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

Christopher D. Harvey[1], Christopher D. Williams[2], Aoife B. Dillon[3] and Christine T. Griffin[1]

[1]Department of Biology, Maynooth University, Maynooth, County Kildare, Ireland

[2]School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UK.

[3]Coillte, Dublin Road, Newtownmountkennedy, County Wicklow, Ireland

Current addresses: Christopher D. Harvey: School of Human and Life Sciences, Canterbury Christ Church University, Canterbury, UK. Aoife B. Dillon Exosect Ltd, Colden Common, Winchester, Hampshire, SO21 1TH, UK

Corresponding author: chris.harvey@canterbury.ac.uk

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.

Key Words: Risk assessment, Inundative biological control, Entomopathogenic nematodes, Pine weevil, Foresry

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 & 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 & 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 & Gaugler, 1993; Forst, 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 & Griffin, 1996), they have the potential to cause environmental impacts other than the intended pest reduction.

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 standardize 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; Clerq et al., 2011). A number of reviews summarize the results of risk assessment studies on both classical and inundative biological control organisms (e.g. Hokkanen and Lynch, 1995; Ehlers & Hokkanen, 1996; Barratt et al.,

2006 & 2010; van Lenteren et al., 2006). 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 & Fuzy, 2006; Susurluk & Ehlers, 2008) and dispersal (e.g. Lacey et al., 1995; Jabbour & Barbercheck, 2008), as well as host range (Peters, 1996). Direct and indirect non-target 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 & 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).

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., 2013). 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 $3.5x10^6$ 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) *S. feltiae*, *Heterorhabditis downesi* Stock, Griffin and Burnell, 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., 2013). *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.

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 of indigenous organisms (Simberloff & Stiling, 1996; van Lenteren et al., 2003; Clerq 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, hedgerows) (Blackshaw, 1988; Hominick & Briscoe, 1990a & 1990b; Boag et al., 1992; Hominick et al., 1995; Gwynn & Richardson, 1996; Chandler et al., 1997; Dillon, 2003). To date, there exist only two records of *S. carpocapsae* in Britain (Georgis & 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 be sure that they are different from the BASF-Becker Underwood strains, which are used commercially (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 both Britain and Ireland (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 & Richardson, 1996;

Chandler et al., 1997; Dillon, 2003), some of which are from coniferous forest soils (Hominick & Briscoe, 1990a; Dillon, 2003; Harvey & 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., 2008) 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 & 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 collected *S. kraussei* from a Sitka spruce (*Picea sitchensis* [Bong.] Carr.) clear-fell from a soil sample around a stump after it had been treated with *H. downesi* in Glendalough (53°03'N 006°28'W, elevation 300 m), which had been felled in 2004. Samples were identified from two separate 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*. The Genbank blast search confirmed the identity to be *S. kraussei* with 98-99% identity. *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 & Briscoe, 1990a; Gwynn & 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).

146 Risk categories for inundative control agents

147 Several methods to standardise risk assessment procedures for inundative control agents have
148 been proposed (van Lenteren et al., 2003; Babendreier et al., 2005; Mills et al., 2006). To meet
149 the criteria for risk assessment of introduced biological control agents recommended by the
150 Organisation for Economic Co-operation and Development (OECD, 2003), van Lenteren et al.
151 (2003) proposed a method of calculating a numerical index based on five risk categories. This
152 method allows for a categorical and quantifiable evaluation of risk. The index value is obtained
153 by estimating risk in each of the five categories based on specific criteria. The likelihood (very
154 unlikely to very likely) and magnitude (minimal to massive) of risk are each assigned a value of
155 1-5; the likelihood and magnitude values within each category are then multiplied and the
156 products are added to arrive at the final index value which can range from 5 to 125, where a
157 higher number indicates a greater environmental risk (van Lenteren et al., 2003). In the present
158 paper, we follow this approach, using results from the pine weevil system complemented by
159 literature from other contexts, to derive risk indices for EPN species *S. carpocapsae* (exotic to
160 Ireland), *S. feltiae* (one strain indigenous and one strain exotic to Ireland) and *H. downesi*
161 (indigenous to Ireland) when used against pine weevil in forestry. We have not included exact
162 risk values for *H. megidis* and *S. kraussei*, the other two species that have been tested against
163 pine weevil and for which fewer data are available. We estimate *H. megidis* to be similar to its
164 close relative *H. downesi*, both being exotic to the habitat, and *S. kraussei* to be similar to *S.*
165 *feltiae*, both species being present in the target habitat.

166

167

168 Risk of EPN application in forest ecosystem

169 a) Establishment

170 In inundative biological control, long-term persistence and establishment of the applied control
171 agent in the target environment is not a desired outcome (Bathon, 1996; van Lenteren et al.,
172 2003). Control agents are applied in large numbers to cause an immediate, but usually transient,
173 reduction in the pest population. EPN have the potential to persist in the soil after application
174 since the applied IJs are the non-feeding, stress-tolerant 'dauer' stage; in addition, they may
175 recycle and multiply in the field by infecting insects (Kaya & Gaugler, 1993; Grewal et al.,
176 2002). The extent and duration of post-application persistence of EPN is expected to vary with
177 the applied species, field conditions and the abundance and suitability of hosts (target and non-
178 target) (Smits, 1996; Barratt et al., 2010; Griffin, 2015). Though EPN numbers may be high in
179 the short term (weeks to months), in most studies numbers decrease rapidly over time and EPN
180 are usually no longer detectable within a year of application (Klein & Georgis, 1992; Wright et
181 al., 1993; Smits, 1996; Kurtz et al., 2007). In a minority of cases however, EPN have been
182 recorded more than a year after application (Shields et al., 1999; Susurluk & Ehlers, 2008;
183 Parkman et al., 1996).

184 Dillon et al. (2008a) investigated the persistence of EPN in soil around pine stumps treated to
185 suppress the large pine weevil in Irish trials. Four species were trialled: *H. megidis, H. downesi,*
186 *S. carpocapsae* and two strains of *S. feltiae*, a commercial strain (EN02) and an indigenous Irish
187 strain isolated from soil in a clear-felled coniferous forest (4CFMO) (Dillon, 2003; Dillon et al.,
188 2008a). EPN corresponding to the genus applied to a stump (i.e. *Steinernema* or *Heterorhabditis*)
189 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 3.5×10^6 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

213 Dillon et al. (2008a), the percentage of soil cores with *S. carpocapsae* decreased significantly
214 within the first two years after EPN application, from up to 12 % of cores after five months to 3
215 % after two years (Harvey & Griffin, 2016). Five years after application, only indigenous
216 *Steinernema* spp. were found around stumps (Harvey & Griffin, 2016). Similar results were
217 obtained for stump bark: *S. carpocapsae* was found under the bark of up to 67 % of stumps one
218 and two years after application, but was not detected there four or five years post application
219 (Harvey & Griffin, 2016). The incidence of *S. carpocapsae* was positively correlated with the
220 size of weevil populations in the stumps, suggesting that persistence of the EPN population was
221 dependent on the population of pine weevils, in which they can reproduce (Pye & Burman, 1978;
222 Dillon, 2003). Since stumps are suitable for pine weevil for only three to four years after felling
223 (Leather et al., 1999), and EPN are usually applied 12 to 18 months after felling (Dillon et al.,
224 2008a), this link between the target pest population and nematode persistence imposes a natural
225 limit on EPN recycling and, therefore, reduces the risk of long-term persistence and
226 establishment. A natural next step would be to extend these experiments to other EPN species,
227 which are potential inundative biological control agents for pine weevil.

