



# Inundative pest control: How risky is it? A case study using entomopathogenic nematodes in a forest ecosystem



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## ABSTRACT

Entomopathogenic nematodes (EPN) are globally important inundative biological control agents. Their widespread use makes environmental risk assessment important, but very few comprehensive post-application risk assessments have been conducted for EPN. We apply a rigorous risk analysis procedure to the use of EPN applied in a forest ecosystem to suppress the large pine weevil (*Hylobius abietis*). In this synthesis, we provide a quantitative evaluation of five risk categories: (a) establishment, (b) dispersal, (c) host range, (d) direct non-target effects and (e) indirect non-target effects. A low level of risk was identified (35–51 out of a possible total of 125). Species exotic to the clear-fell forest ecosystem (*Steinernema carpocapsae* and *Heterorhabditis downesi*) were accorded a lower overall risk status than native species and strains (*Steinernema feltiae*), largely as a result of their shorter persistence in the target environment. We conclude that EPN are a low risk viable alternative control for pine weevil compared to the higher risk conventional control using pyrethroid or neonicotinoid insecticides.

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## 1. Inundative control with EPN and the potential associated risks

Entomopathogenic nematodes (EPN) are lethal insect pathogens that are commercially produced as inundative control agents and used in various regions of the world against a variety of pests (Kaya and Gaugler, 1993; Shapiro-Ilan et al., 2006; Grewal, 2012). There are two genera (*Steinernema* Travassos, 1927 and *Heterorhabditis* Poinar, 1976: Nematoda: Rhabditidae), both of which have global natural distributions (except Antarctica) and are used in biological control (Kaya and Gaugler, 1993; Stuart et al., 2006). The free-living stage of the life cycle, the infective juvenile (IJ), seeks out an insect host, invades it and releases entomopathogenic bacteria from its gut that kill the insect within days (Kaya and Gaugler, 1993; Forst et al., 1997; Lewis et al., 2006). The nematodes feed on the bacteria, reproduce and, typically after a period of two to three weeks, up to several hundred thousand IJs leave the host cadaver to seek out new hosts. Since EPN have a wide potential host range (Peters, 1996), can survive and

reproduce in the field (Bathon, 1996; Smits, 1996) and may disperse, including via phoresy (Eng et al., 2005; Campos-Herrera et al., 2006) or transport by mobile susceptible hosts (Downes and Griffin, 1996), they have the potential to cause environmental impacts other than the reduction of the intended pest.

For assessing the risk of using inundative biological control organisms, van Lenteren et al. (2003) identified five commonly agreed risk categories: host range, dispersal, establishment, and direct and indirect non-target effects. To standardise risk assessment procedures, protocols for assessing the risk of invertebrate biological control organisms in each of these categories have been proposed (e.g. Babendreier et al., 2005; Clercq et al., 2011). A number of reviews summarise the results of risk assessment studies on both classical and inundative biological control organisms (e.g. Hokkanen et al., 1995; Ehlers and Hokkanen, 1996; Barratt et al., 2006, 2010; van Lenteren et al., 2005). For classical and augmentative biological control Hajek et al. (2016) have demonstrated widespread rather trivial effects of introductions and a few cases of direct and indirect impacts at the population and community level, mainly for older (pre 1950) introductions. For EPN, extensive information exists relevant to the risk categories of establishment (or persistence) (e.g. Wright et al., 1993; Shields et al., 1999; Koppenhofer and Fuzy, 2006; Susurluk and Ehlers, 2008) and dispersal (e.g. Lacey et al., 1995; Jabbour and Barbercheck, 2008), as well as host range (Peters, 1996). Direct and indirect non-target

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impacts have received less attention (Bathon, 1996; Somasekhar et al., 2002; de Nardo et al., 2006; Hodson et al., 2012). The available evidence indicates that EPN are generally safe, with little environmental impact (Ehlers and Hokkanen, 1996), though there are very few examples of comprehensive post-application risk assessments investigating multiple risk categories. The only study that has so far investigated all five risk categories is that of van Lenteren et al. (2003) who evaluated the risk of *Steinernema feltiae* (Filipjev, 1934) application in an open field. The present case study summarises risk assessment research carried out on a range of EPN species used to control the large pine weevil (*Hylobius abietis* L., 1758; Coleoptera: Curculionidae) and evaluates the risk for strains that are both native and foreign to the target habitat using the protocol of van Lenteren et al. (2003).

## 2. Large pine weevil control: Target pest, environment and control agents

The large pine weevil is a major forestry pest in 15 European countries, including Ireland and the UK (Långström & Day, 2004). This insect threatens an estimated 3.4 million hectares of forests and would cause up to €140 million in annual damages if not controlled (Långström & Day, 2004). Larvae feed and develop under the bark of stumps and roots of recently dead conifers for one or more years (Leather et al., 1999). Emerging adults feed on the bark of seedlings that are planted to restock such sites, and this can result in up to 100% of the seedlings being killed if the pest is not controlled (Heritage et al., 1989; Leather et al., 1999; Petersson et al., 2005). Forestry practices based on coniferous monoculture with clear-felling have favoured pine weevil, by providing an optimum breeding habitat in stumps, and populations can be very high on clear-fell sites (Leather et al., 1999).

EPN are currently being trialled in Ireland and the UK (including full operational application at selected sites) to evaluate their potential as inundative control agents within an integrated management strategy aimed at replacing pyrethroids (i.e. alpha-cypermethrin and cypermethrin) currently used to control pine weevil (e.g. Brixey et al., 2006; Dillon et al., 2006; Williams et al., 2013a, 2013b). To suppress weevil populations, EPN IJs in aqueous suspension are sprayed onto the soil around the circumference of each tree stump on a site-wide level (recommended rate  $3.5 \times 10^6$  IJs per stump) to target the immature stages (Dillon

et al., 2006). Several EPN species have been tested: *Steinernema carpocapsae* Weiser, 1955, *Steinernema kraussei* Steiner, 1923 *Steinernema feltiae*, *Heterorhabditis downesi* Stock, Griffin and Bunnell, 2002 and *Heterorhabditis megidis* Poinar, Jackson and Klein, 1987 (Table 1) and all have shown potential to significantly reduce weevil populations and/or seedling damage (Brixey et al., 2006; Dillon et al., 2006; Torr et al., 2007; Williams et al., 2013a, 2013b). *Steinernema carpocapsae* is currently the main species in use due to its competitive cost and amenability to mass production, though other species (especially *H. downesi*) have shown better field efficacy.

