



CREaTE

Canterbury Research and Theses Environment

Canterbury Christ Church University's repository of research outputs

<http://create.canterbury.ac.uk>

Please cite this publication as follows:

Harvey, C. and Griffin, C. (2015) Local host-dependent persistence of the entomopathogenic nematode *Steinernema carpocapsae* used to control the large pine weevil *Hylobius abietis*. *BioControl*. pp. 1-9. ISSN 1386-6141.

Link to official URL (if available):

<http://dx.doi.org/10.1007/s10526-015-9709-9>

This version is made available in accordance with publishers' policies. All material made available by CReaTE is protected by intellectual property law, including copyright law. Any use made of the contents should comply with the relevant law.

Contact: create.library@canterbury.ac.uk



1 Local host-dependent persistence of the entomopathogenic nematode *Steinernema carpocapsae*
2 used to control the large pine weevil *Hylobius abietis*

3 Christopher D. Harvey and Christine T. Griffin

4 Abstract

5 Entomopathogenic nematodes (EPN) applied inundatively to suppress insect pests are more
6 likely to persist and establish in stable agroecosystems than in annual crops. We investigated a
7 system of intermediate stability: Tree stumps harbouring the large pine weevil (*Hylobius abietis*
8 L.; Coleoptera: Curculionidae), a major European forestry pest. We tested whether persistence of
9 EPN *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) applied around stumps is
10 maintained by recycling of EPN through pine weevils developing within stumps. *Steinernema*
11 *carpocapsae* was detected in soil around and under the bark of treated tree stumps up to 2 years,
12 but not 4-5 years after application. Differences in nematode presence between sites were better
13 explained by tree species (pine or spruce) than soil type (mineral or peat). Presence of *S.*
14 *carpocapsae* in soil was positively correlated with the number of *H. abietis* emerging from
15 untreated stumps the previous year, which was greater for pine stumps than spruce stumps.

16 Keywords: *Steinernema*; *Hylobius*; entomopathogenic nematodes; pine weevil; persistence; bark

17 1. Introduction

18 Entomopathogenic nematodes (EPN) of the genera *Steinernema* (Rhabditida: Steinernematidae)
19 and *Heterorhabditis* (Rhabditida: Heterorhabdidae) are lethal pathogens of insects with a wide
20 potential host range (Bathon, 1996; Smits, 1996) that are used against pests in horticulture,
21 agriculture and forestry (Grewal et al. 2005). The free living infective juvenile (IJ) invades the

22 haemocoel of insects and releases symbiotic bacteria that cause toxaemia and/or septicaemia,
23 killing the insect within days (Kaya and Gaugler 1993). EPN are mainly used as inundative
24 biological control agents, with insect suppression being effected by the applied IJs. Following
25 application to soil, numbers of IJs typically decrease rapidly and may reach less than 90% of the
26 original inoculum within days (Glazer 1992; Smits 1996; Griffin 2015). Applied nematodes may
27 survive at low numbers for longer periods (Preisser et al. 2005), but longer term persistence of a
28 population depends on recycling – reproduction in target and/or non-target hosts (Campbell et al.
29 1995; Griffin 2015; Koppenhöfer and Fuzy 2009; Peters 1996). Long-term persistence of EPN
30 populations therefore crucially depends on the availability of host insects for reproduction, as
31 well as suitable environmental conditions, and hence varies between agronomic systems. Stable
32 ecosystems such as pasture and alfalfa favour long-term persistence and EPN populations can
33 persist for years after application (Koppenhöfer and Fuzy 2009; Shields et al. 1999), while in
34 annual crops, persistence beyond a year is less common (Susurluk and Ehlers 2008). Tree stumps
35 as a breeding resource for certain forestry pests such as the large pine weevil, *Hylobius abietis* L.
36 (Coleoptera: Curculionidae) represent a moderately stable environment, intermediate between
37 annual and perennial crops.

38 The large pine weevil, *H. abietis*, is one of the most damaging forestry pests in Europe
39 (Långström and Day 2004; Leather et al. 1999). Development takes place under the bark of
40 recently dead conifers, including stumps of recently felled trees, while adults feed on the bark of
41 saplings planted to restock clear-fell sites, often leading to extensive sapling damage and
42 mortality (Leather et al. 1999; Månsson and Schlyter 2004). Clear-felled coniferous forest
43 plantation sites can support large weevil populations (Leather et al. 1999; Örlander et al. 1997).
44 Stumps can remain suitable for pine weevil oviposition for up to three years after felling

45 (Nordenhem 1989) and emergence of adults occurs within one to two years of oviposition
46 (Leather et al. 1999). Traditionally, seedlings are protected by chemical insecticides, but
47 application of EPN to tree stumps, targeting immature weevils developing within, has shown
48 promise for suppression of adult weevil populations (Brixey et al. 2006; Dillon et al. 2006, 2007
49 and 2008a,b; Torr et al. 2007; Williams et al. 2013). Dillon et al. (2008a) investigated the fate
50 over a five year period of four EPN species applied by hand to tree stumps harbouring pine
51 weevil developmental stages. Incidence (percentage of soil cores positive) of all species
52 remained high for the first two years (no difference between months 1, 12 and 24 post
53 application), but declined by year three post application (Dillon et al. 2008a). Only *Steinernema*
54 *feltiae* Filipjev (Rhabditida: Steinernematidae; native to clearfell sites) was recovered in years 4
55 and 5. Pine weevil larvae can support EPN reproduction, yielding up to 98,000 IJs per insect
56 (Dillon 2003). Dillon et al. (2008a) hypothesized that EPN populations initially remained high
57 due to recycling in the target pest, and that the apparent disappearance after 4-5 years of
58 *Steinernema carpocapsae* Weiser and two *Heterorhabditis* species was due to a concomitant
59 decrease in weevil numbers as stumps degraded. The Dillon et al. (2008a) study was conducted
60 on a single site type: stumps of pine (*Pinus sylvestris* L. and *Pinus contorta* Douglas) on a deep
61 peat soil.

