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FOREST APPLICATIONS OF ENTOMOPATHOGENIC NEMATODES

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1. ABSTRACT

The application of entomopathogenic nematodes (EPNs) to the control of insect pests of trees in the broadest sense is reviewed. First considered are pests of trees outside of woodlands and forests, typically in urban environments. Next, pests of deciduous forests and their control is dealt with before moving on to pests of coniferous forests. It should be noted that out of the 99 serious pests of forestry identified by Day and Leather (1997) only 16 (16.2%) have had any research on applications of EPNs for their control. These examples and others are presented in this chapter as well as more broadly addressing the challenges faced in controlling these pests. Finally, a case study of the control of the most serious pest of forestry in Europe, *Hylobius abietis* is presented, which takes account of life cycle, current control methods, factors affecting efficacy of EPNs, environmental safety of EPNs and interaction with other biological control agents.

2. INTRODUCTION

There has been much basic and applied research on entomopathogenic nematodes (EPNs) since the publication of the first major edited volume on all aspects of their biology (Gaugler and Kaya, 1990). Forest applications are no exception to this trend, and it is the aim of this chapter to consider forestry in its widest sense and to show how continuous research on the application of EPNs to the control of pests of trees and forests is making advances towards more sustainable forest management systems throughout the world.

In this chapter I will first consider EPN applications to tree pests outside of woodlands and forests. I will then consider pests in deciduous forests and then pests of coniferous forests. Finally, I will consider, as a case study, the application of EPNs in the control of the large pine weevil (*Hylobius abietis*) – the most serious insect pest of clear fell forest restocking in Europe. Throughout the chapter I will highlight not only where there has been successful application of EPNs to forest pests, but also where there is potential to apply EPN to pests that have not currently been explored in any detail. There will inevitably be some cross-over between other chapters in the present volume, most notably between the chapter on orchard applications of EPN, the chapter on behavioural ecology and the chapter on population dynamics. It is hoped, however, that such cross-over will enhance the integration of the chapter in the volume as a whole and not be an unwanted distraction to the reader.

Biological control can take one of four forms. The first, and least intrusive, is so-called "conservation biocontrol". In this method habitats and parts of habitats are modified to promote natural enemies of the pest that are already resident in the ecosystem. The populations of such natural ecosystem service providers may be boosted by appropriate management. One of the most successful examples of this method has been the establishment of "beetle banks" in tillage field systems. The fields are split with strips of perennial grasses, which are sown to provide overwintering habitats for insect natural enemies of agricultural pests. Another type of biocontrol is "augmentative biological control". In this system, native populations of natural enemies are supplemented by release of additional individuals of the same species. Release of predatory mites and parasitoids under glasshouse conditions have been a successful application of this method. Classical biological control involves the inoculative release of non-native natural enemies of pest species into a geographical area where the agent would not naturally occur. Although there have been examples of major environmental impacts, e.g., the displacement and local extinction of native ladybirds following the escape of *Harmonia axyridis* from glasshouses, when adequate screening and impacts are assessed the method can be very effective e.g. the control of a non-native psyllid pest of Eucalyptus plantations in south west Ireland, through the release of an exotic parasitoid has been both effective and environmentally benign. Also, the release of the predator *Rhizophagus grandis* of the bark beetle pest *Dendroctonus micans* has been a major success in the UK. The final type of biological control, and the method dealt with in the research described in the present chapter is that of "inundative biological control" or "biopesticides". For this method, the aim is not to establish a population of control agents in the ecosystem, but to rather apply vast numbers of agents that will knock-down the pest at the critical time and then no longer persist in the environment. Entomopathogenic nematodes and fungi (EPF) are prime examples of organisms used in inundative biological control. With that said EPNs have been used as classical (inoculative) biological control agents e.g., in the control of mole crickets (Gryllotalpidae) by *Steinernema scapterisci* (Parkman et al., 1996).

It is instructive to consider EPN applications to the 99 major pests of forestry in Europe identified by Day and Leather (1997) for two reasons. Firstly, Europe has one of the best-known insect faunas in the world and knowledge of the pest status of insects there is probably higher than in any other part of the world. Secondly, Europe is a major producer of EPN products and their likelihood of uptake is high. Nevertheless, out of the 99 serious pests of forestry identified by Day and Leather (1997) only 16 (16.2%) have had any research on applications of EPNs for their control and in some cases this has been restricted to identification of natural nematode infection (Table 25.1). We may, therefore, tentatively, conclude that there remains a huge opportunity to apply EPN to these, and other serious forest pests.

3. EPN APPLICATIONS TO TREE PESTS OUTSIDE OF WOODLANDS AND FORESTS

Trees do not just occur in woodlands and forests. A large proportion of trees are found in urban environments – in parks, gardens and as street trees. These trees have great amenity value and provide many ecosystem services for people who live and share their urban environment with them (Livesley et al., 2016). These urban trees are just as susceptible to pests and pathogens as trees that are found in woodlands and forestry. It has been suggested that urban trees could act as sentinels for emerging pests and pathogens in a country (Hugh Evans, *Pers Comm*.). This, combined with an active citizen scientist programme of surveillance (Pocock et al., 2020) could be important in early reporting of exotic non-native serious pests and pathogens of woody plants in a country. As an example, of the increasing pests and pathogens on a national scale, O'Hanlon et al. (2021) document the establishment of non-native pests of trees on the island of Ireland showing that even in a country with a modest forestry estate, protected zone status for many pests and a relatively isolated biogeographical area, invasive species are a serious problem, which is only getting worse.