## 3. Natural distribution of entomopathogenic nematode species used for pine weevil control

Organisms exotic to a particular environment may pose risks that differ in quality and scale from those posed by indigenous organisms (Simberloff and Stiling, 1996; van Lenteren et al., 2003; Clercq et al., 2011; van Lenteren, 2012). Ehlers and Hokkanen (1996) recommended that, unlike the release of indigenous EPN, the release of exotic EPN species (but not exotic strains of indigenous species) should be regulated due to greater potential risk. Thus, a discussion of the risks posed by EPN must take into consideration the known geographical distribution and natural habitats of the applied nematodes.

Surveys of EPN in Britain and Ireland have screened >3000 soil samples collected from a variety of habitats (e.g. grassland, woodland, heathland and hedgerows) (Blackshaw, 1988; Hominick and Briscoe, 1990a, 1990b; Boag et al., 1992; Hominick et al., 1995; Gwynn and Richardson, 1996; Chandler et al., 1997; Dillon, 2003). To date, there exist only two records of *S. carpocapsae* in Britain (Georgis and Hague, 1979, 1981), which have since been disputed (D. Hunt, CABI Europe UK, pers. comm.), and no record of this species in Ireland. A recent, as yet unpublished, study by Rae and colleagues has isolated *S. carpocapsae* from a gorse hedge and a wooded layby, both in Cornwall. Both these isolates were far away from forestry with nematode applications, but the authors are sequencing the mitochondrial DNA to confirm that they are different from commercially produced strains (R. Rae, LJMU UK, pers. comm.). While failure to detect a species does not confirm absence, based on the available evidence we consider *S. carpocapsae* to be exotic to Ireland, but not Britain (Table 1)

**Table 1**

EPN species and strains for which risk assessment studies have been carried out in relation to pine weevil suppression. For each species and strain, status (exotic or indigenous) is given for Britain (Br) and Ireland (Irl) in general, and coniferous forest soils in these islands in particular. Risk categories after van Lenteren et al. (2003) are E = establishment, D = dispersal, DNT = direct non-target effects and INT = indirect non-target effects.

EPN species	Strain and origin	Species/strain present in Br/Irl <sup>a</sup>	Species/strain present in coniferous forest soils <sup>a</sup>	Risk categories Evaluated <sup>a</sup>
<i>Steinernema carpocapsae</i>	All strain, USA	<b>Yes<sup>b</sup></b> (1, 2, 3, 5, 7, 8, 11, 12, 22)	<b>No</b> (2, 8, 12, 13)	<b>E, D, DNT, INT</b> 15, 16, 18, 19, 20, 21
<i>Steinernema feltiae</i>	4CFMO, Ireland	<b>Yes</b> (1, 4, 5, 7, 8, 11, 12)	<b>Yes</b> (2, 12, 13)	<b>E, D, INT</b> 15
<i>Steinernema feltiae</i>	EN02, Germany	<b>Yes<sup>c</sup></b> (1, 4, 5, 7, 8, 11, 12, 15)	<b>No<sup>c</sup></b> (15)	<b>E, D, INT</b> 15
<i>Steinernema kraussei</i>	Not specified (Torr et al., 2007)	<b>Yes</b> (7, 8, 11, 13)	<b>Yes</b> (8, 13)	<b>E</b> 14
<i>Heterorhabditis downesi</i>	K122, Ireland	<b>Yes</b> (6, 11)	<b>No</b> (2, 4, 8, 12)	<b>E, D, DNT, INT</b> 15, 16, 17, 18, 19, 20
<i>Heterorhabditis megidis</i>	UK211, UK; NL-HF85, Netherlands	<b>Yes<sup>d</sup></b> (7,11)	<b>No</b> (2, 4, 8, 12)	<b>E, D, INT</b> 15

<sup>a</sup> References: [1] Blackshaw (1988); [2] Hominick and Briscoe (1990a); [3] Hominick and Briscoe (1990b); [4] Griffin et al. (1991); [5] Boag et al. (1992); [6] Griffin et al. (1994); [7] Hominick et al. (1995); [8] Gwynn and Richardson (1996); [9] Chandler et al. (1997); [10] Griffin et al. (1999); [11] Hominick (2002); [12] Dillon (2003); [13] Harvey (unpublished data); [14] Torr et al., 2007; [15] Dillon et al. (2008a); [16] Dillon et al. (2008b); [17] Everard et al. (2009); [18] Harvey et al. (2012); [19] Harvey and Griffin (2012); [20] Dillon et al. (2012); [21] Harvey and Griffin (2016); [22] R. Rae, pers. comm. (2016).

<sup>b</sup> *S. carpocapsae* has been found in Britain, but not Ireland.

<sup>c</sup> *S. feltiae* is present in UK and Ireland, but strain EN02 originated in Germany (Dillon et al., 2008a).

<sup>d</sup> *H. megidis* has been found in Britain, but not Ireland.

*Steinernema carpocapsae* has not yet been found to occur naturally in forest habitats in Ireland or Britain, however (Table 1).