62 The present study complements the Dillon et al. (2008a) report of EPN persistence in a forest
63 ecosystem, focussing on a single EPN species (*S. carpocapsae*) but extending the investigation
64 to sites with diverse characteristics (soil type and tree species). For this study, nematodes were
65 applied to stumps not by hand, but on a site-wide operational scale using spray nozzles
66 connected to a tank of nematode suspension mounted on a forwarder. Specific objectives are: (1)
67 confirm that the restricted spatial and temporal distribution of EPN reported by Dillon et al

68 (2008a) for *Pinus* spp. on peat in small scale trials is also applicable to commercial scale trials on
69 sites with mineral soil and sites planted with Sitka spruce (*Picea sitchensis* Carr.), the species
70 predominating in Irish and UK plantation forests (Anon. 2003 & 2007). (2) Investigate the
71 occurrence of EPN within the stump. Multiplication of EPN in pine weevils located in or under
72 the bark is expected to release IJs into the space between the bark and the woody material of the
73 tree-stump, but this has not been previously reported. (3) Investigate the relationship between
74 EPN incidence in soil around stumps and the size of weevil populations within stumps. If EPN
75 depend mainly on pine weevil as host, a positive correlation between weevil and EPN
76 populations across sites is expected.

77 2. Material and methods

78 2.1 Study sites

79 In 2007 and 2008, *S. carpocapsae* (All strain; Becker Underwood; Littlehampton, England) at a
80 rate of 3.5 million IJs per stump applied in 500 ml water was applied to several clear-fell sites
81 (Table 1) on an operational, site-wide scale (Williams et al. 2013). *Steinernema carpocapsae* was
82 chosen for the study as it is the only species to date that has been applied operationally against
83 pine weevil (Williams et al. 2013). On each site, a small number of stumps were marked and not
84 treated, to serve as controls. These control stumps allowed an assessment of weevil populations
85 within the stumps, based on the number of adult weevils caught in emergence traps erected over
86 them (Williams et al. 2013). The soil type on each site (peat or mineral soil) was based on
87 records of Coillte Teoranta (the site owner) and confirmed by visual evaluation on site. Conifer
88 forests in northern Europe (including 44% of Irish forests) are frequently planted on former peat

89 bog, having high levels of organic matter; for our purposes, all other soils were classed as
90 mineral soils, having lower organic content.

91 2.2 Soil sampling

92 Soil and bark samples were taken from stumps spaced at 5 to 15 m intervals along a diagonal
93 transect; for n of stumps sampled see Table 2. Soil cores were collected at four aspects (at right
94 angles) around each sampled stump. One soil core each was taken at the bole (0 cm) and at 20,
95 40 and 60 cm along each aspect, resulting in 16 soil cores per sampled stump. Cores were taken
96 to a depth of approximately 5 cm using a 50 ml plastic tube (2.9 cm inner diameter; Sarstaedt;
97 Nürnberg, Germany) which was also used for transport and baiting. Soil cores were baited with
98 final instar waxmoth larvae (*Galleria mellonella* L; Lepidoptera: Pyralidae) at room temperature
99 (Dillon et al. 2006, 2007). Each core was baited twice for 7 days with one bait insect each time.
100 Live insects were incubated at 20°C for a further seven days after removal from soil. Insects that
101 showed signs of *Steinernema* infection (cadaver consistency and cream/tan colour) were
102 incubated at 20°C until IJs emerged. IJs were then measured for length (10 per cadaver) and
103 scored as either *S. carpocapsae* (mean length: 558µm, length range: 438-650µm) (Adams and
104 Nguyen 2002) or a native *Steinernema* sp. (*S. feltiae*: mean: 849, range: 736-950; *Steinernema*
105 *kraussei* Steiner: mean: 951, range: 797-1102) (Adams and Nguyen 2002). *Steinernema feltiae*
106 and (rarely) *S. kraussei* are the only species so far detected in Irish conifer forests (Griffin et al.
107 1991; Gwynn and Richardson 1996; Dillon 2003; C. Harvey unpublished). For samples collected
108 2 years post-application (p.a.) or where no IJs emerged, cadavers were dissected, and spicules of
109 male adult nematodes were used for identification (Adams and Nguyen 2002). Cadavers
110 containing no adults or only females were scored as inconclusive and not included in analysis.

111 2.3 Baiting of bark samples from tree stumps treated with *S. carpocapsae*

112 At each sampled stump, approximately 100 cm² of bark was stripped from the bole of the stump
113 at the soil horizon at each aspect. Bark from each stump was pooled into a bulk sample, and
114 placed in a 250 ml plastic cup for baiting. Ten wax moth larvae were added per cup and cups
115 were covered with Parafilm (Bemis; Soignies, Belgium) and incubated at 20°C. After three days,
116 bait insect mortality was recorded. For sites A1-A4 in addition, 4 small pieces of bark (approx. 4
117 cm² each), one from each of the four sampled aspects, were individually baited with a single wax
118 moth larva. The insect was placed in a well (0.9 cm diam.) and covered by the piece of bark as
119 described by Harvey and Griffin (2012), so that it was in contact with only the under surface of
120 the bark. Since previous studies indicated that infection of insects under bark of tree stumps with
121 native steinernematids is extremely rare (Dillon 2003; Dillon et al. 2008; C. Harvey, personal
122 observation), dead insects with cream colouration were scored as infected by *S. carpocapsae*.

