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- The large pine weevil is the most serious pest of conifer reforestation in Ireland.
- The adult weevil causes damage by feeding on the bark of young transplants.
- With up to 100,000 adult weevils emerging per hectare on recently felled conifer sites, it is not uncommon for 100% of young plants to be killed.
- Pine weevils are susceptible to attack from a number of natural enemies such as fungi, parasites and predators. Of these, insect killing nematodes (microscopic worms) are the most promising biological control agent: capable of reducing weevil populations by up to 70%.
- The practicalities of using nematodes, their environmental safety and their potential for use as part of an integrated pest management strategy are discussed.

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# Controlling the large pine weevil, *Hylobius abietis*, using natural enemies

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The large pine weevil, *Hylobius abietis* (Figure 1) is the most important pest of replanted conifer sites in Ireland. This beetle has been recognised as a pest in Europe since the nineteenth century, but reports of damage were relatively rare in Ireland and Britain until the start of the twentieth century. Adult weevils are brown, with yellow patches on their body, and are approximately 1.5 cm in length.



Figure 1: An adult large pine weevil, Hylobius abietis.

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### Life cycle

Adult weevils are attracted onto a site by the smell of freshly cut timber. The female lays her eggs in stumps of recently cut conifers, where immature weevils develop under the bark. Eggs hatch into larvae that are cream with a brown head capsule and look like legless caterpillars (Figure 2). The larvae develop into pupae which are immobile, cream in colour and soft bodied. Stumps of most conifer species support weevil development, but the numbers developing in and emerging from pine (Pinus species) are higher than from spruce (Picea species) (Figure 3). It is estimated that over 100,000 adult weevils per hectare can emerge in a single year on a pine site in Ireland (Dillon et al. 2006). The size of the weevil population developing in a standing forest is limited by the availability of recently dead wood in which to breed, but the practice of clearfelling gives weevils abundant breeding sites.

Weevils develop from egg to adult in 12-36 months in Ireland and the UK, but development can take up to five years in colder Northern Europe. Development takes longer in spruce than in pine. In Ireland, the number of adult weevils emerging from stumps peaks in September. Emerging adults can either remain on the site, or move to nearby sites that have been recently felled. Adult weevils are normally active between March and October. They can live for up to four years, hibernating in the leaf litter during the winter (Leather et al. 1999).



Figure 3: The number of adult pine weevil (*Hylobius abietis*) emerging from pine (*Pinus* species) and Sitka spruce (*Picea sitchensis*) stumps in Ireland in a single year.



Figure 2: A pine weevil larva feeding underneath the bark in a recently felled stump.

### Damage caused

In mature forests, adult weevils feed in the canopy without causing any significant damage. When a site is replanted, adult weevils emerging from the stumps feed on the bark of young transplants from the root collar up (Figure 4). Adult weevils have a broad host range, feeding on a wide variety of conifer and broadleaved trees, but pine is preferred as a food source. Young transplants can withstand a small amount of feeding, but extensive feeding causes needle loss, reduces plant growth, and can lead to death when plants are completely ring-barked. In the absence of control measures, up to 100% of transplants can be killed.

Heritage and Moore (2001) assessed the key plant and site factors that determine the severity of pine weevil damage. Factors include previous crop, felling date and system, proximity to other clearfelled sites, site preparation and choice of planting stock for reforestation. Damage is expected to be most severe when small conifer plants are used to restock a site within five years of felling; where the previous crop was lodgepole pine (*Pinus contorta*); the felled area is large (> 1 ha); the site is less than 5 km from a recent clearfell (< 4 years old) and grassy weed control and ground preparation are not practised.



Figure 4: Pine weevil feeding damage on a young Sitka spruce (*Picea sitchensis*) seedling.

Until recently, the principal method of preventing pine weevil damage was to spray or dip young transplants in insecticides prior to planting. An additional top-up spray was often required in the second year following planting. Many of the chemicals previously used to protect transplants against pine weevil feeding are no longer available to foresters. Furthermore, as part of the commitment to Sustainable Forest Management, European Member States agreed to reduce the use of chemicals and increase biodiversity in forests. Therefore, there is a real need to find an environmentally sustainable, non-toxic, way of reducing the numbers of adult weevils on a site, and/or reducing the level of damage they cause.

# **Biological control**

Various natural enemies attack immature and adult pine weevils in the wild. Natural enemies include predatory beetles, insect killing fungi, microscopic insect killing worms (entomopathogenic nematodes) and a parasitic wasp, *Bracon hylobii* (Figures 5 to 8). Ground beetles, nematodes and fungi can kill both immature and adult weevils. The parasitic wasp attacks pine weevil larvae only.