228 We conclude that exotic *S. carpocapsae* and *H. downesi* as well as exotic strain *S. feltiae* EN02
229 used against the large pine weevil on clear-fell sites can persist by recycling in the target host in
230 the short term, but that establishment four years or more post-infection is 'unlikely' (likelihood =
231 2; Hickson et al., 2000; van Lenteren et al., 2003) (Table 2). Moreover, we consider the potential
232 non-target habitat on coniferous clear-fell sites where these exotic EPN may establish to be
233 'transient in time and space' (van Lenteren et al., 2003), due to the apparent dependence of EPN
234 on pine weevils for recycling (magnitude = 1; van Lenteren et al., 2003; Table 2). This agrees
235 with similar studies on persistence in other, often very different settings (Smits, 1996; Susurluk

& 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 more than 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) (Table 2). 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 & 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., 20013). 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.

b) 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 & Hom, 1986; Downes & 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., 2013). In addition, IJs may follow lateral roots ('routeways') to locate and infect pine weevil larvae situated more than 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 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 & 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 & Hom, 1986; Smits, 1996; Barratt et al., 2006; Jabbour & 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 & Griffin, 1996; Griffin, 2015). The phoretic route is the most likely explanation for reports of rapid short-range dispersal (Jabbour & 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 & 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 & 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 x 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).

c) 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 at 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).

<u>d) Direct non-target effects</u>

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 & 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; Hodson et al., 2002; Barratt et al., 2006). 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, 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.

346 Saproxylic beetles, which develop in or feed on decomposing wood for at least part of their life
347 cycle, are considered beneficial in forest management and are, therefore, worth protecting
348 (Speight, 1989). These beneficial non-target insects may be at risk of infection as they occupy a
349 similar habitat to the pine weevil. The two-banded longhorn beetle *Rhagium bifasciatum*
350 Fabricius 1775 (Coleoptera: Cerambycidae) is an important wood-decomposing insect on clear-
351 fell sites in Europe (Duffy, 1953; Twinn & Harding, 1999). It develops over several years in
352 fallen deadwood and wood debris but, as tree stumps only become suitably decomposed for this
353 species three to four years after felling (Duffy, 1953), it usually does not co-occur with pine
354 weevils, which are present in stumps one to three years after felling (Leather et al., 1999). These
355 longhorns may, however, be impacted by misdirected spray during nematode application or by
356 EPN dispersing from treated stumps. Harvey et al. (2012) demonstrated that larvae, pupae and
357 adults of *R. bifasciatum* could be infected by both *S. carpocapsae* and *H. downesi* within
358 decomposing deadwood logs, though infection was significantly lower in field experiments than
359 in the laboratory. High rates of infection (> 30 % of insects) were typically only observed in logs
360 that had been directly drenched with a dose of 1.8 million IJs, half the number applied per stump
361 for pine weevil suppression (Dillon et al., 2008a). *Rhagium bifasciatum* infected with EPN were
362 also found in deadwood 1-12 months after application of *S. carpocapsae* to stumps on an
363 operational, site-wide scale, but fewer than 10% of logs contained infected insects, and infected
364 insects represented less than 4% of the overall population sampled. Both *S. carpocapsae* and *H.*
365 *downesi* reproduced in *R. bifasciatum* larvae, so it is possible that some of the infection was as a
366 result of recycling within the logs. The number of logs with infected *R. bifasciatum*, and number
367 of infected longhorns per log declined significantly with increasing distance of logs from treated
368 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 & Day, 2001). Parasitism rates of pine weevil by this gregarious ectoparasitoid are typically in the range of 15 – 30 % (Dillon et al., 2008; 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 & 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 to 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 & Shapiro-Ilan, 2012)..

390 Tree stumps can harbour a large diversity of invertebrates, both in the decomposing wood and
391 bark, and in the soil around them (Wallace, 1953; Abrahamsson & Lindbladh, 2006; Hedgren,
392 2007). Since this is where EPN are applied (Dillon et al., 2008a), impacts on non-target insects
393 are most likely to occur in this area. When debarking tree stumps to record infection of pine
394 weevil after application of EPN, infected non-target insects (e.g. Elateridae) were occasionally
395 found (Harvey, Dillon, pers. obs.). To monitor effects of EPN on non-target Coleoptera, Dillon et
396 al. (2012) placed insect emergence traps over stumps treated with *S. carpocapsae* or *H. downesi*
397 and over untreated stumps. EPN did not affect species diversity, richness, abundance or
398 community composition, either in the year of application or one year later (Dillon et al., 2012).
399 In particular, EPN application had no significant effect on wood-associated species including the
400 abundant saproxylic cerambycid, *Asemum striatum* L. 1758 (Dillon et al., 2012). The authors
401 concluded that the impact on non-target Coleoptera in and around tree stumps is probably
402 negligible for the two species tested to date.

403 Based on the available data summarized here, direct non-target impacts of the EPN species
404 investigated are 'unlikely' when applied against pine weevil (likelihood = 2; Hickson, 2000; van
405 Lenteren et al., 2003) (Table 2). In addition, data for both wood debris-associated and stump-
406 associated non-target insects suggest mortality of these insects is < 5 % of the total available
407 non-target population on site (magnitude = 1; van Lenteren et al., 2003). These assessments,
408 while supported by the limited data available for some EPN species, should be considered
409 tentative until further experimental data become available, especially for species whose non-
410 target risks have not yet been studied in detail in forest ecosystems.

411

e) 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., 2002) 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 (Miller and Barbercheck, 2001; Duncan et al., 2003). For example, Millar and Barbercheck (2001) tested whether indigenous *S. carpocapsae* and *H. bacteriophora* 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 & Barbercheck 2001). *Steinernema feltiae* was 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), though *S. kraussei* has also been detected (Harvey, unpublished). While *S. carpocapsae* was detected for at least 2 years following application, 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