There are numerous records of *Steinernema feltiae* in Britain and Ireland (Blackshaw, 1988; Griffin et al., 1991; Boag et al., 1992; Hominick et al., 1995; Gwynn and Richardson, 1996; Chandler et al., 1997; Dillon, 2003), some of which are from coniferous forest soils (Hominick and Briscoe, 1990a; Dillon, 2003; Harvey and Griffin, 2016). *Steinernema feltiae* strain 4CFMO was isolated by Dillon (2003) from a coniferous clear-fell site in Ireland and we thus consider it indigenous to this environment (Table 1). *Steinernema feltiae* strain EN02 is a commercially produced strain (e-nema GmbH, Germany) that was originally isolated in Germany (Dillon et al., 2008a, 2008b) and, though the species is indigenous to the UK and Ireland, we treat this strain as exotic to Irish coniferous forest (Table 1). *Steinernema kraussei* has likewise been recorded in Britain (Hominick et al., 1995), including in coniferous forest soil (Gwynn and Richardson, 1996). There is one unpublished record of *S. kraussei* from a coniferous clear-fell site in Ireland, confirmed by sequencing the rDNA internal transcribed spacer region (Harvey, unpublished data; Genbank Accession numbers: KU847415, KU847416). Harvey isolated *S. kraussei* from a soil sample taken on a clear-fell in Glendalough (53°03'N 006°28'W, elevation 300 m) at a Sitka spruce stump (*Picea sitchensis* [Bong.] Carr.) that had been treated with *H. downesi* in 2006. Samples were identified from two separate DNA extractions from bulk samples of several hundred to several thousand nematodes. There was some polymorphism detected, but this is not unusual for the ITS region and has been observed before for *S. feltiae*. A Genbank BLAST search of the two obtained sequences showed 98–99% identity with *S. kraussei* ITS DNA sequences in the database. *Heterorhabditis downesi* is indigenous to Britain and Ireland, but has so far been isolated only from sandy coastal soils (Griffin et al., 1994; 1999). *Heterorhabditis megidis* has been isolated in Britain (Hominick et al., 1995; Hominick, 2002), but has likewise not been reported in forest soils (Hominick and Briscoe, 1990a; Gwynn and Richardson, 1996; Dillon, 2003). We therefore consider *H. downesi* and *H. megidis* indigenous to Britain (and, in the case of *H. downesi*, also Ireland), but exotic to coniferous forest plantations in the context of this case study (Table 1).

#### 4. Risk categories for inundative control agents

Several methods to standardise risk assessment procedures for inundative control agents have been proposed (van Lenteren et al., 2003; Babendreier et al., 2005; Mills et al., 2006). To meet the criteria for risk assessment of introduced biological control agents recommended by the Organisation for Economic Co-operation and Development (OECD, 2003), van Lenteren et al. (2003) proposed a method of calculating a numerical index based on five risk categories. This method allows for a categorical and quantifiable evaluation of risk. The index value is obtained by estimating risk in each of the five categories based on specific criteria. The likelihood (very unlikely to very likely) and magnitude (minimal to massive) of risk are each assigned a value of 1–5; the likelihood and magnitude values within each category are then multiplied and the products are added to arrive at the final index value which can range from 5 to 125, where a higher number indicates a greater environmental risk (van Lenteren et al., 2003). In the present paper, we follow this approach, using results from the pine weevil system complemented by literature from other contexts, to derive risk indices for EPN species *S. carpocapsae* (exotic to Ireland), *S. feltiae* (one strain indigenous and one strain exotic to Ireland) and *H. downesi* (indigenous to Ireland) when used against pine weevil in forestry. We have not included exact risk values for *H. megidis* and *S. kraussei*, the other two species that have been tested against pine

weevil and for which fewer data are available. We estimate *H. megidis* to be similar to its close relative *H. downesi*, both being exotic to the target habitat, and *S. kraussei* to be similar to *S. feltiae*, both species being present in the target habitat.

#### 5. Risk of EPN application in forest ecosystem

##### 5.1. Establishment

In inundative biological control, long-term persistence and establishment of the applied control agent in the target environment is not a desired outcome (Bathon, 1996; van Lenteren et al., 2003). Control agents are applied in large numbers to cause an immediate, but usually transient, reduction in the pest population. EPN have the potential to persist in the soil after application since the applied IJs are the non-feeding, stress-tolerant 'dauer' stage; in addition, they may recycle and multiply in the field by infecting insects (Kaya and Gaugler, 1993; Grewal et al., 2002). The extent and duration of post-application persistence of EPN is expected to vary with the applied species, field conditions and the abundance and suitability of hosts (target and non-target) (Smits, 1996; Barratt et al., 2010; Griffin, 2015). Though EPN numbers may be high in the short term (weeks to months), in most studies numbers decrease rapidly over time and EPN are usually no longer detectable within a year of application (Klein and Georgisi, 1992; Wright et al., 1993; Smits, 1996; Kurtz et al., 2007). In a minority of cases however, EPN have been recorded more than a year after application (Shields et al., 1999; Susurluk and Ehlers, 2008; Parkman et al., 1996).

Dillon et al. (2008a) investigated the persistence of EPN in soil around pine stumps treated to suppress the large pine weevil in Irish trials. Four species were trialled: *H. megidis*, *H. downesi*, *S. carpocapsae* and two strains of *S. feltiae*, a commercial strain (EN02) and an indigenous Irish strain isolated from soil in a clear-felled coniferous forest (4CFMO) (Dillon, 2003; Dillon et al., 2008a). EPN corresponding to the genus applied to a stump (i.e. *Steinernema* or *Heterorhabditis*) were recovered up to three years after application (Dillon et al., 2008a), though recovery rates decreased significantly over time: approximately 30% of soil cores scored positive for EPN one month after application, but only approximately 9% did so after three years. Four and five years after application, only *S. feltiae* was found, and it was recovered even around stumps treated with other EPN species. When these *S. feltiae* isolates were compared to the applied strains (indigenous 4CFMO and commercial EN02) using genome-wide molecular analysis (Amplified Fragment Length Polymorphism, AFLP), they were found to be more closely related to the indigenous strain 4CFMO than the exotic strain EN02 (Dillon et al., 2008a). Mesocosm experiments with more controlled conditions by Dillon et al. (2008a) also showed greater persistence of *S. feltiae* 4CFMO compared to *S. feltiae* EN02. Similarly, in a study conducted on UK coniferous forest sites, Torr et al. (2007) compared the persistence of exotic *S. carpocapsae* to that of indigenous *S. kraussei* (Table 1). One year after application, soil was sampled around tree stumps treated with  $3.5 \times 10^6$  IJs of either of the two species. There was a significant decrease in levels of both species over time, though less rapidly for *S. kraussei* (Torr et al., 2007). In addition, densities of *S. kraussei* were consistently higher than those of *S. carpocapsae* from six months after application. Thus, both Torr et al. (2007) and Dillon et al. (2008a) found that EPN species and strains exotic to the habitat persisted on clear-fell sites for shorter periods than indigenous species or strains, possibly due to the latter being better adapted to the target environment (Dillon et al., 2008a).