Figure 5: Adult carabid beetles, including the strawberry ground beetle (*Pterostichus madidus*), predate on the pine weevil. *Photograph courtesy of Roy Anderson.* 



Figure 6: An adult pine weevil killed by an entomopathogenic fungus.



Figure 7: The entomopathogenic nematode *Steinernema carpocapsae*.

### Parasitic wasp, Bracon hylobii

Adult wasps are approximately 0.5 cm in length and are active from May to November. The female wasp uses the vibrations caused by weevil larvae feeding under the bark to find a host. Having located a weevil larva, she drills through the bark and injects venom into it, causing paralysis. The wasp then lays her eggs on or near the weevil larva. Eggs hatch and the tiny wasp larvae begin to feed on the weevil (Figure 9), sucking its contents until nothing is left but the cuticle. Having fed, they spin cocoons and either emerge as adults 7-10 days later, or remain in cocoons until temperatures increase. These parasitic wasps occur throughout Europe where pine weevil occurs. In Ireland, the



Figure 8: A parasitic wasp, *Bracon hylobii*, which attacks pine weevil larvae developing within stumps.

percentage of weevil larvae on a site attacked by this species of wasp ranges from 1 to 40%. Unfortunately, this level of natural parasitism is too low to reduce the number of weevil adults emerging from stumps to below economically damaging levels.

Supplementing wild populations of natural enemies with laboratory-reared individuals has long been practiced in agriculture when the level of natural control in the field is insufficient to protect against pest damage (e.g. releasing ladybirds for aphid control). Researchers at the University of Ulster and Galway-Mayo Institute of Technology investigated the possibility of rearing *Bracon hylobii* for release in Ireland. Rearing the wasps is labour intensive, as



Figure 9: Larvae of the parasitic wasp *Bracon hylobii* feeding on a pine weevil larva, adjacent to a healthy weevil larva.

they require weevil larvae as hosts, and results of releases have been disappointing. Mass production and release of *Bracon hylobii* would require improved production and delivery for success.

### Insect killing nematodes

Insect killing or entomopathogenic nematodes are soilinhabiting insect parasites. These microscopic worms have a worldwide distribution and have been recovered from diverse habitats including grassland, forest, and tilled soils. Entomopathogenic nematodes can infect the soil dwelling stages of a variety of insects, including beetles, butterflies and flies, depending on the nematode species. Nematodes are already used in horticulture in Ireland against the black vine weevil (*Otiorhynchus sulcatus*) and mushroom flies (*Sciaridae*).

Entomopathogenic nematodes actively seek out insects in the soil, using cues such as carbon dioxide and heat to detect their host. Having located a potential host, the nematodes invade the insect through natural openings such as the mouth and anus. In some soft bodied insects nematodes may also enter directly through the insect's cuticle. The insect dies within days. The nematodes reproduce inside, and once the nutrients of the dead insect have been exhausted, their offspring emerge and disperse locally in search of new suitable hosts.

Nematodes are ideal biological control agents because they:

- only attack insects, so pose no risk to fish, birds or mammals;
- are not harmful to humans, so unlike chemical pesticides, there is no risk of poisoning due to accidental exposure;
- actively seek out insect hosts within the soil;
- are fast acting, with insect death often occurring within 48 hours;
- have limited dispersal, so remain close to where they were applied;
- can be mass produced and are commercially available;
- can be applied using conventional spraying equipment;
- > are compatible with a number of herbicides.

Pine weevil larvae, pupae and adults are all susceptible to entomopathogenic nematodes, although adults are the least susceptible stage. Rather than applying nematodes around the young plant to target adult weevils, as is the practice with chemical insecticides, nematodes are applied to the soil around stumps to target the immature weevils. For effective control, nematodes are applied 12-24 months after felling, by which time the bark on the stump has degraded slightly, facilitating nematode entry. The large larvae in stumps 12-24 months after felling are more easily located by nematodes than smaller younger larvae, so applying nematodes immediately after felling is not effective. Nematodes applied to stumps in the summer, when temperatures are warm, quickly invade weevil larvae and four weeks later up to 100,000 nematodes can emerge from each dead insect. These nematode offspring can then go on to infect immature weevils that were not killed by the initial nematode application.