There has been some uptake of EPN applications to urban tree protection, particularly in China. Wang and Li (2017) reviewed the status of EPNs in integrated pest management in China. In a subsection of their chapter, they considered the operational application of EPNs in street trees. They note that Carpenter Worm (*Holcocerus insularis*) – a serious pest that attacks ash (*Fraxinus pennsylvannica*), the Chinese Scholar Tree (*Sophora japonica*) and Willows (*Salix* spp.) mostly in Northern China – have been treated at an operational level by the injection of a *Steinernema feltiae* suspension into boring tunnels left by the pest. Yang and Zhang (1990) report 97% larval mortality following such treatment. Wang and Li (2017) note that several other cities in the region used *Steinernema carpocapsae* with similar success. Citrus and Asian long horn beetles (*Anoplophora chinensis* and *Anoplophora glabripennis*, respectively) are serious boring pests which pose a threat to European forestry – see reports of a local outbreak in Kent (Straw et al., 2015). These pests are also economically important in China. Zhang et al. (2007) used two strains of *Heterorhabditis bacteriophora* and *S. carpocapsae* against these pests. They report over 80% control. Such control methods could be brought into Europe and North America in eradication efforts targeting these pests as and when they emerge. Although it should be noted that these potential programmes may not be approved.

Another pest which threatens street trees is the red palm weevil (*Rhynocophorus ferrugineus*), which attacks palm trees in cities throughout the Mediterranean. Although not currently treated operationally, there has been a detailed laboratory and field trial. Atwa and Hegazi (2014) compared the susceptibility of different life stages of *R. ferrugineus* to EPNs. Twelve EPN species were tested under no choice and five stage choice experiments. The five-stage choice experiment is described as follows: "[t]o test the hypothesis that nematode infestation is related to the life stage of red palm weevil, each box was provided with five weevils: three larval stages (one young "second-third instar," one medium "fifth-sixth instar," and one full grown larva), a newly formed cocoon with live pupa, and an adult." (Atwa and Hegazi, 2014). These EPNs were *H. bacteriophora* (HP88), *Steinernema abbasi*, *Steinernema anomali*, *S. carpocapsae* (All), *S. feltiae*, *Steinernema glaseri*, *Steinernema riobrave*, *Steinernema* sp. (EGG4), *Steinernema ritterai* (EGBS), *Steinernema egyptens* (EBNGE), *Steinernema kushidai* and *Heterorhabditis* sp. (EKB20). The highest mean total mortality was in those treated with *Steinernema* sp. (EGG4) and *S. egyptens*. These species along with *Heterorhabditis* sp. (EKB20) were used in curative assays on infected palms with percentage palm recovery being highest (88.1%) on those treated with *Steinernema* sp. (EGG4). There is obviously huge potential in using EPN against this serious pest of palms.

Another pests of street trees that pose a public health hazard because of urticating hairs on the pests is the oak processionary moth (*Thaumetopoea processionea*). This pest is currently in the Greater London area and current control strategies include spraying with pesticide. There is potential to use EPN against this pest though studies on this have not (to the author's knowledge) yet been undertaken. However, given the success of EPN usage on other street trees and trees in an urban situation, there is huge potential to extend the use of EPN to other emerging pests.

4. EPN APPLICATIONS TO PESTS OF TREES IN DECIDUOUS FORESTS

EPN have been identified from deciduous forest soils throughout the world. In the Czech Republic, Mráček et al. (2005) identified 88 strains of EPN from fruit tree soils and 105 strains from "other" deciduous forest tree soils. These consisted of nine species of EPN – all Steinernematidae. In Italy, Tarasco et al. (2014) identified 38 strains of EPN from deciduous forest soils. These consisted of six species of EPN – again all Steinernematidae. In Nepal, Khatri-Chhetri et al. (2010) identified strains of EPN from a wide variety of habitats 9.09% of their strains were identified from deciduous forests and 5.26% from orchards. Of the species identified from deciduous forests and orchards, three were as yet undescribed species of Steinernematidae (*Steinernema* sp. A, *Steinernema* sp. B and *Steinernema* sp. E). Also, strains of the Heterorhabditidae, *Heterorhabditis indica* were isolated, though the authors note that this species was not a habitat specialist. A point made previously by Griffin et al. (2000).

One of the major pests of forestry identified by Day and Leather (1997) is the Cherry blackfly (*Myzus cerasi*). In Turkey Kepenekci et al. (2014) used local isolated EPNs of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* against adult blackfly. They applied these nematodes at concentrations of 25, 50 and 100 IJs at 15 and 25°C. After 96h, mortality remained below 50% with *S. feltiae* being the most efficacious. At 25°C, mortality increased to over 50% for *H. bacteriophora*, but stayed low for *S. feltiae* and lower still for *S. carpocapsae*. Further laboratory screening and field trials are recommended to test for other strains of EPN against this pest.

In applications against the Beech seed moth (*Cydia fagiglandana*), Clausi et al. (2014) tested the pathogenicity of seventeen strains of the following species: *S. feltiae*, *Steinernema ichusae*, *S. carpocapsae*, *Steinernema vulcanicum*, *S. kraussei*, *H. bacteriophora* and *H. megidis*. The most efficacious strains were *S. feltiae* ESA and *S. feltiae* EPP.

The serious foliar pest of forests known as the brown tail moth (*Euproctis chrysorrhoea*) was investigated by Nikdel et al. (2010) in laboratory bioassays using *S. carpocapsae* and *H. bacteriophora*. Third, fourth and fifth instar *E. chrysorrhoea* were exposed to 0, 500, 1000, 1500, 3000 and 5000 IJs per ml. *S. carpocapsae* caused significantly higher mortality of fourth and fifth instars compared to *H. bacteriophora*.

Gorgadze et al. (2020) reported mortality of 98.4% when an unidentified *Steinernema* sp. was used against the gypsy moth (*Lymantria dispar*). Yu et al. (2017) reported the use of

S. kraussei against the clay-coloured weevil (*Otiorhynchus singularis*). However, this application was in blueberry crops rather than forests where this weevil is also a serious pest.