435 new colonisation of the sites. Dillon et al. (2008a) found that the exotic species *S. carpocapsae*
436 and *H. downesi* and the exotic strain *S. feltiae* EN02 did not displace native strain *S. feltiae*
437 4CFMO on Irish clear-fell sites treated for pine weevil control. When applying an exotic strain of
438 an indigenous species, there is a risk of introgression (Roderick & Navajas, 2003; Hopper et al.,
439 2006), but there was no evidence of hybridization between indigenous and applied strains of *S.*
440 *feltiae* (Dillon et al., 2008a). These findings suggest that indigenous EPN species are unlikely to
441 be displaced in the long term by exotics that are not adapted to the target environment (Grewal et
442 al., 1994), but tests on further EPN species that may be used in pine weevil suppression activities
443 should be considered as the next step in the assessment of indirect non-target effects.
444 As previously noted, inundatively applied EPN may have direct effects on the parasitoid *B.*
445 *hylobii* by killing various life stages. We also consider the possibility of competition between
446 nematodes and this parasitoid for pine weevil larvae. *Bracon hylobii* cannot develop to adulthood
447 on hosts that have been infected with EPN; females oviposited on healthy host larvae, but not on
448 larvae killed by *H. downesi* or *S. carpocapsae*, which should reduce the negative impact on the
449 parasitoid (Everard et al., 2009; Harvey & Griffin, 2012). Female *B. hylobii*, especially those
450 with prior experience, did parasitize live hosts infected with EPN, as long as they were still
451 moving (Everard et al., 2009; Harvey & Griffin, 2012). While this means there is a possibility of
452 competition between EPN and *B. hylobii* (modulated by wasp experience), complementary
453 (additive or synergistic) control effects by the two agents may also emerge (Harvey & Griffin,
454 2012). Dillon et al. (2008b) reported an additive effect of *H. downesi* and *S. carpocapsae* with *B.*
455 *hylobii* on mortality of pine weevil in stumps across three sites. Larger-scale and longer-term
456 monitoring of *B. hylobii* populations is necessary to draw more definite conclusions about
457 population-scale effects of competition between EPN and *B. hylobii*.

458 We estimate that indirect non-target effects of exotic EPN species and strains used for large pine
459 weevil control (i.e. *S. carpocapsae, S. feltiae* EN02 and *H. downesi*) are 'unlikely' (likelihood =
460 2; Hickson, 2000; van Lenteren et al., 2003) (Table 2), and we expect these exotics to have only
461 a 'minor' impact on non-target organisms (magnitude = 2; van Lenteren et al., 2003) (Table 2).
462 Furthermore, we consider indirect non-target impacts to be 'very unlikely' for the native *S.*
463 *feltiae* 4CFMO (likelihood = 1; Hickson, 2000; van Lenteren et al., 2003) as it is already a
464 natural component of coniferous forest soils in Ireland and thus inundative application should not
465 have a qualitative impact on the soil organism community. It should be stressed, however, that
466 these assessments are based on the different aspects of indirect non-target impact investigated for
467 each of the species and that results for one species are not necessarily representative of others.
468 While we have not included exact risk values for *H. megidis* and *S. kraussei*, the other two
469 species that have been tested against pine weevil and for which fewer data are available, we
470 estimate *H. megidis* to be similar to its close relative *H. downesi*, both being exotic to the habitat,
471 and *S. kraussei* to be similar to *S. feltiae*, both species being present in the target habitat.
472

473 Conclusions and risk evaluation

474 Both exotic and indigenous EPN trialled against the large pine weevil persisted in the soil for up
475 to four years after application (Dillon et al., 2008a; Harvey & Griffin, 2016), but the evidence
476 suggests that persistence was driven by recycling through the target pest as intended.
477 Consequently, EPN levels decreased to background levels (for an indigenous strain) or
478 undetectable levels (for exotic species/strains) along with the natural decrease in pest population
479 (Torr et al., 2007; Dillon et al., 2008a; Harvey & Griffin, 2016). Moreover, the exotic applied

480 strain of *S. feltiae* did not displace an indigenous strain (Dillon et al., 2008a). Active horizontal
481 dispersal appeared to be limited to a zone of less than 1 m from the point of application and,
482 while phoresis or some other long-range mechanism of dispersal resulted in movement of EPN
483 outside the treated areas, there is no evidence that they established there (Dillon et al., 2008a;
484 Harvey & Griffin, 2016). Direct non-target effects are limited by the targeted application of
485 exotic EPN (Harvey et al., 2012) and coleopteran communities around tree stumps were
486 unaffected by exotic EPN (Dillon et al., 2012). Moreover, while the parasitoid *B. hylobii* is
487 susceptible to infection by and competition with EPN, there is no indication that this negatively
488 impacts on *B. hylobii* parasitism in the field (Dillon et al., 2008b; Everard et al., 2009; Harvey &
489 Griffin, 2012). Thus, both exotic and indigenous EPN seem to be well-suited as a low-risk
490 alternative to chemical pesticides. While most of the risk assessment studies carried out in our
491 target forest ecosystem focussed on just two species, *S. carpocapsae* and *H. downesi*, we have
492 extrapolated our conclusions to *S. feltiae*. We feel this is acceptable, as *S. feltiae* as the species
493 indigenous to the system can be considered *a prior*i of low risk.

494 Current risk considerations and regulatory restrictions on exotics have resulted in a trend to
495 favour indigenous inundative control agents over exotic ones, reversing the past emphasis on use
496 of exotics (van Lenteren, 2012). The results presented here do not suggest that risk, as defined by
497 van Lenteren et al. (2003), is increased by using exotic species. In fact, using EPN that are not
498 well-adapted to the environment where they are applied might reduce the risk of long-term
499 establishment (Grewal at al., 1994). The indexing method devised by van Lenteren et al. (2003),
500 when applied strictly, is only valid for the environment and setting in which the risk for the
501 control agent has been evaluated. In the setting of large pine weevil control using EPN, we
502 estimate the risk index of the exotic *H. downesi* and *S. carpocapsae* to be 35, as also for the

503 exotic strain of *S. feltiae*, EN02 (Table 2). We arrived at a somewhat higher index value of 51 for
504 *S. feltiae* 4CFMO (native) in a forestry setting in Ireland (Table 2). The main risk category
505 contributing to the differences in indices is establishment; we assign higher scores to the native
506 Irish species *S. feltiae*, particularly the native strain 4CFMO, as it has the potential to persist for
507 longer in coniferous clear-fell soils after application (Dillon et al. 2008a). However, since this
508 species already occurs naturally in this ecosystem, in this case a higher risk index value does not
509 necessarily imply a greater environmental hazard due to application. If we take the establishment
510 risk of *S. feltiae* to be the less conservative 1, then its index value becomes 36. By comparison,
511 van Lenteren et al. (2003) assign an index value of 53 to *S. feltiae* when released in Finland
512 (where it is indigenous) in an open field environment. The slightly different indices between the
513 two studies for application of a native *S. feltiae* are accounted for by higher estimates for
514 establishment and dispersal, and lower estimates for direct and indirect non-target effects in our
515 system compared to that of van Lenteren et al.

516 Of course, no risk assessment can ever be complete and offer a guarantee of safety – risks and
517 benefits must therefore always be weighed in sensible proportion to each other (Clerq et al.,
518 2011; Simberloff, 2012). The pine weevil has been controlled in Ireland and elsewhere mainly by
519 applying chemical pesticide (most recently cypermethrin or α–cypermethrin) to replanted
520 seedlings before and/or after planting (e.g. Torstensson et al., 1999; Willoughby et al., 2004).
521 EPN, as part of an integrated pest management strategy, are intended to help replace
522 cypermethrin and α–cypermethrin as their use is phased out in the European Union under
523 sustainable forest management (SFM) policies. An extensive body of research investigating
524 environmental impacts of pyrethroid pesticides in forestry shows that they can affect a much
525 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 & 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 & 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 & 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.

Acknowledgments: CDH was funded by the EPA STRIVE programme (project 2007-PhD-B-6) and CDW was funded by INTERREG IVA (IMPACT Project), co-funded by the Department of Agriculture, Food and the Marine (DAFM). Field trials were conducted under licence from the Pesticide Control Service of DAFM.