Nematodes are living animals and so are easily killed by desiccation, exposure to the ultraviolet radiation in direct sunlight, lack of oxygen and high temperatures. In order to ensure high quality nematodes, storage and handling instructions printed on the packet must be strictly followed. Commercially available nematodes are formulated in powder, and must be refrigerated until use. Immediately prior to use, the product is mixed with water to activate the nematodes. This mixture must be continuously aerated in order to prevent nematode death due to lack of oxygen. Application rates vary depending on the insect pest and crop, but for pine weevil control 3.5 million nematodes per stump are recommended. Although this seems high, the chances of any of these tiny worms finding their way through 50 cm or more of soil and then under the bark and into a weevil are small, especially as they have to do this before they die of starvation, or are killed by one of the many living and non-living threats to them in the soil.

Researchers at the National University of Ireland Maynooth (NUIM) and Forest Research UK collaborated to select the most effective nematode for weevil control, and to develop methods for applying nematodes on an operational scale. Forest Research UK have developed a forwarder-mounted spray rig fitted with water tanks to apply nematodes to clearfelled sites (Figure 10). Using this rig, the nematode species *Steinernema carpocapsae* has been used on a semi-operational level against the large pine weevil in the UK for a number of years. Seedling death due to adult weevil



Figure 10: Application of entomopathogenic nematodes to stumps using a forwardermounted spray rig devised by Forest Research UK.

feeding in the UK was reported less than 5% on *S. carpocapsae* treated sites, compared with 45-85% on sites where nematodes were not applied (Stuart Heritage pers. comm. 2008). In 2007, the nematode was applied to 150 hectares of Coillte forests. The cost in 2007 of applying 3.5 million *Steinernema carpocapsae* nematodes per stump using this technology was approximately  $\in$ 500 per hectare. Researchers at NUIM have demonstrated that another

nematode species, *Heterorhabditis downesi*, consistently performs better than *Steinernema carpocapsae* on both pine and spruce stumps (Dillon et al. 2006, 2008a). The average reduction in the number of adult pine weevil emerging from stumps treated with *Heterorhabditis downesi* and *Steinernema carpocapsae* was 70 and 37%, respectively (Figure 11). The commercialisation of this the nematode is being assessed.



Figure 11: Percentage reduction in the number of adult pine weevil emerging from spruce or pine stumps in a single year following treatment of stumps with entomopathogenic nematodes at a dose of 3.5 million nematodes/stump.

# Environmental safety of nematodes in forestry

Entomopathogenic nematodes have been trialled and used in agriculture and forestry in many countries for several decades and they have an excellent record of environmental safely. However, before nematodes are widely used in Irish forests it is wise to assess any potential negative impacts. This is considered under two headings:

## Will nematodes negatively impact on stumpinhabiting insects other than the large pine weevil?

In the laboratory, certain stages of the parasitic wasp, *Bracon hylobii*, are susceptible to infection by entomopathogenic nematodes, but infected *Bracon* have rarely been found in excavated stumps in nematode trials. Fully formed cocoons are not susceptible, so careful timing of nematode application should minimise any negative impact on populations of this wasp.

Other stump-inhabiting insects, such as longhorn beetles (*Cerambycidae*), play an important role in nutrient recycling and forest biodiversity. To date no significant negative effects have been observed on longhorn beetle

populations developing in nematode-treated stumps, and researchers at NUIM are continuing to monitor trial sites and test the susceptibility of longhorn beetles.

### Will nematodes establish within the forest ecosystem and negatively impact on populations of non stump-inhabiting insects?

As nematodes reproduce within insects, they can remain around the stump as long as there are suitable insect hosts. Following their application to pine stumps in Ireland, nematodes persisted at high levels for 2 years, but began to decrease by year 3 (Figure 12). By this time (4.5 years after felling), stumps no longer contained immature pine weevils as hosts for nematode multiplication, so nematodes had probably started to die of starvation. Four years after application, nematodes were recovered from only 8% of stumps, which corresponds to the natural level of nematode prevalence in untreated forests in Ireland (Dillon et al. 2008b). Nematodes were never recovered outside the treated area, confirming that dispersal is limited. When nematodes are applied as a spot-treatment around the stump for pine weevil control, the risk to non stump-inhabiting insects such as bees and ladybirds must be considered minimal.



# Conclusions

Insect killing nematodes are the most promising environmentally sustainable way of reducing the number of adult pine weevils emerging onto a site. The nematode *Steinernema carpocapsae* has been successfully used against the large pine weevil in the UK for a number of years. Whether the more virulent species *Heterorhabditis downesi* can be developed commercially is under investigation.

On lodgepole pine sites with exceptionally high weevil populations, nematodes alone may not be sufficient in every case to prevent unacceptable levels of damage to seedlings, though they undoubtedly have a role to play in an integrated pest management programme. Combined with delayed planting and the use of robust planting stock, nematodes may offer a realistic affordable alternative to chemical insecticides as a means of controlling pine weevil populations.

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