123 2.5 Statistics

124 Statistical analysis was carried out using MiniTab Release 15 (MiniTab Solutions; Coventry,
125 UK). To compare the proportion of samples scoring positive for the presence of *S. carpocapsae*
126 over time (successive years after application) or between sites, these binary data
127 (positive/negative) were compared using 2x2 contingency tables with Pearson's χ^2 – test or,
128 where the expected count of at least one cell in the table was < 5, with Fisher's exact test ($\alpha =$
129 0.05). Yates' correction was used for χ^2 – tests on 5x2 tables with expected counts <5.
130 Significance levels of multiple pairwise comparisons of binary data between sampling time
131 points and/or sites were adjusted for type-I family error rate after Bonferroni, with the
132 significance level for n pairwise comparisons involving the same data set adjusted to 0.05/n

133 (Rice 1989). The Mantel-Haenszel-Cochran (MHC) test was used to calculate odds ratios and
134 detect effects of soil type on presence of EPN (in soil cores, at stumps and under bark) while
135 controlling for the effect of tree stump species and vice versa. To investigate whether *S.*
136 *carpocapsae* presence in soil cores and in bark samples was correlated with the size of
137 previously recorded weevil populations in stumps, the percentage of soil cores and bark samples
138 scoring positive for *S. carpocapsae* 2 years p.a. (2009) was regressed in binary logistic
139 regression models for each response variable against the $\log_{10}(x+1)$ of the mean number of adult
140 weevils emerging from untreated control stumps on each of the corresponding sites in 2008
141 (predictive variable; see supplementary data, Dillon et al. 2012), which represented an indicator
142 for the size of pine weevil populations within stumps on each site. The Wald-test was used to test
143 the model coefficient for significant difference from 0. Data for spruce and pine stumps at site
144 A1 (Lackenrea) were used separately in the model. Pearson's Goodness-of-Fit test ($\alpha = 0.05$)
145 was used to confirm validity of the Logit link function used in the model and Pearson residuals
146 were tested for normality (Anderson-Darling test, $\alpha = 0.05$). To test for differences between the
147 percentage of soil cores scoring positive for *S. carpocapsae* at four distances from the bole of
148 stumps, a two-way Chi-square test was used on data combined for each distance across sites A1
149 to A4.

150 3 Results

151 3.1 Presence of entomopathogenic nematodes in soil samples

152 *Steinernema carpocapsae* was detected in soil samples from stumps at all four sites that were
153 sampled up to two years p.a., but not on any of the six sites sampled 4-5 years p.a. (Table 2).
154 Conversely, other *Steinernema* spp. were found at five out of six sites sampled 4-5 years p.a., but

155 not earlier (Table 2). At the sites where soil was sampled in each of years 1 and 2 p.a. (A1 and
156 A2), incidence of *S. carpocapsae* decreased significantly over time in the three samples taken
157 five months, one year and two years after nematode application (A1: cores: $\chi^2_2 = 8.769$, $P =$
158 0.012 ; stumps: $\chi^2_2 = 12.115$, $P = 0.002$ [data combined for pine and spruce]; A2: cores $\chi^2_2 =$
159 19.627 , $P < 0.001$; stumps: $\chi^2_2 = 16.586$, $P < 0.001$; Table 2).

160 Two years p.a., *S. carpocapsae* was recovered from 0.9 to 5.2% of soil cores, representing 11-
161 43% of sampled stumps (Table 2). Differences between sites (A1-A4, treating spruce and pine on
162 A1 separately) were significant based on both cores ($\chi^2_4 = 27.311$, $P < 0.001$) and stumps (Yates'
163 $\chi^2 = 12.522$, d.f = 4; $p = 0.014$). Two years p.a., soil type had a significant effect on the
164 proportion of soil cores scoring positive for EPN when controlling for the effect of stump species
165 (MHC = 7.825, odds ratio [peat:mineral] = 2.494, $P = 0.005$), but the odds ratio for the effect of
166 stump species when controlling for soil type was more than three times as great, with soil cores
167 from pine stumps eight times as likely to score positive for *S. carpocapsae* as cores from spruce
168 stumps (MHC = 17.935, odds ratio [pine:spruce] = 8.009, $P < 0.001$) (Table 3). When data for
169 stumps were used instead, soil type had no significant effect, though the effect of stump species
170 remained highly significant (soil: MHC = 1.067, odds ratio [peat:mineral] = 1.887, $P = 0.302$;
171 stump species: MHC = 17.680, odds ratio [pine:spruce] = ∞ , $P < 0.001$) (Table 3). There was a
172 significant positive relationship between the incidence of *S. carpocapsae* in soil cores and the
173 number of adult weevils emerging the previous year (Wald-test; Coef. = 1.66, $Z = 5.62$, $P <$
174 0.001 ; Fig. 1a).