Despite the wide prevalence of EPN (especially steinernematids) in deciduous forest soils, there has been limited application of EPN as biocontrol agents of boring or foliar pests of deciduous forests where the primary commercial interest is wood. There has, however, been some application of EPN control in fruit bearing deciduous trees in orchards. Chapter 19 in the present volume deals, in greater detail than given here, with applications of EPNs to berry pests. Lakatos and Tóth (2006) investigated the use of *Steinernema glaseri* and *Heterorhabditis downesi* to control the European cockchafer (*Melolontha melolonatha*) – a serious pest of the roots of fruit trees. Kuske et al. (2005) investigated the application of EPN in the control of serious pests of nut trees and orchards. They applied *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, *H. indica* and *Heterorhabditis megidis* to the European Cherry fruit fly (*Rhagoletis cerasi*), *Rhagoletis indifferens*, the hazelnut weevil (*Balaninus nucum*) and the chestnut pest *Curculio elephas*. Şahin and Gözel (2021) investigated EPN efficacy against *Capnodis tenebrionis*, a serious pest of peach orchards in Turkey. They found *H. bacteriophora* to be more effective than *S. feltiae*, *Steinernema affinae* and *S. carpocapsae*. They did not speculate as to whether this was due to different foraging strategies of the applied EPNs. Lacey et al. (2006) investigated the efficacy of *S. feltiae* and *S. carpocapsae* in controlling the codling moth (Lepidoptera: Tortricidae). Despite this active programme of research, Belien (2018) in a CABI review has noted low uptake among growers and attributes this to reduced control activity and persistence due to low temperature regimes and desiccation. Orchard applications of EPNs are reviewed by Shapiro-Ilan et al. (2005) and elsewhere in the present volume and so will not be considered further in this chapter.

5. EPN APPLICATIONS TO PESTS OF TREES IN CONIFEROUS FORESTS

There is a long history of application of EPNs to the control of pests of conifer forests. Back in 1963, Schmiege investigated the feasibility of using an as then undescribed species of *Neoaplectana* (= *Steinernema*) nematode in the control of some forestry pests. Schmiege (1963) investigated, as part of this study, the susceptibility of the red headed pine sawfly (*Neodiprion lecontei*) and the large larch sawfly (*Pristiphora erichsonii*). The study incorporated an analysis of the temperature and moisture requirements of the nematode. However, since this study there has been no follow up work on *N. lecontei* or *P. erichsonii*.

EPNs have been identified from coniferous forest soils from around the world. In a comprehensive study (270 soil samples) from 30 different habitats in 10 geographic regions of California, Stock et al. (1999) recovered 7 species of rhabditid nematode, which were isolated from 26.3% of their samples. In this study of the distribution of EPN in natural habitats in California, they note the following: "Most of the *Steinernema* species were recovered from coniferous and oak forests". *Steinernema kraussei* was isolated above 700 ft from coniferous forests and a grassland in the Sierra Nevada mountains. Also, Mracek et al. (1992) and Steiner (1996) found this species in high-elevation coniferous forests. They also found *Steinernema longicaudum* from lodgepole pine (*Pinus contorta*) forests in the Sierra Nevada range.

In Jordan, Stock et al. (2008) recovered four EPN species (three *Steinernema* and one *Heterorhabditis*). In loamy soil of a coniferous forest plot, the authors recovered *Steinernema anatoliense* (EU 200356). Associated with this species was the symbiotic bacterium (*Xenorhabdus nematophila*).

Tumialis et al. (2019), in laboratory bioassays infected pine lappet moth (*Dendrolimus pini*) with isolates of *S. feltiae* and *H. megidis*. In general, they found that *S. feltiae* was more efficacious than *H. megidis* in terms of percentage parasitism (86.7 – 100% for *S. feltiae* compared to 20 – 100% for *H. megidis*). There was no temperature or concentration-dependent effects on parasitism for *S. feltiae* while there was concentration-dependence in *H. megidis*. However, it should be noted that the authors used high concentrations of IJs, which is not ideal to determine concentration effects. The authors suggest further studies including field trials to help apply EPNs to this serious pest of pine.

Another serious pest of pine is the pine processionary moth (*Thaumetopoea pityocampa*). As well as defoliating trees, the urticating hairs associated with this pest make it a considerable public health hazard as well. Gözel and Gözel (2019) conducted temperature-controlled laboratory bioassays on the susceptibility of this pest to various species of EPN. The EPN species tested were *S. carpocapsae*, *S. feltiae* and *H. bacteriophora*. At 10°C larvae treated with 200 IJs of *S. carpocapsae* and *S. feltiae* reached 80% mortality by day 6. In contrast, the two strains of *H. bacteriophora* reached a maximum mortality of 70% by day 7. At 25 °C all species and strains had reached 100% mortality by day 3. These promising early results could pave the way for field trials of these nematodes against this serious pest.

The great spruce bark beetle (*Dendroctonus micans*) is a serious boring pest of spruce trees (*Picea* spp.). Current control methods for this pest rely sanitation felling, restriction of transport of infected trees and on inoculative classical biological control using the predatory beetle *Rhizophagus grandis* (Evans and Fielding, 1994). Despite this rather successful approach in the UK, in Turkey there has been some investigation of the possible application of EPNs to the control of this pest. Kepenekci and Atay (2014) tested, under temperature-controlled laboratory conditions, the efficacy of strains of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* against larval *D. micans* isolated from Turkey. At 1000 IJs concentration and at 25°C, they report 98.04 and 94.04% mortality for *S. feltiae* and *H. bacteriophora*, respectively. *S. carpocapsae* did not attain higher than 40% mortality under any combination of temperature and IJ concentration.

Grucmanová and Holuša (2013) describe phoretic relationships between nematodes and various species of *Ips* – the most frequent phoretic nematodes being *Bursaphelenchus eidmanni* and *Micoletzkya buetschlii*. Endoparasitic nematodes were also identified including *Cotortylenchus*, *Cryptaphelenchus*, *Ektaphelenchus*, *Parasitaphelenchus*, *Parasitorhabditis* and *Parasitylenchus*. The most frequent endoparasite being *Parasitylenchus dispar* and *Contortylenchus diplogaster*. It is doubtful whether any of these associated nematodes could act as an effective biological control of the various species of *Ips*, but the phoretic nature of the association does bring to mind the possibility of using *Ips* as a “living insect bomb” *sensu* Gumus et al. (2015), especially given the cryptic nature of the life history of *Ips* spp.

Triggiani (1983) conducted field trials in Italy using *H. bacteriophora* against the pine shoot beetle *Tomicus piniperda*. Triggiani showed that adults excavating their nuptial chambers in pine trunks could be infected by nematodes if a suspension was injected into the tunnels.