545 Table 1: EPN species and strains for which risk assessment studies have been carried out in
546 relation to pine weevil suppression. For each species and strain, status (exotic or indigenous) is
547 given for Britain (Br) and Ireland (Irl) in general, and coniferous forest soils in these islands in
548 particular. Risk categories after van Lenteren et al. (2003) are E = establishment, D = dispersal,
549 DNT = direct non-target effects and INT = indirect n**o**n-target effects.

550

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

551

552 [1]References : [1] Blackshaw, 1988, [2] Hominick & Briscoe, 1990a; [3] Hominick & Briscoe, 1990b; [4]
553 Griffin et al., 1991; [5] Boag et al., 1992; [6] Griffin et al., 1994; [7] Hominick et al., 1995; [8] Gwynn &
554 Richardson,1996; [9] Chandler et al., 1997; [10] Griffin et al., 1999; [11] Hominick, 2002; [12] Dillon,
555 2003; [13] Harvey (unpublished data); [14] Torr et al., 1997; [15] Dillon et al., 2008a; [16] Dillon et al.,
556 2008b; [17] Everard et al., 2009; [18] Harvey et al., 2012; [19] Harvey & Griffin, 2012; [20] Dillon et al.,
557 2012; [21] Harvey & Griffin, 2016.

[2] *S. feltiae* is present in UK and Ireland, but strain EN02 originated in Germany (Dillon et al., 2008a).

[3] *H. megidis* has been found in Britain, but not Ireland

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 to 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					
		Establishment	Dispersal	Host range	Direct non-target effects	Indirect non-target effects	Risk index
S. carpocapsae	Likelihood	2	2	5	2	2	
	Magnitude	1	1	5	1	2	
	L x M	**2**	**2**	**25**	**2**	**4**	**35**
H. downesi	Likelihood	2	2	5	2	2	
	Magnitude	1	1	5	1	2	
	L x M	**2**	**2**	**25**	**2**	**4**	**35**
S. feltiae (EN02)	Likelihood	2	2	5	2	1	
	Magnitude	2	1	5	1	2	
	L x M	**4**	**2**	**25**	**2**	**2**	**35**
S. feltiae (4CFMO)	Likelihood	4	2	5	2	1	
	Magnitude	5	1	5	1	2	
	L x M	**20**	**2**	**25**	**2**	**2**	**51**
S. feltiae[1]	Likelihood	3	1	5	4	4	
	Magnitude	5	1	5	2	1	
	L x M	**15**	**1**	**25**	**8**	**4**	**53**

[1] 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.

584 References

585 Abrahamsson, M. & Lindbladh, M. J. (2006). A comparison of saproxylic beetle occurrence
586 between man-made high- and low-stumps of spruce (Picea abies). *Forest Ecology and*
587 *Management*, *226*(1-3), 230–237.

588 Anderson, R. L. (1982). Toxicity of Fenvalerate and Permethrin to Several Nontarget Aquatic
589 Invertebrates. *Environmental Entomology*, *11*, 1251–1257.

590 Babendreier, D., Bigler, F. & Kuhlmann, U. (2005). Methods used to assess non-target effects of
591 invertebrate biological control agents of arthropod pests. *BioControl*, *50*(6), 821–870.

592 Bale, J. S., van Lenteren, J. C. & Bigler, F. (2008). Biological control and sustainable food
593 production. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
594 *363*(1492), 761–776.

595 Barratt, B. I. P., Blossey, B. & Hokkanen, H. M. T. (2006). Post-release evaluation of non-target
596 effects of biological control agents. In: *Environmental impact of invertebrates for*
597 *biological control of Arthropods*, pp.166-186. Bigler, E., Babendreier, D. & Kuhlmann, U.
598 (Eds.). CABI International, Wallingford, UK.

599 Barratt, B. I. P., Howarth, F. G., Withers, T. M., Kean, J. M. & Ridley, G. S. (2010). Progress in
600 risk assessment for classical biological control. *Biological Control*, *52*(3), 245–254.

601 Bathon, H. (1996). Impact of entomopathogenic nematodes on non-target hosts. *Biocontrol*
602 *Science and Technology*, *6*(3), 421–434.

603 Battisti, A. (1994). Effects of entomopathogenic nematodes on the spruce web-spinning sawfly
604 *Cephalcia arvensis* Panzer and its parasitoids in the fields. *Biocontrol Science and*
605 *Technology*, *4* , 95–102.

606 Bengtsson, J., Nilsson, S. G., Franc, A. & Menozzi, P. (2000). Biodiversity, disturbances,
607 ecosystem function and management of European forests. *Forest Ecology and Management*,
608 *132*(1), 39–50.

609 Bigler, F., Bale, J. S., Cock, M. J. W., Dreyer, H., Greatrex, R., Kuhlmann, U., Loomans, A.J.M.
610 & van Lenteren, J. C. (2005). Guidelines on information requirements for import and
611 release of invertebrate biological control agents in European countries. *Biocontrol News and*
612 *Information*, *26*(4), 115N–123N.

613 Blackshaw, R. P. (1988). A survey of insect parasitic nematodes in Northern Ireland. *Annals of*
614 *Applied Biology*, *113*(3), 561–565.

615 Boag, B., Neilson, R. & Gordon, S. C. (1992). Distribution and prevalence of the
616 entomopathogenic nematode *Steinernema feltiae* in Scotland. *Annals of Applied Biology*,
617 *121*(2), 355–360.

618 Boivin, G., Kölliker-Ott, U. M., Bale, J. & Bigler, F. (2006). Assessing the establishment
619 potential of inundative biological control agents. In: *Environmental impact of invertebrates*
620 *for biological control of Arthropods*, pp. 98-113. Bigler, E., Babendreier, D. & Kuhlmann,
621 U. (Eds.). CABI International, Wallingford, UK.
622
623 Brixey, J. M., Moore, R. & Milner, A. D. (2006). Effect of entomopathogenic nematode
624 (*Steinernema carpocapsae* Weiser) application technique on the efficacy and distribution of
625 infection of the large pine weevil (*Hylobius abietis* L.) in stumps of Sitka spruce (*Picea*
626 *sitchensis* Carr.) created at different times. *Forest Ecology and Management*, *226* (1-3),
627 161–172.

628 Brockerhoff, E., Jactel, H., Parrotta, J., Quine, C. & Sayer, J. (2008). Plantation forests and
629 biodiversity: oxymoron or opportunity? *Biodiversity and Conservation, 17*(5), 925–951.

630 Caltagirone, L. E. (1981). Landmark examples in classical biological control. *Annual Review of*
631 *Entomology*, *26*(1), 213–232.

632 Campos-Herrera, R., Trigo, D. & Gutiérrez, C. (2006) Phoresy of the entomopathogenic
633 nematode *Steinernema feltiae* by the earthworm *Eisenia fetida*. *Journal of Invertebrate*
634 *Pathology, 92*, 50–54.

635 Chandler, D., Hay, D. & Reid, A. P. (1997). Sampling and occurrence of entomopathogenic
636 fungi and nematodes in UK soils. *Applied Soil Ecology*, *5*(2), 133–141.