175 3.2 Presence of entomopathogenic nematodes in bark samples

176 *Steinernema carpocapsae* was recovered from bark one and two years p.a., but no EPN were
177 found there after 4 or 5 years (Table 2). For most of the stumps on sites A2 and A3 where *S.*
178 *carpocapsae* was recovered from bulk bark samples it was also detected when only the inside of
179 the bark was baited (site A2, one year p.a.: 15/20 stumps; site A2, two years p.a.: 6/11; site A3,
180 two years p.a.: 9/12). The proportion of stumps where bark samples were positive for *S.*
181 *carpocapsae* at sites A1 and A2 decreased from one to two years of application (Table 2),
182 significantly so for site A2 ($\chi^2_1 = 6.944$, $P = 0.008$; Table 2). Two years p.a., the proportion of
183 stumps with *S. carpocapsae* in bulk bark samples ranged from 0 to 67%, a highly significant
184 difference between sites (A1-A4, treating spruce and pine on A1 separately) (Yates' $\chi^2 = 19.203$,
185 d.f = 4, $P < 0.001$). Two years p.a., stump species had a significant effect on the proportion of
186 stumps with EPN detected under their bark (MHC = 15.108, odds ratio [pine:spruce] = ∞ , $P <$
187 0.001), but soil type did not (MHC = 0.204, odds ratio [peat:mineral] = 1.424, $P = 0.651$) (Table
188 3). The percentage of stumps with positive bark samples tended to be higher on sites with high
189 weevil emergence compared with sites with low emergence (Fig. 1b). However, link functions of
190 binary logistic models regressing the proportion of tree stumps with bark positive for *S.*
191 *carpocapsae* against mean number of weevils emerging from untreated stumps in 2008 did not
192 provide an adequate fit for the data (Pearson's Goodness-of-Fit test, $P < 0.05$).

193 3.3 Dispersal of EPN from treated stumps

194 At all sites where *S. carpocapsae* was recovered, and at all sampling times, the percentage of
195 cores positive for *S. carpocapsae* tended to be highest directly at the bole of the stump (0 cm
196 distance) and lowest at 60 cm distance from the bole of a stump. Nematodes were detected 60 cm
197 from the stump bole within 5 months p.a. (data not shown). The percentage of soil cores positive

198 for *S. carpocapsae* (sites A1 to A4) decreased significantly with increasing distance from the
199 bole of the stump two years p.a. ($\chi^2_3 = 68.57$, $P < 0.001$; Fig. 2).

200 4. Discussion

201 Our results confirm the finding by Dillon et al. (2008a) that *S. carpocapsae* declines to
202 undetectable levels 4-5 years after application to coniferous tree stumps for pine weevil control.
203 They also support Dillon et al.'s (2008a) suggestion that this is due to a concomitant decrease in
204 the availability of weevils for reproducing as stumps degraded. We did find other steinernematid
205 nematodes 4-5 years after application, probably *S. feltiae* or *S. kraussei*, the only other
206 *Steinernema* spp. so far detected in Irish conifer forests or clear-fells (Dillon 2003; Griffin et al.
207 1991; C. Harvey unpublished data). Similarly, Dillon et al. (2008a) found that the only EPN
208 recovered 4-5 years after application was *S. feltiae*, either an indigenous applied strain or a native
209 strain that naturally colonised the site. It is possible that the abundance of these native EPN
210 species is linked to the availability of soil-associated insect hosts, which may increase in
211 diversity and number as clear-fell sites proceed through stages of succession (Butterfield 1997;
212 Irwin et al. 2014; Niemelä et al. 1993; Pawson et al. 2006).

213 Though inundatively applied IJs can survive in soil for months (Dillon et al. 2008a; Kung et al.
214 1990; Poinar and Hom 1986), up to 90 % of them are expected to die within hours of application
215 (Smits 1996). Therefore, most (if not all) of the *S. carpocapsae* found at our first sampling time
216 five months p.a. and beyond had likely originated from reproducing through insect hosts in the
217 field (Gaugler 1988; Smits 1996; Susurluk and Ehlers 2008). Nematodes were applied in July
218 2007 to coincide with the occurrence of late instar larvae and pupae of pine weevil in the stumps.
219 *Steinernema carpocapsae* can reproduce in immature pine weevil in the field and a single

220 infected weevil larva can yield more than 85,000 IJs (Dillon 2003; Pye and Burman 1978). Our
221 data support the hypothesis that *S. carpocapsae* was reproducing in immature pine weevils:
222 incidence of IJs in soil and bark was positively associated with the size of weevil populations in
223 stumps (as indicated by adult weevil emergence from stumps the year previous). Infection rates
224 of pine weevil by *S. carpocapsae* in the weeks after application are proportionately similar for
225 pine and spruce stumps (Brixey et al. 2006; Dillon et al. 2006 and 2008b) and appear not to be
226 affected by weevil population size per stump (Williams et al. 2013). Thus, differences in *S.*
227 *carpocapsae* reproduction between spruce and pine stumps are likely driven by long-term
228 differences in overall weevil population size between the two stump species, with higher weevil
229 populations in pine (Dillon 2003; Leather et al. 1999; Williams et al. 2013). While soil type is
230 more important than tree species in determining effectiveness of *S. carpocapsae* against the pine
231 weevil (Williams et al. 2013), tree species (as a predictor of weevil populations in stumps) has a
232 greater effect on continued presence of the nematodes in this ecosystem.