Dillon et al.'s (2008a) study compared various species in a uniform setting (pine stumps on deep peat soil), while Harvey and

Griffin (2015) monitored persistence of a single species (*S. carpocapsae*) under varied conditions: lodgepole pine (*Pinus contorta* Douglas) and Sitka spruce stumps on peat (nearly pure organic matter) or mineral soil. Similar to the results obtained by Dillon et al. (2008a), the percentage of soil cores with *S. carpocapsae* decreased significantly within the first two years after EPN application, from up to 12% of cores after five months to 3% after two years (Harvey and Griffin, 2016). Five years after application, only indigenous *Steinernema* spp. were found around stumps (Harvey and Griffin, 2016). Similar results were obtained for stump bark: *S. carpocapsae* was found under the bark of up to 67% of stumps one and two years after application, but was not detected there four or five years post application (Harvey and Griffin, 2016). The incidence of *S. carpocapsae* was positively correlated with the size of weevil populations in the stumps, suggesting that persistence of the EPN population was dependent on the population of pine weevils, in which they can reproduce (Pye and Burman, 1978; Dillon, 2003). Since stumps are suitable for pine weevil for only three to four years after felling (Leather et al., 1999), and EPN are usually applied 12–18 months after felling (Dillon et al., 2008a), this link between the target pest population and nematode persistence imposes a natural limit on EPN recycling and, therefore, reduces the risk of long-term persistence and establishment. A natural next step would be to extend these experiments to other EPN species that are potential inundative biological control agents for pine weevil.

We conclude that exotic *S. carpocapsae* and *H. downesi* as well as exotic strain *S. feltiae* EN02 used against the large pine weevil on clear-fell sites can persist by recycling in the target host in the short term, but that establishment four years or more post-infection is 'unlikely' (likelihood = 2; Hickson et al., 2000; van Lenteren et al., 2003) (Table 2). Moreover, we consider the potential non-target habitat on coniferous clear-fell sites where these exotic EPN may establish to be 'transient in time and space' (van Lenteren et al., 2003), due to the apparent dependence of EPN on pine weevils for recycling (magnitude = 1; van Lenteren et al., 2003; Table 2). This agrees with similar studies on persistence in other, often very different settings (Smits, 1996; Susurluk and Ehlers, 2008). The indigenous strain *S. feltiae* 4CFMO, however, was originally isolated from a coniferous clear-fell site and so is likely to be adapted to this habitat and to hosts there, other than pine weevil. Therefore, if it were applied to sites where it is not

already present, it may persist for longer and in a greater area compared to exotic EPN. We therefore conclude that establishment of *S. feltiae* 4CFMO on coniferous clear-fell sites is 'likely' (likelihood = 4; Hickson, 2000; van Lenteren et al., 2003) and, because >50% of the area of coniferous clear-fell sites is soil available for colonisation by EPN, the potential area of establishment is 'massive' (magnitude = 5; van Lenteren et al., 2003). However, since it appears that native EPN may colonise clear-fell sites as part of a natural ecological succession, following colonisation by native grasses and the associated insect fauna (Harvey and Griffin, 2016), this 'risk' is essentially no different to that of a natural recolonisation event. A less conservative view would be that the risk of establishment for indigenous species necessarily represents the lowest risk possible and would therefore better fit the category of 'very unlikely' establishment, resulting in a numerical risk value of 1 for *S. feltiae* (van Lenteren et al., 2003) (Table 2). While establishment risk of EPN in coniferous clear-fell soils can be considered low overall based on these results, persistence for up to four years after application still provides a window of time in which they can disperse to other areas, potentially creating additional risk.

## 5.2. Dispersal

EPN disperse through soil as IJs which are typically about 0.5–1 mm in length. Depending on soil type, moisture content etc., the rate of horizontal dispersal of IJs after inundative application is usually a few centimetres per day and limited to a scale of meters overall (Poinar and Hom, 1986; Downes and Griffin, 1996; Barratt et al., 2006). IJs of both *Steinernema* and *Heterorhabditis* species can move through mineral and peat soils like those found on coniferous clear-fell sites (Kruitbos et al., 2010; Williams et al., 2013a, 2013b). In addition, IJs may follow lateral roots ('routeways') to locate and infect pine weevil larvae situated >50 cm from the point of application (Dillon et al., 2006; Ennis et al., 2012).

Dillon et al. (2008a) investigated the dispersal of EPN in the field and in mesocosms containing peat, simulating the type of soil typical of many coniferous plantations in Ireland and Britain. In mesocosms, a very low incidence of three EPN species (*S. carpocapsae*, *S. feltiae* 4CFMO and *H. downesi*) was detected 20 cm from the point of application, the maximum distance that was sampled. In the field, soil samples were three to four times more likely to score

**Table 2**

Risk indices for *Steinernema carpocapsae*, *Heterorhabditis downesi* and *Steinernema feltiae* when used against the large pine weevil. Values for likelihood of risk are determined on a scale of 1–5 (1 = very unlikely, 2 = unlikely, 3 = possible, 4 = likely, 5 = very likely), as are values for magnitude (1 = minimal, 2 = minor, 3 = moderate, 4 = major, 5 = massive), based on criteria outlined in van Lenteren et al. (2003). Within each risk category, the values for likelihood and magnitude of effects are multiplied, and the products are added to give the risk index (van Lenteren et al. 2003).