Hylobius abietis is one of the most serious pests of clearfelled forests in Europe. As such, there has been much research on the use of the following EPN species: *Steinernema carpocapsae*, *S. kraussei*, *S. feltiae*, *H. dowensi* and *H. megidis*. There have also been many studies of efficacy, a meta-analysis on factors driving success, much work on interactions with other biological control agents and investigations of environmental safety and non-target effects. Given this body of research and given the lessons that can be learned in application to other pests, the next section deals with *Hylobius abietis* as a case study.

6. CASE STUDY: APPLICATION OF EPN IN THE CONTROL OF THE LARGE PINE WEEVIL (*HYLOBIUS ABIETIS*) – THE MOST SERIOUS PEST OF CLEAR FELL FORESTS IN EUROPE

6.1 Life cycle of the Large Pine Weevil and its pest status

From the outset it is important to note that the problems associated with the large pine weevil (*Hylobius abietis*) are essentially and fundamentally man-made problems. Silvicultural practices which promote the use of clearfell (clearcut) forestry and large areas of breeding sites (stumps) with even aged plantings provide an optimum ecological niche for *Hylobius*.

Ever since the establishment of plantation forestry in Europe, *H. abietis* L. has been a major pest (Munro, 1927). In newly clear-felled stands adult female weevils are attracted by the volatiles and oviposit just under the bark of the stumps. Weevil larvae subsequently develop in the protected environment under the bark for one to three years depending on temperature (Leather et al., 1999; Inward et al., 2012). Following emergence, adults feed on the bark of young trees and replanted sites can suffer up to 100% mortality of newly planted trees if no control measures are taken. *H. abietis* is estimated to cost the UK economy £2 million *per annum* (Weslien et al., 1998; Leather et al. 1999). The set of environmental conditions provided by clearfell forestry – large amounts of volatile cues which attract gravid females, copious breeding habitats in the form of stumps and fresh food for newly emerging adults is the ideal niche for pine weevil and so it is important to note that *H. abietis* is in fact a man-made problem (Evans et al., 2004): there is very little impact of pine weevil in mixed aged stands subject to selective felling. However, as long as plantation forestry, subject to clear-felling remains an important part of the forestry estate, control of the large pine weevil will be necessary. Damage to saplings occurs on weevil emergence and when the weevil undergoes a period of maturation feeding (Figure 25.1).

6.2 Control of the Large Pine Weevil with EPN

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

Alternatives to the chemical control of pine weevil include changes in silviculture practices, including mounding, planting later in the season and leaving sites fallow for a number of years (Von Sydow, 1997; Örlander and Nilsson, 1999; Örlander and Nordlander; 2003). Another cultural tactic is the application of the fungus *Phlebiopsis gigantea* (Fr. : Fr.) Juelich in the biocontrol of the fungus *Heterobasidion annosum* (Fr.) Bref, which has the additional benefit of making stumps unsuitable for weevil oviposition and development (Skrzecz, 1996, 2001). Another approach to weevil control is to manage forest blocks in a landscape context with regard to pine weevil meta-population dynamics; to this end a *Hylobius* integrated management system using GIS technology has been developed (Wainhouse et al., 2001; Evans et al., 2004).

EPNs are widely used in the UK to control pine weevil and, until recently, were also widely used in Ireland. At operational level, *S. carpocapsae* is applied by pressure hose, from a tank mixer mounted on a modified forwarder, at an average rate of 3.5 million nematodes per stump (Torr et al., 2005). These nematodes are targeted to kill the weevil larvae, pupae and callow adults that are developing in the stump under the bark. For experiments, however, EPNs are usually applied by hand as a sub-surface drench at the same rate. For experiments, weevil control is measured either, as percentage parasitism by destructively sampling stumps (Figure 25.2), or through monitoring percentage reduction in emergence relative to control, using emergence traps (Figure 25.3). There have been many studies assessing the efficacy of EPN in the control of pine weevil (Brixey et al., 2006; Dillon et al., 2006; Dillon et al., 2007; Dillon et al., 2008; Torr et al., 2007; Williams et al., 2013a)

Brixey et al. (2006), reporting on experiments carried out in 1997, were the first to report on EPN efficacy in clear-fell forestry. They used a sub-surface drench and a surface spray to apply *S. carpocapsae* IJs to Sitka spruce stumps. They report a higher susceptibility of callow adults than either larvae or older adults and this is consistent with Williams et al.

(2015) who showed that pupae that are infected with nematodes, will metamorphose into callow adults before being killed by IJs. This is the case even if pupae are washed in tween after infection, suggesting that IJs are waiting in spiracles or some other orifice. Brixey et al. (2006) report relatively poor rates of control (5.2-18.3%) and suggest that timing of applications is important.

Dillon et al. (2006), in field trials, tested the efficacy of the three commercially available EPNs against pine weevil – namely: *H. megidis*, *S. feltiae* and *S. carpocapsae* – and also *H. downesi*. All EPNs parasitized larvae 40-49cm from the bole of the stump and to a depth of 30-39cm below soil surface. *Heterorhabditis downesi* was the most efficacious EPN used parasitizing 55-63% of developing weevils. Dillon et al. (2007) showed that even at half doses of EPN applied to Pine stumps still maintained efficacies of 75-79% reduction relative to control for *H. downesi*. In contrast to Brixey et al. (2006), Dillon et al. (2007) reported higher percentage of larvae were parasitized (60%) compared to pupae (36%) or callow adults (18%).

Torr et al. (2007) compared *S. carpocapsae* with *S. kraussei* since the latter has been isolated from soils in Northern England, is efficacious at a lower temperature and adopts a “cruiser” rather than “ambusher” foraging strategy. Laboratory and field trials showed no difference in efficacy though *S. kraussei* did persist longer in the field. Overall, the authors note very little advantage to swapping control strategies from *S. carpocapsae* to *S. kraussei*.