233 Since weevil populations in stumps may remain high for 2-3 years after nematodes are applied
234 (Leather et al. 1999) they may facilitate repeated cycles of reproduction of applied *S.*
235 *carpocapsae* under the bark. Longer term presence of *S. carpocapsae* in the soil (1-2 years p.a.)
236 may thus be explained by IJs migrating into the soil following reproduction under stump bark.
237 Nematode populations fluctuate with the target pest population (Campbell et al. 1995), but
238 reproduction in non-target insects may also be important. For example, Hodson et al. (2012)
239 found a positive correlation between persistence of *S. carpocapsae* in pistachio orchards and
240 pitfall catches of non-target tenebrionid beetles. Plantation forests and clear-fell sites can harbour
241 a considerable diversity and number of potential non-target insects (Dillon et al. 2012; Fahy and
242 Gormally 1998; Niemelä and Koivula 2007; Sippola et al. 2002). However, Dillon et al. (2012)

243 found no evidence of an impact of EPN on non-target beetle populations in a study that included
244 some of the sites sampled for this study (sites A1 to A4). Consequently, reproduction of *S.*
245 *carpocapsae* through such non-target hosts is unlikely to contribute significantly to the EPN
246 persistence we observed.

247 On all of the sites in our study, *S. carpocapsae* was applied within two years of felling, when the
248 number of weevils per tree stump tends to peak (Dillon et al. 2007; Leather et al. 1999; Williams
249 et al. 2013). As weevils emerge and stumps degrade, the pest population inevitably drops to a
250 level that no longer supports large nematode populations (Leather et al. 1999). Thus, the
251 ephemeral and contained nature of weevil populations in tree stumps should provide a natural
252 limit to EPN presence on clear-fell sites, especially if reproduction in non-target insects is
253 infrequent. We detected *S. carpocapsae* up to 60 cm from the bole of treated tree stumps, but
254 incidence dropped steeply with increasing distance, a trend similar to previous studies (Torr et al.
255 2007; Dillon et al. 2008a). On the clear-fell sites we sampled, the absence of a stable pool of
256 suitable hosts for reproduction outside of tree stumps may have prevented *S. carpocapsae* from
257 establishing. However, there is potential for local pockets of nematode recycling where dead-
258 wood with susceptible longhorn beetles occurs (Harvey et al. 2012).

259 Dispersal and prolonged persistence of control agents is usually not a desired outcome of
260 inundative biological control, mainly because of the increased risk of non-target impacts (Bathon
261 1996; Smits 1996; van Lenteren et al. 2003). Where EPN reproduction is primarily restricted to
262 the target pest habitat, however, as appears to be the case in our studied system, recycling in the
263 pest may enhance and extend the controlling effect, thereby reducing the need for repeated
264 application while minimising damage to non-target hosts outside of this habitat (Klein and
265 Georgis 1992; Smits 1996). We conclude that, for the large pine weevil the persistence of

266 inundatively applied EPN is dependent on the target pest population, resulting in limited risk of
267 dispersal and longer term establishment while at the same time potentially enhancing control
268 efficacy. The same may be true of other pests with transient populations occurring in cryptic
269 habitats.

270

271

272

273

274

275 References

276 Adams BJ, Nguyen, KB (2002) Taxonomy and systematics. In: Gaugler R (ed)

277 Entomopathogenic Nematology, 1st edn. CAB publishing, Wallingford, England, pp 1-35.

278 Anon. (2003) National inventory of woodland and trees. Forestry Commission, Wexford, Ireland

279 Anon. (2007) National forest inventory Republic of Ireland. Forest Service, Midlothian, UK

280 Bathon H (1996) Impact of entomopathogenic nematodes on non-target Hosts. *Biocontrol Sci*

281 *Technol* 6:421–434

282 Brixey JM, Moore R, Milner AD (2006) Effect of entomopathogenic nematode (*Steinernema*

283 *carpocapsae* Weiser) application technique on the efficacy and distribution of infection of

284 the large pine weevil (*Hylobius abietis* L.) in stumps of Sitka spruce (*Picea sitchensis* Carr.)
285 created at different times. For *Ecol Manage* 226:161–172

286 Butterfield J, (1997) Carabid community succession during the forestry cycle in conifer
287 plantations. *Ecography (Cop.)* 20:614–625

288 Campbell JF, Lewis E, Yoder F, Gaugler RJ (1995) Entomopathogenic nematode
289 (*Heterorhabditidae* and *Steinernematidae*) seasonal population dynamics and impact on
290 insect populations in turfgrass. *Biol Control* 5:598–606

291 Dillon AB (2003) Biological control of the large pine weevil, *Hylobius abietis* L., (Coleoptera:
292 Curculionidae) using entomopathogenic nematodes. PhD thesis submitted at National
293 University of Ireland Maynooth, Ireland

294 Dillon AB, Ward D, Downes MJ, Griffin CT (2006) Suppression of the large pine weevil
295 *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps by entomopathogenic
296 nematodes with different foraging strategies. *Biol Control* 38:217–226

297 Dillon AB, Downes MJ, Ward D, Griffin CTJ (2007) Optimizing application of
298 entomopathogenic nematodes to manage large pine weevil, *Hylobius abietis* L.
299 (Coleoptera:Curculionidae) populations developing in pine stumps, *Pinus sylvestris*. *Biol*
300 *Control* 40:253–263

301 Dillon AB, Rolston AN, Meade CV, Downes MJ, Griffin CT (2008a) Establishment, persistence,
302 and introgression of entomopathogenic nematodes in a forest ecosystem. *Ecol Appl* 18:735–
303 747

