.