6.3 Soil, tree stump species and pest density effects on efficacy

Williams et al. (2013b) analysed existing data together with previously unpublished data from the COFORD funded project ABATE and the INTERREG IVA project IMPACT, to determine which factors are most important in determining the efficacy of EPN in the control of the large pine weevil. Altogether, there were 28 field trials in the dataset. They had data for the efficacy of two nematode species: *S. carpocapsae* – a so-called “ambush forager” which is typically thought to wait and stand on its tail to latch on to passing insects (this is also the species used at an operational level) and *H. downesi* – a so-called “cruise forager” which is typically thought to seek out insect prey (this species was reported by Dillon et al. [2006; 2007] to be the most efficacious of the nematodes that they tested). Efficacy was measured by two methods in the datasets. Firstly, by measuring the reduction in weevil emergence from treated compared to untreated control stumps by placing tent-like emergence traps, which collect emerging weevils, over the stumps (Fig 3). Secondly, levels of parasitism between treated and untreated stumps were compared by removing the bark and counting the number of infected pine weevils in the stumps (Fig 2). General linear models were constructed with percentage parasitism and percentage reduction as responses and soil type (peat versus mineral), tree species (spruce versus pine) and weevil population as explanatory variables. The most parsimonious models, for both nematode species, both measures of efficacy and at both a site and stump level consistently showed that the most significant factor was soil type, with EPNs being more efficacious on sites with peaty soils than on sites of mineral soil. Neither tree species nor weevil population significantly affected EPN efficacy. Also, they found that *H. downesi* was significantly more efficacious than *S. carpocapsae*, but the latter did surprisingly well. A number of practical recommendations can be made from this meta-analysis. Firstly, it may be worthwhile for biological control companies to investigate the feasibility of mass-producing *H. downesi*. Secondly, EPN applications should be focused on peaty sites where they are likely to be more effective. However, peaty sites have their own difficulties in terms of ease of access. Since efficacy was independent of both the tree species of the stumps and the weevil population in the stumps, it would be beneficial to focus on spruce, which typically has lower populations of weevils so that equivalent percentage reductions are more likely to reduce the weevil population below the absolute economic threshold of about five weevils per stump. The results of this analysis are presented in detail in Williams et al. (2013b).

In a follow up study, Kapranas et al. (2017) tested for soil type and weevil density effects in field trials using *S. carpocapsae* and *H. downesi*. Whereas Williams et al. (2013b) used general linear models, Kapranas et al. (2017) used: “generalized linear models (GLMs) (Crawley 1997). We assumed quasi-binomial error variance for parasitism (proportional) data, and significance of effects was assessed by the change in deviance when a variable was removed from the full model. Williams et al. (2013b) also used a mixed effect logistic regression analysis to explore parasitism rates in relation to depth below soil surface and horizontal distance from the bole of the stump.” (Kapranas et al., 2017). Kapranas et al. (2017) reports a significant negative density dependence of parasitism rate when soil type is taken into account.

6.4 EPN use in combination with other control agents

The first study to combine EPN with other control strategies in *H. abeitis* control efforts was that of Dillon et al. (2008). They used both "top down" (EPNs *S. carpocapsae* and *H. downesi*) and "bottom up" methods of population reduction (the wood colonising fungus *Trichoderma koingii*). They also monitored the levels of natural control by the braconid *Bracon hylobii*. Experiments were conducted in Sitka spruce stumps. *Heterorhabditis downesi* reduced the amount of emergence relative to controls and EPNs had no effect on parasitism rates by the braconid. *Trichoderma koingii* did not suppress weevils but did advance weevil development. Whereas the fungus had a negative effect on EPN parasitism it positively affected parasitism by the braconid suggesting that it interfered with competition between EPNs and *Bracon hylobii*.

Other potential biological control agents of pine weevil are entomopathogenic fungi (EPF) of the genera *Beauveria* and *Metarhizium*. Ansari and Butt (2012) have shown with laboratory assays that larvae, pupae and adults of pine weevil are all susceptible to EPF. The efficacy of EPF, EPN and mixtures of EPN and EPF were investigated in five field trials over three years (Williams et al., 2013a). These trials focused on Pine stumps on peaty substrates, but similar trials were undertaken on spruce stumps in Wales (Evans, *Pers. Comm.*). In general, treatments that included EPNs were more efficacious than those that did not and, in only one trial did an EPF-only treatment provide a reduction in weevil populations, relative to untreated controls, approaching significance (P=0.058). Half-dose treatments allowed the assessment of synergy between agents. When agents are more efficacious together than each is when applied alone and the effect is significantly greater than an additive effect, they are said to act synergistically. In all of the trials that allowed this analysis, the effects were additive. Interestingly, half dose treatments were often seen to be as, if not more, effective than full doses of EPNs. This suggests that wider operational-level field trials of reduced dose EPN applications may be an avenue for future study. All these results are qualitatively similar to those of our Welsh colleagues (McAlister, *Pers. Comm.*). When a biological control agent fails to reduce pest populations to an economically acceptable level, a number of processes may be occurring: firstly, it may be that the agent is getting to the target, but not killing it; secondly it may be that the agent is not getting to the target and so has no opportunity to kill it and, thirdly, it may be that although some of the agent is getting to some of the target and killing it, not enough is getting to enough targets to reduce them below the level required. Given the results of Ansari and Butt (2012), it is unlikely that EPF are getting to the weevils and not killing them, so either all or none of the EPF is reaching the target. Williams et al. (2013a) developed a molecular protocol using restriction fragment length polymorphism (RFLPs) to distinguish between the native species of *Beauveria* commonly found on pine weevil under bark in untreated forests, *Beauveria caledonica*, and *Beauveria bassiana*, the species that was applied. They found that there was a high association between *B. bassiana* infection and application of this agent. Since they measured the depth in the soil and distance from the bole of the stump of recovered weevils, they also showed that weevils could be infected by applied fungus up to a depth of 18cm below the soil surface and up to 28cm distant from the bole of the stump, under the bark. Interestingly, native *B. caledonica* infected weevils up to 30cm below the soil and up to 33cm distant from the stump. Future research should, therefore, perhaps focus on native EPF and agent delivery technology to improve the efficacy of EPF in weevil control. Soil from around stumps (EPF-treated and untreated control) at three of the field sites were monitored for EPF presence. Soil samples were baited with wax moth larvae and EPF mycelia from killed moths were grown on agar that was selective for EPF. We then extracted DNA and sequenced the Internal Transcribed Spacer region (*ITS*) to identify EPF species. It was apparent that applied EPF persisted for at least three years in soil around stumps, but there was no apparent spread of inoculum from fungus-treated stumps to untreated control stumps. We also identified a number of interesting EPF – *Pochonia bulbillosa* (a species previously isolated from forest soils in New Zealand [Ali, 2012]) and *Beauveria pseudobassiana* (a species previously isolated from South America). All of these native isolates have potential for future study as biocontrols of pine weevil (Williams, unpublished).