EPN species/strain		Risk category					Risk index
		Establishment	Dispersal	Host range	Direct non-target effects	Indirect non-target effects	
<i>S. carpocapsae</i>	Likelihood	2	2	5	2	2	<b>35</b>
	Magnitude	1	1	5	1	2	
	L × M	<b>2</b>	<b>2</b>	<b>25</b>	<b>2</b>	<b>4</b>	
<i>H. downesi</i>	Likelihood	2	2	5	2	2	<b>35</b>
	Magnitude	1	1	5	1	2	
	L × M	<b>2</b>	<b>2</b>	<b>25</b>	<b>2</b>	<b>4</b>	
<i>S. feltiae</i> (EN02)	Likelihood	2	2	5	2	1	<b>35</b>
	Magnitude	2	1	5	1	2	
	L × M	<b>4</b>	<b>2</b>	<b>25</b>	<b>2</b>	<b>2</b>	
<i>S. feltiae</i> (4CFMO)	Likelihood	4	2	5	2	1	<b>51</b>
	Magnitude	5	1	5	1	2	
	L × M	<b>20</b>	<b>2</b>	<b>25</b>	<b>2</b>	<b>2</b>	
<i>S. feltiae</i> <sup>a</sup>	Likelihood	3	1	5	4	4	<b>53</b>
	Magnitude	5	1	5	2	1	
	L × M	<b>15</b>	<b>1</b>	<b>25</b>	<b>8</b>	<b>4</b>	

<sup>a</sup> The risk index for *S. feltiae* when applied to an open field in Finland from van Lenteren et al. (2003) is given here for comparison.

positive for EPN when taken at a treated tree stump compared to a distance of 20 cm from the stump (Dillon et al., 2008a). The distance from the stump at which EPN were found was not influenced by species: exotic species *S. carpocapsae* and *H. downesi* dispersed at a rate comparable to the indigenous *S. feltiae* 4CFMO. Harvey and Griffin (2016) likewise observed that the probability of detecting *S. carpocapsae* decreased significantly as distance from the stump increased from 0 cm to 60 cm. These findings are in general agreement with previous studies in different settings, where EPN presence decreases rapidly with distance from the point of application (Poinar and Hom, 1986; Smits, 1996; Barratt et al., 2006; Jabbour and Barbercheck, 2008).

Long-distance dispersal can occur, however, when facilitated by infected or externally contaminated host insects or other carriers. Transport in wind and water may also occur, though considered rare (Downes and Griffin, 1996; Griffin, 2015). The phoretic route is the most likely explanation for reports of rapid short-range dispersal (Jabbour and Barbercheck, 2008) or long-range dispersal over several hundred meters up to kilometres (Barratt et al., 2006). Following application of *Steinernema scapterisci* Nguyen and Smart, 1990 to control mole crickets in Florida, infected insects were collected as far as 23 km from the nearest site of application (Parkman et al., 1993; Parkman and Smart, 1996). Lacey et al. (1995) reported dispersal of *Steinernema glaseri* Steiner, 1929 IJs on the cuticle or within the haemocoel of *Popillia japonica* Newman, 1841. Infected beetles in many cases contained enough nematodes to allow reproduction, and dispersal in the field over at least 50 m was reported. The potential for dispersal of EPN by adult pine weevil has been demonstrated in the laboratory (Kruitbos et al., 2009).

Dillon et al. (2008a) tested for wider dispersal of EPN from treated stumps but found no EPN at distances ranging from 1 to 10 m from the nearest treated stump. Harvey (2010) extended the sampling up to 100 m off-site. *Steinernema carpocapsae* was detected in a small proportion of samples collected 5–10 m from two of three sites where it had been applied 1–2 years previously (Harvey, 2010). When the areas at which each of these positive samples was detected were extensively re-sampled (40 bulk soil samples, each comprised of 5 subsamples at each previously positive spot) five years after application, only native *Steinernema* spp. were isolated (Harvey & Griffin, unpublished data). Failure to detect *S. carpocapsae* does not guarantee that no spread and/or establishment of this species off-site has occurred, but it does suggest that any *S. carpocapsae* populations that may have remained after five years are most likely small and isolated. Similar tests for other EPN should be undertaken to establish their potential for off-site spread.

The natural host range and the mechanisms underlying the persistence and patchy distribution of EPN populations in the wild are poorly understood (Stuart and Gaugler, 1994; Peters, 1996; Smits, 1996; Griffin, 2015). However, given the results discussed here, the distance of dispersal within and off clear-fell sites is unlikely to exceed 100 m (likelihood = 2; van Lenteren et al., 2003) for any of the EPN investigated and, given the large number of IJs applied per stump (approx.  $3.5 \times 10^6$ ), the magnitude of any such dispersal will probably be 'minimal' (i.e. <1% of the applied EPN dispersing, magnitude = 1; van Lenteren et al., 2003), which is similar to previous evaluations of EPN dispersal risk (Smits, 1996; Barratt et al., 2006) (Table 2).

### 5.3. Host range

In laboratory assays, EPN have a broad host range: for example, *S. carpocapsae* was reported to kill >200 species of insects from 10 orders in close-contact laboratory assays (Poinar, 1979); however, the realised host range in the field is expected to be much

narrower, and the range of insects affected to vary between species (Peters, 1996). Due to the wide potential host range, however, van Lenteren et al. (2003) assigned maximal risk values of 5 to both likelihood and magnitude of risk to *S. feltiae* when applied to an open field in Finland (>30 species host range and taxon range > order level, respectively; van Lenteren et al., 2003). We have adopted this evaluation of host range for all EPN species used against the large pine weevil in our risk index estimation (Table 2).