304 Dillon AB, Moore CP, Downes MJ, Griffin CT (2008b) Evict or infect? Managing populations
305 of the large pine weevil, *Hylobius abietis*, using a bottom-up and top-down approach. For
306 *Ecol Manage* 255:2634–2642

307 Dillon AB, Foster A, Williams CD, Griffin CT (2012) Environmental safety of
308 entomopathogenic nematodes – effects on abundance, diversity and community structure of
309 non-target beetles in a forest ecosystem. *Biol Control* 63: 107–114

310 Fahy O, Gormally M (1998) A comparison of plant and carabid beetle communities in an Irish
311 oak woodland with a nearby conifer plantation and clearfelled site. For *Ecol Manage*
312 110:263–273

313 Gaugler R (1988) Ecological considerations in the biological control of soil-inhabiting insects
314 with entomopathogenic nematodes. *Agric Ecosyst Environ* 24:351–360

315 Glazer I (1992) Survival and efficacy of *Steinernema carpocapsae* in an exposed environment.
316 *Biocontrol Sci Technol* 2:101–107

317 Grewal PS, Ehlers RU, Shapiro-Ilan DI (eds) (2005) *Nematodes as biocontrol agents*, 1st edn.
318 CAB Publishing, Wallingford, England

319 Griffin CT, Moore JF, Downes MJ (1991) Occurrence of insect-parasitic nematodes
320 (*Steinernematidae*, *Heterorhabditidae*) in the Republic of Ireland. *Nematologica* 37:92–100

321 Griffin CT (2015) Behaviour and population dynamics of entomopathogenic nematodes
322 following application. In: Campos Herrera R (ed) *Nematode pathogenesis of insects and*
323 *other pests – ecology and applied technologies for sustainable plant and crop protection*, 1st

324 edn. Springer, Berlin, Germany, pp 57-95.

325 Gwynn RL, Richardson PN (1996) Incidence of entomopathogenic nematodes in soil samples
326 collected from Scotland, England and Wales. *Fundam appl Nematol* 19:427–431

327 Harvey CD, Alameen KM, Griffin CT (2012) The impact of entomopathogenic nematodes on a
328 non-target, service-providing longhorn beetle is limited by targeted application when
329 controlling forestry pest *Hylobius abietis*. *Biol Control* 62:173-182

330 Harvey CD, Griffin CT (2012) Host activity and wasp experience affect parasitoid wasp foraging
331 behaviour and oviposition on nematode-infected larvae of the forestry pest *Hylobius abietis*.
332 *Ecol Entomol* 37:269–282

333 Hodson AK, Siegel JP, Lewis EE (2012) Ecological influence of the entomopathogenic
334 nematode, *Steinernema carpocapsae*, on pistachio orchard soil arthropods. *Pedobiologia*
335 55:51–58

336 Irwin S, Pedley S, Coote L, Dietzsch A, Wilson M, Oxbrough A, Sweeney O, Moore K, Martin
337 R, Kelly D, Mitchell FG, Kelly T, O’Halloran J (2014) The value of plantation forests for
338 plant, invertebrate and bird diversity and the potential for cross-taxon surrogacy. *Biodivers*
339 *Conserv* 23:697–714

340 Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. *Annu Rev Entomol* 38:181–206

341 Klein MG, Georgis R (1992) Persistence of control of Japanese Beetle (Coleoptera:
342 Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. *J Econ Entomol*
343 85:727–730

344 Koppenhöfer AM, Fuzy EM (2009) Long-term effects and persistence of *Steinernema scarabaei*
345 applied for suppression of *Anomala orientalis* (Coleoptera: Scarabaeidae). *Biol Control*
346 48:63–72

347 Kung SP, Gaugler R, Kaya HK (1990) Soil type and entomopathogenic nematode persistence. *J*
348 *Invertebr Pathol* 55:401–406

349 Långström B, Day KR (2004) Damage, control and management of weevil pests, especially
350 *Hylobius abietis*. In: Leutier F (ed) *Bark and wood boring insects in living trees in*
351 *Europe, a synthesis*, 1st edn. Springer, Berlin, Germany, pp 415-444.

352 Leather SR, Day KR, Salisbury AN (1999) The biology and ecology of the large pine weevil,
353 *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bull Entomol Res*
354 89:3–16

355 Månsson PE, Schlyter F (2004) *Hylobius* pine weevils adult host selection and antifeedants:
356 feeding behaviour on host and non-host woody scandinavian plants. *Agr Forest Entomol*
357 6:165-171

358 Niemelä J, Langor D, Spence JR (1993) Effects of clear-cut harvesting on boreal ground-beetle
359 assemblages (Coleoptera: Carabidae) in Western Canada. *Conserv Biol* 7:551–561

360 Niemelä J, Koivula MKDJ (2007) The effects of forestry on carabid beetles (Coleoptera:
361 Carabidae) in boreal forests. *J Insect Conserv* 11:5–18

362 Nordenhem, H (1989) Age, sexual development, and seasonal occurrence of the pine weevil
363 *Hylobius abietis* (L.). *J Appl Entomol* 108:260–270

364 Örlander G, Nilsson U, Nordlander G (1997) Pine weevil abundance on clear-cuttings of
365 different ages: A 6-year study using pitfall traps. *Scand J For Res* 12:225–240

366 Pawson SM, Brockerhoff EG, Norton DA, Didham RK (2006) Clear-fell harvest impacts on
367 biodiversity: past research and the search for harvest size thresholds. *Can J For Res* 36:
368 1035–1046