McNamara et al. (2018) also reported additive results when using commercially available *Metarhizium brunneum* and *Beauveria bassiana* and a strain of *Beauveria caledonica* isolated from the pest's habitat along with *S. carpocapsae* and *H. downesi*. They also note that applied EPF can survive in the soil for up to two years (especially *M. brunneum*).

6.5 Environmental safety of EPN in forest ecosystems

Steinernema carpocapsae is native to Britain, but to date has not been isolated from natural populations in Ireland. Obviously, many environmentalists have legitimate concerns over the release of a non-native species at a rate of 3.5 million per stump over vast tracts of the forestry estate in Ireland. A number of studies have been undertaken at NUI, Maynooth, to assess the significance of the risk posed by EPN. These studies consider the following risks: firstly, what is the risk to important ecosystem service providers; secondly, what are the risks to non-target stump-inhabiting beetles; and, thirdly, what are the effects on native EPNs i.e., do applied EPNs displace the native EPN community. Ecosystem services is currently a "hot button topic" in ecology with the realisation that services provided by nature, and traditionally regarded as economic externalities of zero value, are actually worth $33 trillion per year to the world economy (Costanza et al., 1997)[1]. *Rhagium bifasciatum* is a native long horn beetle of plantation forests in Ireland. The species typically inhabits logs and branches of brash rather than stumps, where it develops and breaks up the interior of the wood allowing clearing of the brash and recycling of nutrients into the forest soil. Harvey et al. (2011) showed in the laboratory that realistic doses of nematodes can easily penetrate logs of brash and will subsequently infect and kill *R. bifasciatum* inhabiting logs. Field collections of brash logs, however, showed a relatively low infection rate (<10% of logs infected and <4% of *R. bifasciatum* individuals) from sites that had been operationally treated with EPN and that levels of infection quickly dropped off as distance to the nearest stump increased. So, whereas there is potential impact here, this is largely mitigated through targeted application of control agents.

Another ecosystem service provider is *Bracon hylobii*, a native parasitoid of pine weevil. Any effect of nematode infection on wasp populations is likely to be detrimental to the provision of this service. In a series of elegant experiments, reported in Harvey and Griffin (2012), it was shown that *B. hylobii* uses vibration cues to select host larvae under bark and wasps will parasitise nematode-infected hosts as long as they move. The non-specificity of host choice was further demonstrated by eliciting oviposition even on abnormal hosts (i.e. larvae of insects not usually found under the bark). Hence there is potential here for competition between *B. hylobii* and inundatively applied EPNs. It was suggested that EPN be applied just after most adult wasps have eclosed in late spring, but when parasitism of weevils is still quite low to mitigate competition between the two species.

One might predict that applying millions of insect-killing nematodes onto a stump is likely to have a major effect on non-target insect species. Indeed, EPN have a wide host range and laboratory experiments suggest potential non-target susceptibility. Surprisingly, however, Dillon et al. (2012) showed that this is actually not the case. Non-target beetles were collected from a number of sites, one year and two years after application of EPN. All beetles that emerged from both nematode-treated and untreated control stumps were identified to species. There was no significant effect of nematode treatment on the total numbers of beetles, the

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[1] At the time (1997) world total Gross National Product was estimated at $18 trillion.

number of beetle species, their diversity or the beetle community composition. Neither was there an effect on these metrics for the sub-set of stump-inhabiting (saproxylic) species. There was, however, not surprisingly, a large effect of both site and tree species on these metrics. It is, therefore, important to note that “potential” effects identified under controlled conditions in the laboratory need not necessarily become “realised” effects in the field.

Another set of organisms potentially disrupted by the application of EPNs is the native EPN species that would ordinarily inhabit the site. Harvey and Griffin (2016) showed that applied EPNs only persist at a very low levels three years after application and that persistence decreased with increasing distance from the stump. He also showed a very limited amount of off-site spread of EPNs. Five years after application, sampling at the same sites, showed no persistence of the applied EPNs at all and the recolonisation of sites by the native species *Steinernema feltiae*, probably as a result of ecological succession of sites with native vegetation and the associated insect fauna suitable to the native EPN species (Harvey and Griffin, 2016). We may, therefore, conclude that there are many potential negative impacts in releasing the non-native *S. carpocapsae* to control pine weevil in Ireland, but in fact, most of these risks have not been realised in the field. Harvey et al. (2016) provide a rigorous quantitative evaluation of five risk categories in the use of EPNs to control *H. abietis*. These categories were: establishment, dispersal, host range, direct non-target effects and indirect non-target effects. Overall, EPNs were given a lower score (35-51 out of 125) compared to conventional control with pyrethroid or neonicotinoid insecticides.

7. CONCLUSIONS

EPN are a classic example of inundative (biopesticide) biological control. Although there has been some work on trees outside of forests, deciduous forests and coniferous forests, of the 99 serious pests of European Forests only 16.2% have had any investigation of the applicability of EPN to sustainably controlling these pest populations. EPN (especially Steinernematidae) have been isolated from forestry soils throughout the world so the dangers posed by introducing EPN *en masse* appear to be negligible, but it is important to consider environmental safety and non-target effects. One area that has received a lot of attention and has been scaled up to operational levels is the biological control of the large pine weevil (*H. abietis*). This is the most serious insect pest of coniferous forests in Europe. I have reviewed the various aspects of EPN research in relation to its control, effects of stump species, soil type and weevil populations on EPN efficacy, the use of EPN with other control agents and the environmental safety of EPN in Forestry. It is hoped that in the future such studies will become common on the other serious pests of forestry throughout the world.