637 Clercq, P., Mason, P. & Babendreier, D. (2011). Benefits and risks of exotic biological control
638 agents. *BioControl*, *56*(4), 681–698.

639 Cock, M. J. W., Lenteren, J. C., Brodeur, J., Barratt, B. I. P., Bigler, F., Bolckmans, K., Consoli,
640 F.L., Haas, F., Mason, P.G. & Parra, J.R.P., Parra, J. R. P. (2010). Do new access and
641 benefit sharing procedures under the convention on biological diversity threaten the future
642 of biological control? *BioControl*, *55*(2), 199–218.

643 Collier, T. & Van Steenwyk, R. (2004). A critical evaluation of augmentative biological control.
644 *Biological Control*, *31*(2), 245–256.

645 DeBach P. & Rosen D. (1991). Biological control by natural enemies. 2nd edition, 440 pp.,
646 Cambridge University Press, Cambridge, England.

DeLorenzo, M.E. & Fulton, M.H. (2012). Comparative risk assessment of permethrin, chlorothalonil, and diuron to coastal aquatic species. *Marine Pollution Bulletin*, 64(7):1291–9.

De Nardo, E. A. B., Grewal, P. S., McCartney, D. & Stinner, B. R. (2006). Non-target effects of entomopathogenic nematodes on soil microbial community and nutrient cycling processes: A microcosm study. *Applied Soil Ecology*, *34*(2-3), 250–257

Dillon, A.B. (2003). Biological control of the large pine weevil, *Hylobius abietis* L., (Coleoptera: Curculionidae) using entomopathogenic nematodes. PhD thesis submitted at NUI Maynooth, Ireland.

Dillon, Aoife B, Ward, D., Downes, M. J., & Griffin, C. T. (2006). Suppression of the large pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes with different foraging strategies. *Biological Control*, *38* (2), 217–226.

Dillon, A B, Rolston, A. N., Meade, C. V, Downes, M. J. & Griffin, C. T. (2008a). Establishment, persistence, and introgression of entomopathogenic nematodes in a forest ecosystem. *Ecological Applications*, *18*(3), 735–747.

Dillon, Aoife B, Moore, C. P., Downes, M. J. & Griffin, C. T. (2008b). Evict or infect? Managing populations of the large pine weevil, *Hylobius abietis*, using a bottom-up and top-down approach. *Forest Ecology and Management*, *255*(7), 2634–2642.

Dillon, A. B., Foster, A., Williams, C. D., & Griffin, C. T. (2012). Environmental safety of entomopathogenic nematodes – Effects on abundance, diversity and community structure of non-target beetles in a forest ecosystem. *Biological Control*, *63*(2), 107–114.

Downes, M. J. & Griffin, C. T. (1996). Dispersal behaviour and transmission strategies of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema. Biocontrol Science and Technology*, *6* (3), 347–356.

Duffy E.A.J. (1953). A Monograph of the immature stages of British and imported timber beetles (Cerambycidae). 350 p., British Museum (Natural History), London.

Duncan, L. W., Graham, J. H., Dunn, D. C., Zellers, J., McCoy, C. W., & Nguyen, K. (2003). Incidence of endemic entomopathogenic nematodes following application of *Steinernema riobrave* for control of *Diaprepes abbreviatus*. *Journal of Nematology*, *35*(2), 178–186.

Ehlers, R.-U. & Hokkanen, H. M. T. (1996). Insect biocontrol with non-endemic entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.): Conclusions and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. *Biocontrol Science and Technology*, *6*(3), 295–302.

Eng, M. S., Preisser, E. L. & Strong, D. R. (2005) Phoresy of the entomopathogenic nematode Heterorhabditis marelatus by a non-host organism, the isopod *Porcellio scaber*. *Journal of Invertebrate Pathology*, *88*, 173–176.

Ennis, D. E., Dillon, A. B. & Griffin, C. T. (2012). Simulated roots and host feeding enhance infection of subterranean insects by the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Invertebrate Pathology*, *103*(2), 140–143.

Evans, H. Moore, R., Heritage, S. & Wainhouse D. (2004). Developments in the integrated management of pine weevil, a pest of restocking in conifer plantations. *Forest Research Annual Reports and Accounts* 2003-2004. Forestry Commission, England.

Everard, A., Griffin, C. T. & Dillon, A. B. (2009). Competition and intraguild predation between the braconid parasitoid *Bracon hylobii* and the entomopathogenic nematode *Heterorhabditis downesi*, natural enemies of the large pine weevil, *Hylobius abietis. Bulletin of Entomological Research*, *99*(02), 151–161.

Ferron, P. (1978). Biological control of insect pests by entomogenous fungi. *Annual Review of Entomology 23*:409-442.

Forst, S., Dowds, B., Boemare, N. & Stackebrandt, E. (1997). *Xenorhabdus* and *Photorhabdus* spp.: Bugs that kill bugs. *Annual Review of Microbiology*, *51*(1), 47–72.

Gaugler, R., Campbell, J. F., Selvan, S. & Lewis, E. E. (1992). Large-scale inoculative releases of the entomopathogenic nematode *Steinernema glaseri*: Assessment 50 years later. *Biological Control*, *2*(3), 181–187.

Georgis, R. & Hague, N.G.M. (1979). A steinernematid nematode in the web-spinning larch sawfly, *Cephalcia lariciphila* (Wachtl). *Plant Pathology*, *28*, 98-99.

Georgis, R, & Hague, N.G.M. (1981) A neoaplectanid nematode in the larch sawfly *Cephalcia lariciphila* (Hymenoptera : Pamphiliidae). *Annals of Applied Biology 99*, 171-177.

Georgis, R., Koppenhöfer, A. M., Lacey, L. A., Bélair, G., Duncan, L. W., Grewal, P. S., Samish, M., Tan, L., Torr, P. & van Tol, R. W. H. M. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, *38*(1), 103–123.

Glazer, I. (1996). Survival mechanisms of entomopathogenic nematodes. *Biocontrol Science and Technology*, *6*(3), 373–378.

Grewal, P. S., Selvan, S. & Gaugler, R. (1994). Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology*, *19*(4), 245–253.

Grewal, P. S., Wang, X. & Taylor, R. A. J. (2002). Dauer juvenile longevity and stress tolerance in natural populations of entomopathogenic nematodes: is there a relationship? *International Journal for Parasitology*, *32*(6), 717–725.

Grewal, P.S. (2012). Entomopathogenic nematodes as tools in integrated pest management. In: *Integrated Pest Management: Principles and Practice,* p. 162-236. Dharam, P. A., Shankar, U. (Eds.). CABI International, Wallingford, UK.

Griffin, C T, Moore, J. F. & Downes, M. J. (1991). Occurrence of Insect-Parasitic Nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. *Nematologica*, *37*, 92–100.

Griffin, C. T., Joyce, S. A., Dix, I., Burnell, A. M. & Downes, M. J. (1994). Characterisation of the entomopathogenic nematode *Heterorhabditis* (Nematoda: Heterorhabditidae) from Ireland and Britain by molecular and cross-breeding techniques, and the occurrence of the genus in these islands. *Fundamental and applied nematology*, *17*(3), 245–253.