369 Peters A (1996) The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact
370 on insect populations. *Biocontrol Sci Technol* 6:389–402

371 Poinar GO, Hom A (1986) Survival and horizontal movement of infective stage *Neoaplectana*
372 *carpocapsae* in the field. *J Nematol* 18:34–36

373 Preisser EL, Dugaw CJ, Dennis B, Strong DR (2005) Long-term survival of the entomopathogenic
374 nematode *Heterorhabditis marelatus*. *Env Entomol* 34:1501–1506

375 Pye AE, Burman M (1978) *Neoaplectana carpocapsae*: Infection and reproduction in large pine
376 weevil larvae, *Hylobius abietis*. *Exp Parasitol* 46:1–11

377 Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225

378 Shields EJ, Testa A, Miller JM, Flanders KL (1999) Field efficacy and persistence of the
379 entomopathogenic nematodes *Heterorhabditis bacteriophora* Oswego and *H. bacteriophora*
380 NC on Alfalfa Snout Beetle larvae (Coleoptera: Curculionidae). *Environ Entomol* 28:128–
381 136

382 Sippola AL, Siitonen J, Punttila P (2002) Beetle diversity in timberline forests: a comparison
383 between old-growth and regeneration areas in Finnish Lapland. *Ann Zool Fenn* 39:69–86

384 Smits PH (1996) Post-application persistence of entomopathogenic nematodes. *Biocontrol Sci*
385 *Technol* 6:379–388

386 Susurluk A, Ehlers R-U (2008) Field persistence of the entomopathogenic nematode
387 *Heterorhabditis bacteriophora* in different crops. *BioControl* 53:627–641

388 Torr P, Heritage S, Wilson MJ (2007) *Steinernema kraussei*, an indigenous nematode found in
389 coniferous forests: efficacy and field persistence against *Hylobius abietis*. *Agric For*
390 *Entomol* 9:181–188

391 Van Lenteren JC, Babendreier D, Bigler F, Burgio G, Hokkanen HMT, Kuske S, Loomans AJM,
392 Menzler-Hokkanen I, van Rijn PCJ, Thomas MB, Tommasini MG, Zeng QQ (2003)
393 Environmental risk assessment of exotic natural enemies used in inundative biological
394 control. *BioControl* 48:3–38

395 Williams CD, Dillon AB, Girling RD, Griffin CT (2013) Organic soils promote the efficacy of
396 entomopathogenic nematodes, with different foraging strategies, in the control of a major
397 forest pest: A meta-analysis of field trial data. *Biol Control* 65:357–364

398

399

400

401

402

403

404

405

406 Table 1: Coniferous clear-fell sites located in the Republic of Ireland with stumps treated with
 407 *Steinernema carpocapsae* on a site-wide scale (i.e. all stumps treated). SS = Sitka Spruce, LP =
 408 lodgepole pine. For N see Table 2.

409

Site number	Site	Site location	Dimensions of site (m approx.)	Year Felled	Year of application	Soil type	Stump species
A1	Lackenrea	52°08'N 007°48'W 53 m	400 x 400	2005	2007	Mineral	SS, LP (mixed stand)
A2	Glendine	53°05'N 007°34'W 458 m	500 x 400	2005	2007	Peat	LP
A3	Ballymacshaneboy	52°18'N 008°36'W 311 m	200 x 200	2005	2007	Mineral	LP
A4	Knockeen	52°12'N 007°10'W 79 m	200 x 100	2005	2007	Peat	SS
A5	Deerpark	53°09'N 006°12'W 319 m	300 x 300	2005	2007	Mineral	SS
A6	Featherbed	53°14'N 006°19'W 361 m	500 x 500	2007	2008	Mineral	SS
A7	Raheenkyle	52°18'N 008°34'W 426 m	250 x 150	2006	2008	Mineral	SS

410 Table 2: Results of soil and bark sampling on seven coniferous clear-fell sites treated with *S.*
 411 *carpocapsae* to control *H. abietis*. Percentages indicate proportion of all soil cores from the site
 412 that contained *S. carpocapsae* (cores), proportion of tree stumps sampled on a site with at least
 413 one of the soil cores collected at the stump containing *S. carpocapsae* (stumps), proportion of
 414 sampled stumps with bulk bark samples containing *S. carpocapsae* (bulk) and proportion of
 415 sampled stumps with *S. carpocapsae* detected under the bark (under). For sites A1 and A2,
 416 values for a site within a column that share the same letter are not significantly different from
 417 each other (χ^2 or Fisher's Exact test; for multiple comparisons on core and stump data,
 418 Bonferroni $\alpha = 0.017$). LP = lodgepole pine, SS = Sitka spruce. Site details in Table 1.p.a. =
 419 post-application.