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9. TABLES AND FIGURES

Table 25.1: Major pests of Forestry in Europe (from Day and Leather, 1997)

Major forest Pest of Europe	**Common name**	**Use of EPN**
Hemiptera		
Adelges abietis (L.)	Pineapple gall woolly aphid	No
Adelges laricis Vall.	Larch woolly aphid	No
Aradus cinnamomeus (Panz.)	Pine bark bug	No
Cinara piceae (Panz.)	Great black spruce bark aphid	No
Cinara pilicornis (Htg.)	Brown spruce shoot aphid	No
Cinara pineae (Mord.)	Large pine aphid	No
Cryptococcus fagisuga (Lundg.)	Felted beech coccus	No
Drepanosiphum platanoidis (Schr.)	Sycamore aphid	No
Dreyfusia piceae (Ratz.)	Balsam woolly aphid	No
Dreyfusia nordmannianae (Eck.)	Silver fir woolly aphid	No
Gilletteela cooleyi (Gill.)	Douglas fir woolly aphid	No
Elatobium abietinum (Walker)	Green spruce aphid	No

Eucallipterus tiliae (L.)	Lime aphid	No
Eulachnus agilis (Kalt.)	Spotted pine aphid	No
Kermes quercus (L.)	Oak scale or pox	No
Matsucoccus josephi (Bod. & Harp)	Pine bast scale	No
Mindarus abietinus (Horv.)	Balsam twig aphid	No
Myzus cerasi L.	Cherry blackfly	*Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora*
Pachypappella vesicalis (Koch.)	Spruce root aphid	No
Pachypappella lactea (Tullg.)	Spruce root aphid	No
Phyllaphis fagi (L.)	Beech woolly aphid	No
Pineus pini (Macq.)	Pine woolly aphid	No
Schizolachnus pineti (Fab.)	Grey pine needle aphid	No
Stagona pini (Burm.)	Pine root aphid	No
Lepidoptera		
Bupalus piniaria L.	Pine looper moth	No
Choristoneura murinana (Hbn.)	Fir bud moth	No
Coleophora laricella Hbn.	Larch case bearer	No
Cydia conicolana Heyl.	Pine cone moth	No
Cydia fagiglandana Zell.	Beech seed moth	*Steinernema feltiae*, *S. ichusae*, *S. carpocapsae*, *S. vulcanicum*, *S. kraussei*, *Heterorhabditis*

		bacteriophora and *H. megidis*
Cydia strobilella (L.)	Spruce cone tortrix	No
Dendrolimus pini (L.)	Pine lappet moth	*Steinernema feltiae* and *Heterorhabditis megidis*
Dioryctria abietella Schih.	Pine Knothorn moth	No
Epinotia nanana (Treitsch.)	Dwarf spruce bell moth	No
Epinotia tedella (Clerck)	Spruce bell moth	No
Epirrita autumnata (Bkh.)	Autumnal moth	No
Euproctis chrysorrhoea (L.)	Browntail moth	*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*
Lymantria dispar L.	Gypsy moth	*Steinernema* sp., *S. borjomiense*, *S. thesami*, *Parasitorhabditis* sp., *Phasmarhabditis* sp.
Lymantria monacha L.	Nun moth	No
Malacosoma neustria (L.)	Lackey moth	Bacteria isolated from septicaemic *Galleria mellonella*
Operophtera brumata (L.)	Winter moth	No
Orgyia antiqua L.	Vapourer moth	No
Panolis flammea (Den. &Schiff.)	Pine beauty moth	No
Prays fraxinella Don.	Ash bud moth	No
Retinia resinella (L.)	Pine resin gall moth	No

Rhyacionia buoliana (Den. & Schiff.)	Pine shoot moth	No
Thaumetopoea pityocampa (Den. & Schiff.)	Pine processionary moth	*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*
Tortrix viridana (L.)	Green oak tortrix; Oak leaf roller moth	No
Yponomeuta evonymella (L.)	Bird cherry ermine moth	No
Zeiraphera diniana (Gn.)	Grey larch tortrix; Larch bud moth	No
Diptera		
Dasyneura laricis (F. Loew)	Larch bud midge	No
Phytoagromya populicolia Haliday	Poplar leaf miner	No
Resseliella picea Seit	Fir seed gall midge	No
Thecodiplosis brachyntera (Schwardt.)	Pine needle shortening gall midge	No
Hymenoptera		
Andricus quercuscalicis (Burgs.)	Knopper gall wasp	No
Cephalcia abietis (L.)	Web-spinning fir sawfly	No
Cephalcia lariciphila (Wachtl.)	Web-spinning larch sawfly	No
Diprion pini L.	Large pine sawfly	No
Gilpinia hercyniae Htg.	European spruce sawfly	No
Megastigmus pinus Parfitt	Silver fir seed wasp	No
Megastigmus spermotrophus (Wachtl.)	Douglas fir seed wasp	No

Nematus melanaspis Htg.	Gregarious poplar sawfly	No
Nematus pavidus Lepelt.	Lesser willow sawfly	No
Nematus salicis L.	Large willow sawfly	No
Neodiprion lecontei (Fitch.)	Redheaded pine sawfly	Undescribed species of *Neoaplectana*
Neodiprion sertifer (Geoff.)	European pine sawfly; Fox-coloured sawfly	No
Pristiphora abietina Christ.	Gregarious spruce sawfly	No
Pristiphora erichsonii (Htg.)	Large larch sawfly	Undescribed species of *Neoaplectana* (= *Steinernema*)
Pristiphora testacea Jur.	Birch sawfly	No
Pristiphora wesmaeli Tisch.	Large sawfly	No
Sirex noctilio Fab.	Steely blue woodwasp	No
Urocerus gigas (L.)	Giant woodwasp	No
Coleoptera		
Brachyonyx pineti (Payk.)	Pine needle feeding weevil	No
Chrysomela populi (L.)	Large red poplar leaf beetle	No
Dendroctonus micans Kugelaan	Great spruce bark beetle	*Steinernema carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora*
Galerucella lineola F.	Brown willow beetle	No
Hylastes ater Payk.	Black pine beetle	No