### 5.4. Direct non-target effects

Non-target impacts of inundatively applied EPN are of concern for three related reasons. Firstly, negative impacts on biodiversity are considered detrimental in sustainable management of natural resources, as they are likely to reduce the resilience and function of an ecosystem (Bengtsson et al., 2000; Brockerhoff et al., 2008). Secondly, non-target insects that are of particular benefit to sustainable forest management (e.g. wood decomposers) may be at particular risk due to their proximity to the zone of nematode application (Harvey et al., 2012). Thirdly, non-target impacts have the potential to disrupt natural control of the pest if they affect an important natural enemy (van Lenteren, 2012; Harvey and Griffin, 2012). This last point is underlined by the fact that control by natural enemies, without intervention, may make a considerable economic contribution to pest control (Waage et al., 1988; Losey and Vaughan, 2006).

Direct non-target impacts arise when applied EPN infect and kill organisms other than the target pest. Considering the wide potential host range of EPN (Peters, 1996), occasional infection of non-target individuals is probably common when inundatively applying EPN IJs, but this should be distinguished from widespread or pervasive non-target infection that reduces abundance and diversity of non-target species (Bathon, 1996; van Lenteren et al., 2003). Published surveys of non-target impacts at population and community level, before and after EPN application, suggest that such impacts are rare and, if they do occur, tend to be minor (Bathon, 1996; Barratt et al., 2006; Hodson et al., 2012). Nonetheless, plantation forests and the associated clear-fell sites, though not always as diverse as mature and natural forest stands (Grove, 2002; Irwin et al., 2014), may harbour a significant number of insects, particularly saproxylics, including red-listed species (Sippola et al., 2002; Jonsell et al., 2007; Irwin et al., 2014). To assess the impact of EPN on non-target insects in the pine weevil system we looked both for effects on community composition and on two key ecosystem service providers, a parasitoid and a common saproxylic species.

Saproxylic beetles, which develop in or feed on decomposing wood for at least part of their life cycle, are considered beneficial in forest management and are, therefore, worth protecting (Speight, 1989). These beneficial non-target insects may be at risk of infection as they occupy a similar habitat to the pine weevil. The two-banded longhorn beetle *Rhagium bifasciatum* Fabricius 1775 (Coleoptera: Cerambycidae) is an important wood-decomposing insect on clear-fell sites in Europe (Duffy, 1953; Twinn and Harding, 1999). It develops over several years in deadwood but, as tree stumps only become suitably decomposed for this species three to four years after felling (Duffy, 1953), it usually does not co-occur with pine weevils, which are present in stumps one to three years after felling (Leather et al., 1999). These longhorns may, however, be impacted by misdirected spray during nematode application or by EPN dispersing from treated stumps. Harvey et al. (2012) demonstrated that larvae and adults of *R. bifasciatum* could be infected by both *S. carpocapsae* and *H. downesi* within decomposing deadwood logs, though infection was significantly lower in field experiments than in the laboratory. High rates of infection (>30% of insects) were typically only observed in logs that

had been directly drenched with a dose of 1.8 million IJs, half the number applied per stump for pine weevil suppression (Dillon et al., 2008a). *Rhagium bifasciatum* infected with EPN were also found in deadwood 1–12 months after application of *S. carpocapsae* to stumps on an operational, site-wide scale, but fewer than 10% of logs contained infected insects, and infected insects represented less than 4% of the overall population sampled. Both *S. carpocapsae* and *H. downesi* reproduced in *R. bifasciatum* larvae, so it is possible that some of the infection was as a result of recycling within the logs. The number of logs with infected *R. bifasciatum*, and number of infected longhorns per log declined significantly with increasing distance of logs from treated stumps (Harvey et al., 2012). The targeted application of EPN around tree stumps therefore appears to limit direct non-target risks for this and probably also other saproxylic beetles in deadwood and wood debris.

*Bracon hylobii* Ratzeburg 1848 is an important beneficial insect that provides natural control of the large pine weevil (Henry and Day, 2001). Parasitism rates of pine weevil by this gregarious ectoparasitoid are typically in the range of 15–30% (Dillon et al., 2008a, 2008b; Harvey, unpublished data), but can be as high as 90% (Henry, 1995). Any intraguild predation of EPN on *B. hylobii* could potentially be detrimental to this natural control (Rosenheim et al., 1995). Several parasitoid wasps are susceptible to EPN, especially as larvae (Battisti, 1994; Lacey et al., 2003; Mbata and Shapiro-Ilan, 2012). Larvae, pupae and adults of *B. hylobii* were susceptible to *H. downesi* infection in laboratory assays (Everard et al., 2009). Adults emerging from cocoons were most susceptible (80% mortality in close-contact trials) while pupae inside cocoons were infected only rarely (<8% of pupae infected inside cocoons after exposure to 10,000 IJs of *H. downesi* (Everard et al., 2009)). However, such close-contact laboratory assays, with high concentrations of EPN, almost certainly over-represent infection rates in the field. Dillon et al. (2008b) found no reduction in *B. hylobii* parasitism of pine weevil in stumps treated with *H. downesi* or *S. carpocapsae* 18–23 months earlier, but infection of *B. hylobii* itself with EPN was not assessed. Susceptibility of a parasitoid to EPN does not necessarily impact on parasitism of the pest: larvae of the parasitoid *Habrobracon hebetor* Say 1836 are susceptible to infection with *Heterorhabditis indica* Poinar, Karunakar & David, 1992, but when nematode and wasp were used together against Indian meal moth *Plodia interpunctella* Hübner 1813 in laboratory assays, no antagonistic effect was observed (Mbata and Shapiro-Ilan, 2012).