Griffin, Christine T, Dix, I., Joyce, S. A., Burnell, A. M. & Downes, M. J. (1999). Isolation and characterisation of *Heterorhabditis* spp. (Nematoda: Heterorhabditidae) from Hungary, Estonia and Denmark. *Nematology*, *1*, 321–332.

Griffin, C. T. (2012). Perspectives on the behavior of entomopathogenic nematodes from dispersal to reproduction: traits contributing to nematode fitness and biocontrol efficacy. *Journal of nematology*, *44*(2), 177–184.

Griffin, C. T. (2015). Behaviour and population dynamics of entomopathogenic nematodes following application. In: *Nematode pathogenesis of insects and other pests—ecology and applied technologies for sustainable plant and crop protection*, p. 57–95. Campos-Herrera, R., (Ed.). Springer, Berlin.

Grove, S. J. (2002). Saproxylic insect ecology and the sustainable management of forests. *Annual Review of Ecology and Systematics*, *33* (1), 1–23.

Gwynn, R. L. & Richardson, P. N. (1996). Incidence of entomopathogenic nematodes in soil samples collected from Scotland, England and Wales. *Fundam. appl. Nematol.*, *19*(5), 427–431.

Hajek, A. E., Hurley, B. P., Kenis, M., Garnas J. R., Bush, S. J., Wingfield, M. J., van Lenteren, J. C. & Cock, M. J. W. (2016). Exotic biological control agents: a solution or contribution to arthropod invasions? *Biological Invasions, 18*: 953 – 969

Harvey, C. D., Alameen, K. M. & Griffin, C. T. (2012). The impact of entomopathogenic nematodes on a non-target, service-providing longhorn beetle is limited by targeted

746 application when controlling forestry pest *Hylobius abietis*. *Biological Control*, *62*(3), 173–
747 182.

748 Harvey, C. D., & Griffin, C. T. (2012). Host activity and wasp experience affect parasitoid wasp
749 foraging behaviour and oviposition on nematode-infected larvae of the forestry pest
750 *Hylobius abietis*. *Ecological Entomology*, *37*(4), 269–282.

751 Harvey, C. D., & Griffin, C. T. (2016). Local host-dependent persistence of the
752 entomopathogenic nematode *Steinernema carpocapsae* used to control the large pine
753 weevil *Hylobius abietis*. *BioControl*, 61(2), 185-193.

754 Hedgren, P. O. (2007). Early arriving saproxylic beetles (Coleoptera) and parasitoids
755 (Hymenoptera) in low and high stumps of Norway spruce. *Forest Ecology and*
756 *Management*, 241(1-3), 155–161.

757 Henry, C. J. (1995). The effect of a braconid ectoparasitoid, *Bracon hylobii* Ratz. on larval
758 populations of the large pine weevil (*Hylobius aboietis* L.). PhD thesis submitted at the
759 University of Ulster, Coleraine.

760 Henry, C. J. & Day, K. R. (2001). Egg allocation by *Bracon hylobii* Ratz., the principal
761 parasitoid of the large pine weevil (*Hylobius abietis* L.), and implications for host
762 suppression. *Agricultural and Forest Entomology*, *3* (1), 11–18.

763 Heritage, S., Collins, S. & Evans, H. F. (1989). A survey of damage by *Hylobius abietis* and
764 *Hylastes* spp. in Britain. Forestry Canada (Pacific and Yukon region), Victoria, Canada:
765 28-33.
766
767 Hickson, R., Moeed, A. and Hannah, D. (2000). HSNO, ERMA and risk management.
768 *New Zealand Science Review* 57: 72–77.

769 Hodson, A. K., Siegel, J. P. & Lewis, E. E. (2012). Ecological influence of the
770 entomopathogenic nematode, *Steinernema carpocapsae*, on pistachio orchard soil
771 arthropods. *Pedobiologia*, *55*(1), 51–58.

772 Hokkanen, H. M. T. & Sailer, R. I. (1985). Success in classical biological control. *Critical*
773 *Reviews in Plant Sciences*, *3*(1), 35–72.

774 Hokkanen, H. M. T., Lynch J.M., & Robinson, J. (1995). Preface: overview of benefits and risks
775 of biological control introductions. In: *Biological Control: Benefits and Risks*, p. 17-22.
776 Hokkanen, H. M. T. & Lynch J.M. (Eds.). Cambridge University Press, Cambridge, UK.
777
778 Hopper, K. R., Britch, S. C., Wajnberg, E. (2006). Risks of interbreeding between species used in
779 biological control and native species, and methods for evaluating their occurrence and
780 impact. In: *Environmental impact of invertebrates for biological control of arthropods*,

781 p.78-97. Bigler, E., Babendreier, D. and Kuhlmann, U. (Eds.). CABI Publishing,
782 Wallingford, UK.

783 Hominick, W. M. & Briscoe, B. R. (1990a). Survey of 15 sites over 28 months for
784 entomopathogenic nematodes (Rhabditida: Steinernematidae). *Parasitology*, *100*(02), 289–
785 294.

786 Hominick, W. M. & Briscoe, B. R. (1990b). Occurrence of entomopathogenic nematodes
787 (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. *Parasitology*, *100*,
788 295–302.

789 Hominick, W. M., Reid, A. P. & Briscoe, B. R. (1995). Prevalence and habitat specificity of
790 steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and
791 the Netherlands. *Journal of Helminthology*, *69*(01), 27–32.

792 Hominick, W. M. (2002). Biogeography. *Entomopathogenic Nematology*, p. 115-145.
793 Gaugler, R. (Ed.), CABI publishing, Wallingford, England.

794 Inward, D.J.G., Wainhouse, D. and Peace, A. 2012. The effect of temperature on the
795 development and life cycle regulation of the pine weevil *Hylobius abietis* and the potential
796 impacts of climate change. *Agricultural and Forest Entomology* 14: 348-357.

797 Irwin, S., Pedley, S., Coote, L., Dietzsch, A., Wilson, M., Oxbrough, A., Sweeney, O., Moore, K.
798 M., Martin, R., Kelly, D. L., Mitchell, F. J. G., Kelly, T. C. & O'Halloran, J. (2014). The
799 value of plantation forests for plant, invertebrate and bird diversity and the potential for
800 cross-taxon surrogacy. *Biodiversity and Conservation*, *23*(3), 697–714.

801 Jabbour, R. & Barbercheck, M. E. (2008). Soil and habitat complexity effects on movement of
802 the entomopathogenic nematode *Steinernema carpocapsae* in maize. *Biological Control*,
803 *47*(2), 235–243.

804 Jansson, R. K. (1993). Introduction of exotic entomopathogenic nematodes (Rhabditida:
805 Heterorhabditidae and Steinernematidae) for biological control of insects: potential and
806 problems. *The Florida Entomologist*, *76*(1), 82–96.

807 Jonsell, M., Hansson, J. & Wedmo, L. (2007). Diversity of saproxylic beetle species in logging
808 residues in Sweden - Comparisons between tree species and diameters. *Biological*
809 *Conservation*, *138*(1-2), 89–99.