Hylastes brunneus Er.	Black pine beetle	No
Hylastes cunicularius Er.	Black spruce beetle	No
Hylobius abietis (L.)	Large pine weevil	*Steinernema carpocapsae*, *S. kraussei*, *S. feltiae*, *Heterorhabditis downesi* and *H. megidis*
Hylobius pinastri (Gyll.)	Small pine weevil	No
Hylurgopinus rufipes (Eich.)	Canadian elm bark beetle	No
Ips acuminatus Gyll.	Engraver beetle	Natural nematodes isolated
Ips cembrae Heer.	Large larch bark beetle	Natural nematodes isolated
Ips typographus L.	Eight-toothed spruce bark beetle	Natural nematodes isolated
Otiorhynchus singularis L.	Clay-coloured weevil	*Steinernema kraussei*
Phyllobius pyri L.	Common leaf weevil	No
Phratora vitellinae L. – previously *Phyllodecta vitellinae*	Brassy willow beetle	No
Phratora vulgatissima L. – previously *Phyllodecta vulgatissima*	Blue willow beetle	No
Pissodes pini L.	Banded pine weevil	No
Pissodes validirostris Sahl.	Pine cone weevil	No
Pityogenes bidentatus Herbst.	Two-toothed pine beetle	No
Plagiodera versicolora (Laich)	Broader willow leaf beetle	No
Rhynchaenus fagi L.	Beach leaf miner	No

Saperda populnea L.	Small poplar longhorn beetle	No
Scolytus multistriatus Marsham	Small elm beetle	No
Scolytus scolytus Fab.	Large elm beetle	No
Tomicus minor Htg.	Lesser pine shoot beetle	No
Tomicus piniperda (L.)	Pine shoot beetle	*Heterorhabditis bacteriophora*
Xyloterus lineatus (Ol.)	Conifer ambrosia beetle	No

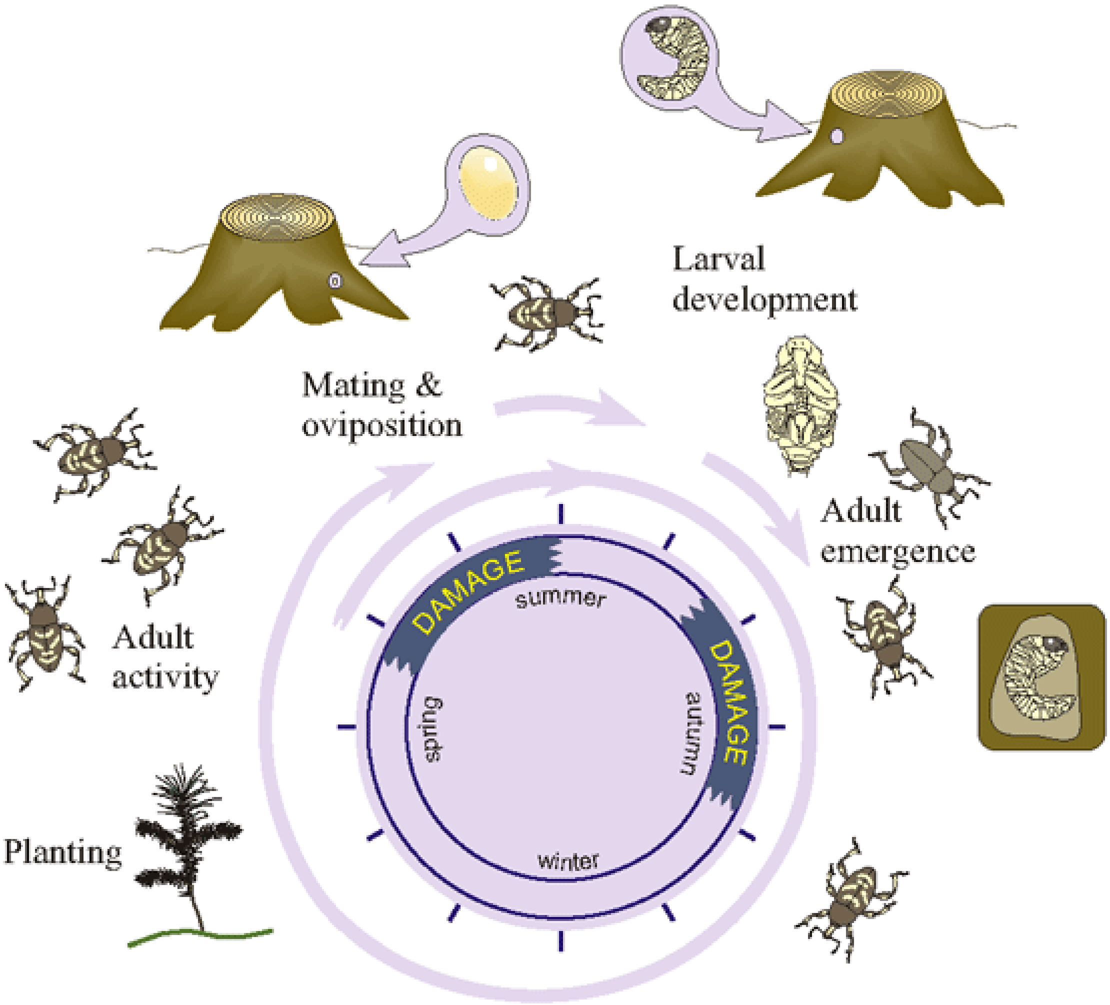

Figure 25.1: The Life Cycle of the Large Pine Weevil (*Hylobius abietis*)

Figure 25.2: Destructively sampled stump after monitoring percentage weevil parasitism under the bark which has been removed.

Figure 25.3: Emergence trap surrounding a stump to monitor percentage reduction of weevil emergence relative to control.