Tree stumps can harbour a large diversity of invertebrates, both in the decomposing wood and bark, and in the soil around them (Wallace, 1953; Abrahamsson and Lindbladh, 2006; Hedgren, 2007). Since this is where EPN are applied (Dillon et al., 2008a), impacts on non-target insects are most likely to occur in this area. When debarking tree stumps to record infection of pine weevil after application of EPN, infected non-target insects (e.g. Elateridae) were occasionally found (Harvey, Dillon, pers. obs.). To monitor effects of EPN on non-target Coleoptera, Dillon et al. (2012) placed insect emergence traps over stumps treated with *S. carpocapsae* or *H. downesi* and over untreated stumps. EPN did not affect species diversity, richness, abundance or community composition, either in the year of application or one year later (Dillon et al., 2012). In particular, EPN application had no significant effect on wood-associated species including the abundant saproxylic cerambycid, *Asemum striatum* L. 1758 (Dillon et al., 2012). The authors concluded that the impact on non-target Coleoptera in and around tree stumps is probably negligible for the two species tested to date.

Based on the available data summarised here, direct non-target impacts of the EPN species investigated are 'unlikely' when applied against pine weevil (likelihood = 2; Hickson et al., 2000; van Lenteren et al., 2003) (Table 2). In addition, data for both wood

debris-associated and stump-associated non-target insects suggest mortality of these insects is <5% of the total available non-target population on site (magnitude = 1; van Lenteren et al., 2003). These assessments, while supported by the limited data available for some EPN species, should be considered tentative until further experimental data become available, especially for species whose non-target risks have not yet been studied in detail in forest ecosystems.

### 5.5. Indirect non-target effects

Indirect effects of biological control are among the most difficult to study and disentangle (Simberloff, 2012), making them the least researched aspect of risk assessment. Applying large numbers of EPN may influence trophic interactions in the soil, thereby potentially changing nematode (Somasekhar et al., 2002) and/or microarthropod assemblages (Hodson et al., 2012) as well as nutrient cycles (De Nardo et al., 2006). Where persistence and dispersal of a control agent are low risk factors, it can be argued that indirect non-target effects are also unlikely (Barratt et al., 2006). Nonetheless they should be assessed for completeness. EPN may compete for hosts with other parasites, pathogens and parasitoids at the same trophic level. In the pine weevil system, we consider indirect effects on native EPN and on *Bracon hylobii*. Studies elsewhere indicate that endemic nematodes may persist in spite of inundative application of EPN (Millar and Barbercheck, 2001; Duncan et al., 2003). For example, Millar and Barbercheck (2001) tested whether indigenous *S. carpocapsae* and *H. bacteriophora* Poinar, 1975 were displaced by the exotic nematode *Steinernema riobrave* Cabanillas, Poinar, and Raulston, 1994 after inundative application to corn fields in the US. Though the exotics persisted for more than two years, no evidence of long-term displacement of either of the endemic species was found (Millar and Barbercheck 2001). *Steinernema feltiae* was previously the only EPN recovered in a survey of coniferous forestry throughout Ireland, being found in 10% of mature standing forests and 7% of replanted clear-felled sites (Dillon, 2003). More recently, however, *Steinernema kraussei* has also been detected (Harvey, unpublished). While *S. carpocapsae* was detected for at least 2 years following its application on coniferous clear fell sites in Ireland, it was replaced on several sites by indigenous steinernematids (Harvey and Griffin, 2016). As the sites had not been sampled for EPN prior to treatment, it is not known whether endemic EPN were temporarily suppressed to undetectable levels, or their later detection was as a result of a new colonisation of the sites. Dillon et al. (2008a) found that the exotic species *S. carpocapsae* and *H. downesi* and the exotic strain *S. feltiae* EN02 did not displace native strain *S. feltiae* 4CFMO on Irish clear-fell sites treated for pine weevil control. When applying an exotic strain of an indigenous species, there is a risk of introgression (Roderick and Navajas, 2003; Hopper et al., 2006), but there was no evidence of hybridization between indigenous and applied strains of *S. feltiae* (Dillon et al., 2008a). These findings suggest that indigenous EPN species are unlikely to be displaced in the long term by exotics that are not adapted to the target environment (Grewal et al., 1994), but tests on further EPN species that may be used in pine weevil suppression activities should be considered as the next step in the assessment of indirect non-target effects.

As previously noted, inundatively applied EPN may have direct effects on the parasitoid *B. hylobii* by killing various life stages. We also consider the possibility of competition between nematodes and this parasitoid for pine weevil larvae. *Bracon hylobii* cannot develop to adulthood on hosts that have been infected with EPN; females oviposited on healthy host larvae, but not on larvae killed by *H. downesi* or *S. carpocapsae*, which should reduce

the negative impact on the parasitoid (Everard et al., 2009; Harvey and Griffin, 2012). Female *B. hylobii*, especially those with prior experience, did parasitise live hosts infected with EPN, as long as they were still moving (Everard et al., 2009; Harvey and Griffin, 2012). While this means there is a possibility of competition between EPN and *B. hylobii* (modulated by wasp experience), complementary (additive or synergistic) control effects by the two agents may also emerge (Harvey and Griffin, 2012). Dillon et al. (2008b) reported an additive effect of *H. downesi* and *S. carpocapsae* with *B. hylobii* on mortality of pine weevil in stumps across three sites. Larger-scale and longer-term monitoring of *B. hylobii* populations is necessary to draw more definite conclusions about population-scale effects of competition between EPN and *B. hylobii*.