Site	Time p.a. (years)	N (cores; stumps)	Presence of <i>S.carpocapsae</i>				Presence of other <i>Steinernema</i> sp	
			Percentage (number)				Percentage (number)	
			soil		bark		cores	stumps
cores	stumps	bulk	under					
A1 LP	0.42	320;20	2.2 (7)a	30 (6)a	-	-	0	0
	1	240;15	3.3 (8)a	40 (6)a	7 (1)	7 (1)	0	0
	2	240;15	1.3 (3)a	13 (2)a	7 (1)	7 (1)	0	0
	5	240;15	0	0	0	0	2.9 (7)	33 (5)
A1 SS	0.42	160;10	3.1 (5)a	40 (4)a	-	-	0	0
	1	240;15	3.3 (8)a	53 (8)a	7 (1)	7 (1)	0	0
	2	240;15	0 b	0 b	0	0	0	0
	5	240;15	0	0	0	0	0.8 (2)	13.3 (2)
A2 LP	0.42	480;30	11.0 (53)a	77 (23)a	-	-	0	0
	1	480;30	13.5 (65)a	87 (26)a	67 (20)a	60 (18)a	0	0

	2	480;30	5.2 (25)b	43 (13)b	37 (11)b	27 (8)b	0	0
	5	480;30	0	0	0	0	0	0
A3 LP	2	480;30	2.9 (14)	37 (11)	40 (12)	33 (10)	0	0
	5	480;30	0	0	-	-	1.3 (6)	20.0 (6)
A4 SS	2	432;27	0.9 (4)	11 (3)	0	0	0	0
A5 SS	5	480;30	0	0	0	0	2.3 (11)	23.3 (7)
A6 SS	4	480;30	0	0	0	0	1.7 (8)	23.3 (7)
A7 SS	4	480;30	0	0	0	0	1.9 (9)	16.7 (5)

420

421

422 Table 3: Summary of *S. carpocapsae* presence in soil around stumps (proportion and number of
 423 soil cores and stumps scoring positive) and under bark of stumps (proportion and number of bulk
 424 bark samples scoring positive) in 2009, two years after application. Data for sites A1 to A4
 425 pooled by soil type and stump species.

426

Data pooled by		soil		bulk bark
		cores	stumps	samples
Soil type	Mineral	1.9 (17/920)	21.7 (13/60)	21.7 (13/60)
	Peat	3.2 (29/912)	22.8 (13/57)	19.3 (11/57)
Tree species	Lodgepole Pine	3.5 (42/1200)	34.7(26/75)	32.0 (24/75)
	Sitka Spruce	0.6 (4/672)	0 (0/42)	0 (0/42)

432

433

434

435

436

437

438

439

440

441

442 **Fig. 1** Scatterplots for the percentage of soil cores scoring positive for *S. carpocapsae* (a) or the
443 percentage of stumps with bark samples scoring positive for *S. carpocapsae* (b) plotted against
444 the $\log_{10}(x+1)$ of the mean number of pine weevils emerging from untreated control stumps on
445 sampled sites A1-A4 (spruce and pine from site A1 used separately). Black points show spruce
446 sites, grey points show pine sites. Horizontal error bars give standard error of mean weevil
447 emergence

448 **Fig. 2** Presence of *S. carpocapsae* in soil cores with increasing distance from bole of sampled
449 stumps 2 years after *S. carpocapsae* application. Bars represent percentage of soil cores positive
450 for *S. carpocapsae* at each distance (data combined at each distance for sites A1-A4; total n =
451 468 cores for each distance).

452

453

454

455

456

457

458

459

460

461

462

463 Figure 1

464

465

466

467

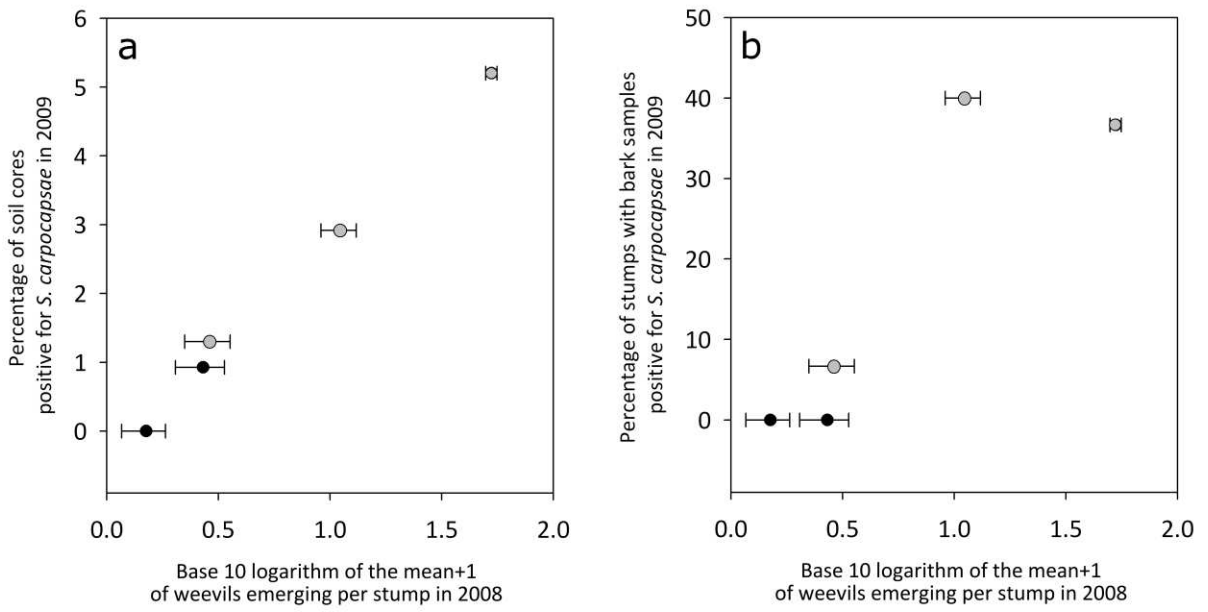
468

469

470

471

472



473 Figure 2

474

475