810 Kaya, H. K. & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of*
811 *Entomology*, *38*(1), 181–206.

812 Kingsbury, P. D. & Kreutzweiser, D. P. (1987). Permethrin treatments in canadian forests. Part 1: Impact
813 on stream fish. *Pesticide Science*, *19*(1), 35–48.

814 Klein, M. G. & Georgisi, R. (1992). Persistence of control of Japanese beetle (Coleoptera:
815 Scarabaeidae) larvae with Steinernematid and Heterorhabditid lematodes. *Journal of*
816 *Economic Entomology*, *85*, 727–730.

817 Koppenhofer, A. M., & Fuzy, E. M. J. (2006). Effect of soil type on infectivity and persistence of
818 the entomopathogenic nematodes *Steinernema scarabaei, Steinernema glaseri,*
819 *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. *Journal of Invertebrate*
820 *Pathology*, *92* (1), 11–22.

821 Kreutzweiser, D. P., & Kingsbury, P. D. (1987). Permethrin treatments in canadian forests. Part
822 2: Impact on stream invertebrates. *Pesticide Science*, *19*(1), 49–60.

823 Kruitbos, L.M., Heritage, S., Wilson, M.J. (2009). Phoretic dispersal of entomopathogenic
824 nematodes by *Hylobius abietis*. Nematology 11, 419–427.

825 Kruitbos, L. M., Heritage, S., Hapca, S.,& Wilson, M. J. (2010). The influence of habitat quality
826 on the foraging strategies of the entomopathogenic nematodes *Steinernema carpocapsae*
827 and *Heterorhabditis megidis. Parasitology*, *137*(02), 303–309.

828 Kurtz, B., Toepfer, S., Ehlers, R.-U., Kuhlmann, U. (2007). Assessment of establishment and
829 persistence of entomopathogenic nematodes for biological control of western corn
830 rootworm. *Journal of Applied Entomology*, *131*(6), 420–425.

831 Lacey, L A, Kaya, H. K. & Bettencourt, R. (1995). Dispersal of *Steinernema glaseri* (Nematoda:
832 Steinernematidae) in adult Japanese beetles, *Popillia japonica* (Coleoptera: Scarabaeidae).
833 *Biocontrol Science and Technology*, *5*(1), 121–130.

834 Lacey, L. & Goettel, M. (1995). Current developments in microbial control of insect pests and
835 prospects for the early 21st century. *BioControl*, *40*(1), 3–27.

836 Lacey, L A, Frutos, R., Kaya, H. K. & Vail, P. (2001). Insect pathogens as biological control
837 agents: do they have a future? *Biological Control*, *21*(3), 230–248.

838 Lacey, Lawrence A, Unruh, T. R. & Headrick, H. L. (2003). Interactions of two idiobiont
839 parasitoids (Hymenoptera: Ichneumonidae) of codling moth (Lepidoptera: Tortricidae) with
840 the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae).
841 *J Invertebr Pathol*, *83* (3), 230–239.

842 Laengle, T. & Strasser, H. (2010). Developing a risk indicator to comparatively assess
843 environmental risks posed by microbial and conventional pest control agents. *Biocontrol*
844 *Science and Technology*, *20* (7), 659–681.
845 Långström, B. & Day, K. R. (2004). Damage, control and management of weevil pests,
846 especially *Hylobius abietis*. In: *Bark and wood boring insects in living trees in Europe, a*
847 *synthesis*, p. 415-444. Lieutier, F., Day, K. R., Battisti, A., Grégoire, J-C., Evans, H. F.
848 (Eds.). Springer, Dordrecht, Netherlands.

Leather, S. R., Day, K. R. & Salisbury, A. N. (1999). The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal? *Bulletin of Entomological Research*, *89* (01), 3–16.

Lewis, E. E., Campbell, J., Griffin, C., Kaya, H. & Peters, A. J. (2006). Behavioral ecology of entomopathogenic nematodes. *Biological Control*, *38* (1), 66–79.

Losey, J.E. & Vaughan, M. (2006). The economic value of ecological services provided by insects. *BioScience*, 56 (4), 311-323.

Louda, S. M., Pemberton, R. W., Johnson, M. T. & Follett, P. A. (2003). Nontarget effects: the Achilles' heel of biological control? Retrospective Analyses to Reduce Risk Associated with Biocontrol Introductions. *Annual Review of Entomology*, *48*(1), 365–396.

Mbata, G. N. & Shapiro-Ilan, D. I. (2012). Compatibility of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) and *Habrobracon hebetor* (Hymenoptera: Braconidae) for biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae). *Biological Control*, *54*(2), 75–82.

McLeesc, D., Metcalfe, C., & Zitko, V. (1980). Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp. *Bulletin of Environmental Contamination and Toxicology*, *25*(1), 950–955.

Meyling, N. V. & Eilenberg, J. (2007). Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control*, *43*(2), 145–155.

Mills, N.J., Babendreier, D. & Loomans, A.J.M. (2006). Methods for monitoring the dispersal of natural enemies from point source releases associated with augmentative biological control. In: *Environmental impact of invertebrates for biological control of Arthropods*, p. 114-131. Bigler, E., Babendreier, D. and Kuhlmann, U. (Eds.). CABI Publishing, Wallingford, UK.

Millar, L. C. & Barbercheck, M. E. (2001). Interaction between endemic and introduced entomopathogenic nematodes in conventional-till and no-till corn. *Biological Control*, *22*(3), 235–245.

Nowell, D. C. & Maynard,G. V. (2005). International guidelines for the export, shipment, import and release of biological control agents and other beneficial organisims (ISPM No. 3). In: *Proceedings, 2nd International Symposium of Biological Control of Arthropods*, 12-16 September 2005, Davos, Switzerland. Hoddle, M. S., Ed.

OECD (2003). Guidance for Registration Requirements for Microbial Pesticides. *OECD Series on Pesticides, 18.* OECD Publications Service, Paris, France.

Parkman, J. P., Frank, J. H., Nguyen, K. B. & Smart, G. C. (1993). Dispersal of *Steinernema scapterisci* (Rhabditida: Steinernematidae) after Inoculative Applications for Mole Cricket (Orthoptera: Gryllotalpidae) Control in Pastures. *Biological Control*, *3*(3), 226–232.

Parkman, J. P., Frank, J. H., Walker, T. J. & Schuster, D. J. (1996) Classical biological control of *Scapteriscus* spp. (Orthoptera: Gryllotalpidae) in Florida. *Environmental Entomology*, *25*(6), 1415–1420.

Parkman, J. P. & Smart, G. C. (1996). Entomopathogenic nematodes, a case study: Introduction of *Steinernema scapterisci* in Florida. *Biocontrol Science and Technology*, *6*(3), 413–420.

Peters, A. (1996). the natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. *Biocontrol Science and Technology*, *6*(3), 389–402.

Petersson, M., Örlander, G. & Nordlander, G. (2005). Soil features affecting damage to conifer seedlings by the pine weevil *Hylobius abietis*. *Forestry*, *78*, 83–92.

Poinar, G. O. (1979). *Nematodes for biological control of insects*. CRC Press, Inc., 1979.

Poinar, G. O. & Hom, A. (1986). Survival and horizontal movement of infective stage *Neoaplectana carpocapsae* in the field. *Journal of Nematology*, *18*(1), 34–36.

