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

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Abstract

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

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

1. Introduction

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

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

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

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

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

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

2. Material and methods

2.1 Study sites

In 2007 and 2008, S. carpocapsae (All strain; Becker Underwood; Littlehampton, England) at a rate of 3.5 million IJs per stump applied in 500 ml water was applied to several clear-fell sites (Table 1) on an operational, site-wide scale (Williams et al. 2013). Steinernema carpocapsae was chosen for the study as it is the only species to date that has been applied operationally against pine weevil (Williams et al. 2013). On each site, a small number of stumps were marked and not treated, to serve as controls. These control stumps allowed an assessment of weevil populations within the stumps, based on the number of adult weevils caught in emergence traps erected over them (Williams et al. 2013). The soil type on each site (peat or mineral soil) was based on records of Coillte Teoranta (the site owner) and confirmed by visual evaluation on site. Conifer 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 Nürnbrecht, 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 cm^2 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 cm^2 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 (α =
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

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

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

3.2 Presence of entomopathogenic nematodes in bark samples

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

3.3 Dispersal of EPN from treated stumps

At all sites where S. carpocapsae was recovered, and at all sampling times, the percentage of cores positive for S. carpocapsae tended to be highest directly at the bole of the stump (0 cm distance) and lowest at 60 cm distance from the bole of a stump. Nematodes were detected 60 cm 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)

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

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

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

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

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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.

			Presence of S.carpocapsae Percentage (number)				Presence of other Steinernema sp Percentage (number)	
Site	Time p.a. (years)	N (cores; stumps)	soil		bark			
			cores	stumps	bulk	under	cores	stumps
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)

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

Data pooled by		soil		bulk bark samples
		cores	stumps	
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)

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

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

Figure 1

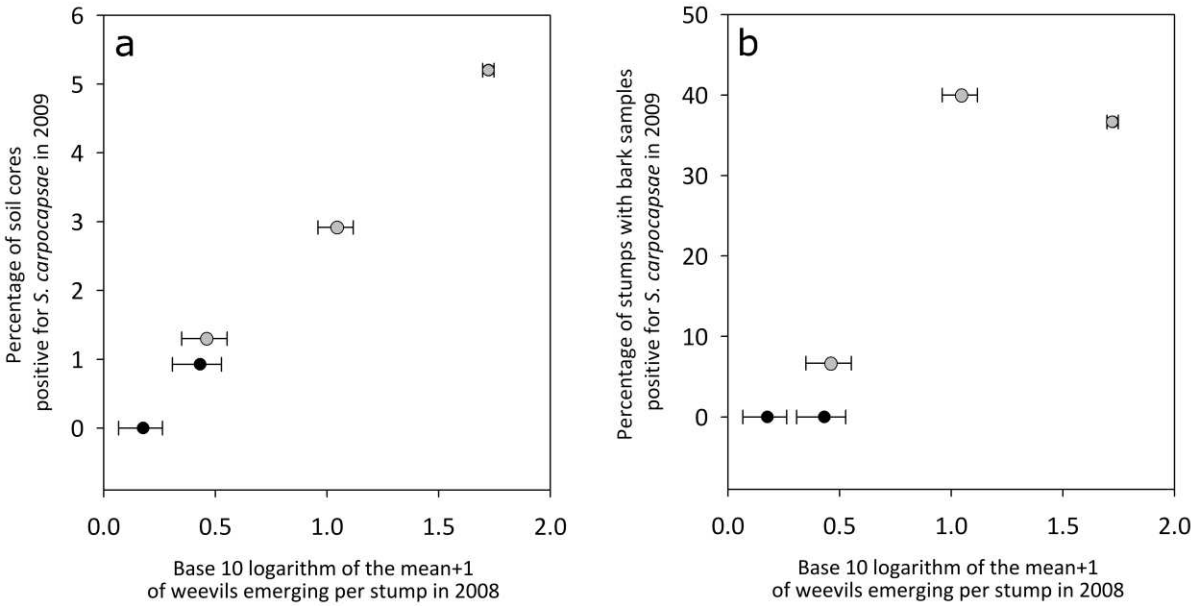

Figure 2