We estimate that indirect non-target effects of exotic EPN species and strains used for large pine weevil control (i.e. *S. carpocapsae*, *S. feltiae* ENO2 and *H. downesi*) are 'unlikely' (likelihood = 2; Hickson et al., 2000; van Lenteren et al., 2003) (Table 2), and we expect these exotics to have only a 'minor' impact on non-target organisms (magnitude = 2; van Lenteren et al., 2003) (Table 2). Furthermore, we consider indirect non-target impacts to be 'very unlikely' for the native *S. feltiae* 4CFMO (likelihood = 1; Hickson et al., 2000; van Lenteren et al., 2003) as it is already a natural component of coniferous forest soils in Ireland and thus inundative application should not have a qualitative impact on the soil organism community. It should be stressed, however, that these assessments are based on the different aspects of indirect non-target impact investigated for each of the species and that results for one species are not necessarily representative of others.

## 6. Conclusions and risk evaluation

Both exotic and indigenous EPN trialled against the large pine weevil persisted in the soil for up to four years after application (Dillon et al., 2008a; Harvey and Griffin, 2016), but the evidence suggests that persistence was driven by recycling through the target pest as intended. Consequently, EPN levels decreased to background levels (for an indigenous strain) or undetectable levels (for exotic species/strains) along with the natural decrease in pest population (Torr et al., 2007; Dillon et al., 2008a; Harvey and Griffin, 2016). Moreover, the exotic applied strain of *S. feltiae* did not displace an indigenous strain (Dillon et al., 2008a). Active horizontal dispersal appeared to be limited to a zone of less than 1 m from the point of application and, while phoresis or some other long-range mechanism of dispersal resulted in movement of EPN outside the treated areas, there is no evidence that they established there (Dillon et al., 2008a; Harvey, 2010; Harvey and Griffin, 2016). Direct non-target effects are limited by the targeted application of exotic EPN (Harvey et al., 2012) and coleopteran communities around tree stumps were unaffected by exotic EPN (Dillon et al., 2012). Moreover, while the parasitoid *B. hylobii* is susceptible to infection by and competition with EPN, there is no indication that this negatively impacts on *B. hylobii* parasitism in the field (Dillon et al., 2008b; Everard et al., 2009; Harvey and Griffin, 2012). Thus, both exotic and indigenous EPN seem to be well-suited as a low-risk alternative to chemical pesticides. While most of the risk assessment studies carried out in our target forest ecosystem focussed on just two species, *S. carpocapsae* and *H. downesi*, we have extrapolated our conclusions to *S. feltiae*. We feel this is acceptable, as *S. feltiae* is a species indigenous to the system and can be considered *a priori* to be of low risk.

Current risk considerations and regulatory restrictions on exotics have resulted in a trend to favour indigenous inundative

control agents over exotic ones, reversing the past emphasis on use of exotics (van Lenteren, 2012). The results presented here do not suggest that risk, as defined by van Lenteren et al. (2003), is increased by using exotic species. In fact, using EPN that are not well-adapted to the environment where they are applied might reduce the risk of long-term establishment (Grewal et al., 1994). The indexing method devised by van Lenteren et al. (2003), when applied strictly, is only valid for the environment and setting in which the risk for the control agent has been evaluated. In the setting of large pine weevil control using EPN, we estimate the risk index of the exotic *H. downesi* and *S. carpocapsae* to be 35, as also for the exotic strain of *S. feltiae*, ENO2 (Table 2). We arrived at a somewhat higher index value of 51 for *S. feltiae* 4CFMO (native) in a forestry setting in Ireland (Table 2). The main risk category contributing to the differences in indices is establishment; we assign higher scores to the native Irish species *S. feltiae*, particularly the native strain 4CFMO, as it has the potential to persist for longer in coniferous clear-fell soils after application (Dillon et al. 2008a). However, since this species already occurs naturally in this ecosystem, in this case a higher risk index value does not necessarily imply a greater environmental hazard due to application. If we take the establishment risk of *S. feltiae* to have a less conservative value of 1, then its overall risk index value becomes 36. By comparison, van Lenteren et al. (2003) assign an index value of 53 to *S. feltiae* when released in Finland (where it is indigenous) in an open field environment. The slightly different indices between the two studies for application of a native *S. feltiae* are accounted for by higher risk estimates for establishment and dispersal, and lower risk estimates for direct and indirect non-target effects in our system compared to that of van Lenteren et al.

Of course, no risk assessment can ever be complete and offer a guarantee of safety – risks and benefits must therefore always be weighed in sensible proportion to each other (Clercq et al., 2011; Simberloff, 2012). The pine weevil has been controlled in Ireland and elsewhere mainly by applying chemical pesticide (most recently cypermethrin or  $\alpha$ -cypermethrin) to replanted seedlings before and/or after planting (e.g. Torstensson et al., 1999; Willoughby et al., 2004). EPN, as part of an integrated pest management strategy, are intended to help replace cypermethrin and  $\alpha$ -cypermethrin as their use is phased out in the European Union under sustainable forest management (SFM) policies. An extensive body of research investigating environmental impacts of pyrethroid pesticides in forestry shows that they can affect a much wider range of organisms than do EPN (e.g. crustaceans and vertebrates), can impact on terrestrial and – unlike EPN – also aquatic non-target organisms and can persist in both soil and freshwater (e.g. McLeesc et al., 1980; Anderson, 1982; Kreutzweiser and Kingsbury, 1987; DeLorenzo and Fulton, 2012). Moreover, by altering the composition of freshwater invertebrate communities, pyrethroids can also have indirect impact on other non-target organisms (Kingsbury and Kreutzweiser, 1987). Though the risk indexing method by van Lenteren et al. (2003) is not designed to incorporate chemical pesticides, the risk of pyrethroids in terms of host range, persistence (analogous to establishment for EPN) and direct and indirect non-target impacts in the context of pine weevil control is likely to be greater than that of the EPN discussed here. This is consistent with Laengle and Strasser (2010), who compared risk factors for biological control agents with pesticides. They report risk factors in the order of thousands for pesticides and in the order of hundreds for biological control agents. Thus, from the perspective of minimizing the risk of environmental impact, EPN appear to be a superior alternative to conventional chemical control methods when managing the large pine weevil.

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