Pye, A. E. & Burman, M. (1978). *Neoaplectana carpocapsae*: Infection and reproduction in large pine weevil larvae, *Hylobius abietis*. *Experimental Parasitology*, *46*(1), 1–11.

Ricciardi, A. & Cohen, J. (2007). The invasiveness of an introduced species does not predict its impact. *Biological Invasions*, *9*(3), 309–315.

Roderick, G.K. & Navajas, M. (2003). Genes in new environments: genetics and evolution in biological control. *Nature Reviews Genetics*, 4(11):889–99.

Rosenheim, J. A., Kaya, H. K., Ehler, L. E., Marois, J. J. & Jaffee, B. A. (1995). Intraguild predation among biological-control agents: theory and evidence. *Biological Control*, *5*(3), 303–335.

Shapiro-Ilan, D. I., Gouge, D. H., Piggott, S. J. & Fife, J. P. (2006). Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control*, *38*(1), 124–133.

Shields, E. J., Testa, A., Miller, J. M. & Flanders, K. L. (1999). Field efficacy and persistence of the entomopathogenic nematodes *Heterorhabditis bacteriophora* Oswego and *H. bacteriophora* NC on Alfalfa Snout Beetle larvae (Coleoptera: Curculionidae). *Environmental Entomology*, *28*, 128–136.

914 Simberloff, D. & Stiling, P. (1996). Risks of species introduced for biological control. *Biological*
915 *Conservation*, *78*, 185–192.

916 Simberloff, Daniel. (2012). Risks of biological control for conservation purposes. *BioControl*,
917 *57*(2), 263–276.

918 Simon, J. G. (2002). Saproxylic insect ecology and the sustainable management of forests.
919 *Annual Review of Ecology and Systematics*, *33*, 1–23.

920 Sippola, A. L., Siitonen, J. & Punttila, P. (2002). Beetle diversity in timberline forests: a
921 comparison between old-growth and regeneration areas in Finnish Lapland. *Ann. Zool.*
922 *Fennici*, *39* , 69–86.

923 Smits, P. H. (1996). Post-application persistence of entomopathogenic nematodes. *Biocontrol*
924 *Science and Technology*, *6*(3), 379–388.

925 Somasekhar, N., Grewal, P. S., De Nardo, E. A. B. & Stinner, B. R. (2002). Non-target effects of
926 entomopathogenic nematodes on the soil nematode community. *Journal of Applied*
927 *Ecology*, *39*, 735–744.

928 Speight, M.C.D. (1989). Saproxylic invertebrates and their conservation. *Nature and*
929 *Environment Series.* Council of Europe, Strasbourg (France), 79 pp.

930 Stiling, P. (1993). Why do natural enemies fail in classical biological control programs.
931 *American Entomologist*, *39*, 31–37.

932 Stuart, R. J. & Gaugler, R. (1994). Patchiness in populations of entomopathogenic nematodes.
933 *Journal of Invertebrate Pathology*, *64*(1), 39–45.

934 Stuart, R. J., Barbercheck, M. E., Grewal, P. S., Taylor, R. A. J. & Hoy, C. W. (2006).
935 Population biology of entomopathogenic nematodes: Concepts, issues, and models.
936 *Biological Control* 38: 80–102.

937 Susurluk, A. & Ehlers, R.-U. (2008). Field persistence of the entomopathogenic nematode
938 *Heterorhabditis bacteriophora* in different crops. *BioControl*, *53*(4), 627–641.

939 Torr, P., Heritage, S. & Wilson, M. J. (2007). *Steinernema kraussei*, an indigenous nematode
940 found in coniferous forests: efficacy and field persistence against *Hylobius abietis*.
941 *Agricultural and Forest Entomology*, *9*(3), 181–188.

942 Torstensson, L., Börjesson, E., & Arvidsson, B. (1999). Treatment of bare root spruce seedlings
943 with permethrin against pine weevil before lifting. *Scandinavian Journal of Forest*
944 *Research*, *14*, 408–415

945 Twinn , P. F. G. & Harding, P .T. (1999). Provisional atlas of the longhorn beetles

(Coleoptera, Cerambycidae) of Britain. Biological Records Centre, Huntingdon, UK.

Van Driesche, R. G., Carruthers, R. I., Center, T., Hoddle, M. S., Hough-Goldstein, J., Morin, L. (2010). Classical biological control for the protection of natural ecosystems. *Biological Control*, *54*(Supplement 1), S2–S33.

Van Lenteren, J. C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H. M. T., Kuske, S. (2003). Environmental risk assessment of exotic natural enemies used in inundative biological control. *BioControl*, *48*, 3–38.

Van Lenteren. J.C., Bale, J., Bigler, F., Hokkanen, H.M.T., Loomans, A.J.M. (2005). Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology*, 51(1):609–34.

Van Lenteren, J. (2012). The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, *57*(1), 1–20.

Vincent, C., Goettel, M. S. & Lazarovits, G. (Eds.). (2007). *Biological Control: A Global Perspective: Case Studies from Around the World*. Cabi International, Wallingford, UK.

Waage, J. K. & Hassell, M. P. (1982). Parasitoids as biological control agents? A fundamental approach. *Parasitology*, *84*(04), 241–268.

Waage, J. K., Greathead, D. J., Brown, R., Paterson, R. R. M., Haskell, P. T., Cook, R. J. & Krishnaiah, K. (1988). Biological control: challenges and opportunities [and discussion]. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* , *318* (1189), 111–128.

Wainhouse, D., Inward, D.J.G. and Morgan, G. 2014. Modelling geographical variation in voltinism of Hylobius abietis under climate change and implications for management. *Agricultural and Forest Entomology* 16: 136-146.

Wallace, H. R. (1953). The ecology of the insect fauna of pine stumps. *Journal of Animal Ecology*, *22*(1), 154–171.

Williams, C. D., Dillon, A. B., Girling, R. D. & Griffin, C. T. (2013). Organic soils promote the efficacy of entomopathogenic nematodes, with different foraging strategies, in the control of a major forest pest: A meta-analysis of field trial data. *Biological Control*, *65*(3), 357–364.

Williams, C. D., Dillon, A. B., Harvey, C. D., Hennessy, R., Namara, L. M. & Griffin, C. T. (2013). Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic nematodes and fungi using eradicant and prophylactic strategies. *Forest Ecology and Management*, *305*(0), 212–222.

Willoughby, I., Evans, H., Gibbs, J., Pepper, H., Gregory, S., Dewar, J., Nisbet, T., Pratt, J., McKay, H., Siddons, R., Mayle, B., Heritage, S., Ferris, R. & Trout, R. (2004). Reducing pesticide use in forestry—practical guide. The Forestry Commission, pp. 25–29

Wilson, M. J., Ehlers, R.-U. & Glazer, I. (2012). Entomopathogenic nematode foraging strategies - is *Steinernema carpocapsae* really an ambush forager? *Nematology*, *14*(4), 389–394.

Wright, R. J., Witkowski, J. F., Echtenkamp, G. & Georgis, R. (1993). Efficacy and persistence of *Steinernema carpocapsae* (Rhabditida: Steinemematidae) applied through a center-pivot irrigation system against larval com rootworms (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, *86*, 1348–1354.